Sjögren's syndrome

Challenges of a multifaceted disease

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CONTENTS

CHAPTER 1

General introduction

Primary Sjögren's syndrome (pSS) is a chronic systemic autoimmune disease, characterised by chronic inflammation of the salivary and lacrimal glands[1]. The prevalence of pSS is estimated to be 61 cases per 100.000 inhabitants, with a strong predominance in women[2]. The highly heterogeneous presentation of pSS includes a wide range of local and systemic symptoms and multi-organ involvement, varying over time. As the presenting symptoms of pSS are often non-specific, a large delay often occurs before patients are correctly diagnosed. Patients with pSS consider sicca symptoms (xerostomia and keratoconjunctivitis sicca), fatigue and pain (arthralgia and tendomyalgia) the main symptoms of their disease[3]. Although these symptoms are sometimes considered 'benign', since they are non-life threatening, these symptoms can be severely disabling. As a result, patients with pSS show markedly reduced health-related quality of life (HR-QoL) and lower employment rates[4].

Extraglandular and systemic manifestations of pSS include arthritis, Raynaud's phenomenon, vasculitis, cytopenia, pulmonary involvement, nephritis, myositis, and neurological involvement. Patients with pSS are at increased risk of developing lymphoma, in particular mucosa-associated lymphoid tissue (MALT) lymphoma in the parotid glands[5].

Pathogenesis

The pathogenesis of pSS is complex and has not been fully elucidated. In genetically and hormonally predisposed individuals, viral infections or endogenous factors are thought to trigger the initiation of an inflammatory autoimmune response, involving both the innate and the adaptive immune system[6]. Besides viral infections, the gut and/or oral microbiome presumably play a role in the development of pSS[7].

Epithelial cells are likely involved in the initiation and maintenance of glandular inflammation in pSS. After being damaged by a viral infection or other trigger, salivary gland epithelial cells act as a target of autoimmune disease, possibly by forming a source of SSA and SSB auto-antigens. Furthermore, epithelial cells serve important immunological functions, by producing cytokines and chemokines, and acting as antigen presenting cells[8]. In response to toll-like receptor stimulation or endogenous triggers, epithelial cells and local dendritic cells produce inflammatory cytokines, including type 1 interferons, and chemokines. Interferon is a major driver of the production of the chemokine CXCL10, and the cytokines B-cell Activating Factor (BAFF) and A Proliferation-Inducing Ligand (APRIL). CXCL10 causes recruitment of lymphoid cells to the glandular tissue. BAFF and APRIL are important cytokines involved in B-cell survival and proliferation. In early stages of the disease, the resulting lymphoid infiltrates consist mostly of $CD4^+$ helper T-cells, which initiate a positive feedback loop by producing pro-inflammatory cytokines and inducing B-cell activation[9]. In this early stage, focal lymphoid infiltrates develop around the striated ducts. In later stages, the peri-ductal infiltrates become organized and form ectopic lymphoid tissue, in response to expression of homeostatic lymphoid chemokines. The ectopic lymphoid tissue contains all elements to

carry out autoimmune responses. A major B-cell attracting chemokine involved in ectopic lymphoid tissue in the formation in the glandular tissue of pSS patients is CXCL13. In more severe disease, T-cell dependent activation of B-cells causes formation of germinal centers in the exocrine glands[8].

B-cell hyperactivity is a hallmark of pSS, reflected amongst others by the presence of increased serum IgG levels, presence of autoantibodies such as antinuclear antibodies (including anti-SSA and SSB antibodies) and rheumatoid factor, and presence of cryoglobulins. pSS is associated with polymorphisms of genes involved in B-cell receptor (BCR) signalling, and pSS patients show higher levels of molecules involved in BCR signalling such as Bruton's tyrosine kinase (BTK)[8].

The mechanisms that cause salivary gland dysfunction in pSS are still unclear. Although the chronic inflammatory infiltrate in the salivary and tear glands contributes to damage and gland hypofunction, the presence of a glandular infiltrate is not significantly associated with the presence of sicca symptoms[10]. Potential mechanisms underlying the glandular dysfunction are the presence of anti-muscarinic autoantibodies, altered mucin expression, nitric oxide-mediated salivary gland dysfunction, altered aquaporin-5 distribution, and presence of anti-aquaporin-5 autoantibodies[11]. Extraglandular manifestations of pSS can be the result of lymphocytic invasion in epithelial tissues, such as in interstitial lung disease, interstitial nephritis, and/or the result of immune complex deposition, such as in vasculitis, peripheral neuropathy and glomerulonephritis[12].

Vaginal dryness and sexual dysfunction

Besides sicca symptoms of the eyes and mouth, dryness of the skin and other mucosal surfaces may be present in pSS. Vaginal dryness and pain during intercourse are common symptoms of pSS[13–19]. Little is known about the pathogenesis of vaginal dryness in pSS. In healthy women, vaginal dryness is often caused by decreased estrogen levels after menopause, leading to vulvovaginal atrophy. In pSS however, vaginal dryness often already occurs before menopause[13,17,20]. These observations suggest that although menopause may worsen symptoms of vaginal dryness in women with Sjögren's syndrome, other factors are likely to be important in the pathogenesis of these symptoms.

Besides vaginal dryness and dyspareunia, other symptoms of pSS may negatively influence sexual function, such as fatigue, myalgia and arthralgia. Previous studies have reported a high prevalence of sexual dysfunction in rheumatologic disorders[21]. Maddali Bongi et al.[17] reported that 62% of pSS patients rated sexual activity as important and 68% stated that symptoms of pSS affected their sexual ability, which shows that the impact of pSS on sexual function should not be overlooked. However, data on sexual function in women with pSS are scarce.

Classification and stratification

Diagnosis and classification of pSS are challenging due to the heterogeneous presentation of the disease. As a gold standard for diagnosis of pSS does not exist, diagnosis and classification of pSS are based on the interpretation of several tests and observations. Many different classification criteria sets for pSS have been in use. Until a few years ago, the 2002 American-European Consensus Group (AECG) criteria were most frequently used by researchers and clinicians[22]. The AECG criteria include 2 subjective items (sicca symptoms of the eyes and mouth) and 4 objective items (presence of functional impairment of the salivary and lacrimal glands, presence of anti-SSA or SSB antibodies and a focus score of ≥1 in the salivary gland biopsy). The focus score is defined as the number of mononuclear cell infiltrates in the salivary gland parenchyma containing at least 50 inflammatory cells in a 4 mm^2 section. In 2012, Shiboski et al.[23] proposed new classification criteria, which were designed to select the right patients for clinical trials with biological disease-modifying anti-rheumatic drugs (DMARDs) and provisionally approved by the American College of Rheumatology (ACR). The provisional ACR criteria included focus score, serology and ocular staining score (OSS). By including only objective items, the authors aimed to increase the specificity of the ACR criteria in comparison to older criteria sets. However, these criteria were less feasible than the AECR criteria, as an ophthalmologist who is qualified to determine the OSS is not always available in rheumatology clinics. In 2016, the International Sjögren's Syndrome Criteria Working Group developed the ACR-European League against Rheumatism (EULAR) criteria for pSS using methodology endorsed by both the ACR and EULAR, to reach international consensus regarding the classification criteria, and allow comparison of results between trials[24,25]. The ACR-EULAR criteria combine items from the AECG and ACR criteria and use a weighted scoring system, which gives three points for a focus score ≥1 and positive anti-SSA antibodies, and one point for a decreased unstimulated whole salivary flow, decreased Schirmer's test, or increased ocular staining score (table 1). Patients with a score of ≥4 are classified as pSS.

Table 1. ACR-EULAR criteria for primary Sjögren's syndrome[23]

Item	Weight
Focal lymphocytic sialadenitis and focus score ≥1	3 points
Anti-SSA/Ro positive	3 points
Ocular staining score ≥5 in at least 1 eye	1 point
Schirmer's test ≤5mm/5min in at least 1 eye	1 point
Unstimulated whole salivary flow rate ≤0.1ml/min	1 point

A point of criticism regarding the ACR-EULAR criteria is that they are not actually new criteria, but a reshuffling of items from older criteria sets, and did not improve classification of pSS in comparison to the AECG criteria[26]. The Schirmer's test and assessment of unstimulated whole saliva flow rate are easy to perform, but do not differentiate between pSS and other causes of xerostomia and keratoconjunctivitis sicca[27,28]. Salivary gland ultrasound (SGUS)

has been proposed as an alternative test to support diagnosis and classification of pSS. Ultrasonography has many advantages; it is non-invasive, non-irradiating, inexpensive and can be repeated for follow up. SGUS has shown good diagnostic properties for diagnosing pSS, with a pooled sensitivity of 69% and specificity of 92%[29,30], and good inter- and intra-observer reliability[31–33]. A simple scoring system, examining hypoechogenic areas in one parotid and submandibular gland, shows sufficient validity to predict classification of pSS patients[34]. Using a simple scoring system increases the feasibility of SGUS. As many rheumatologists already use musculoskeletal ultrasound, use of SGUS can be easily incorporated in rheumatologic outpatient clinics. However, the value of adding salivary gland ultrasound to classification criteria for pSS, or replacing current items with salivary gland ultrasound, has not yet been studied.

Even when the same classification criteria are used in trials, there can be big differences in the characteristics of study populations, depending on the population from which patients are selected, the tests used to diagnose patients with pSS, and other inclusion criteria. A recent study illustrated the heterogeneity of pSS by performing hierarchical cluster analysis to stratify patients based on their symptoms[35]. Four subgroups of pSS patients were identified: low symptom burden, high symptom burden, dryness dominant with fatigue and pain dominant with fatigue. These four phenotypical groups showed distinct clinical and biological profiles. This may explain discrepancies between outcomes of studies which have similar inclusion criteria, as patients with different phenotypes may also respond differently to therapies. In order to be able to compare different study populations, we should therefore search for biomarkers and clinical characteristics which determine the phenotype of a patient and which may predict response to therapy. Ideally, these characteristics should be reliable and easy to evaluate, which makes SGUS a promising tool for clinical phenotyping of pSS patients.

Systemic treatment

Although better understanding of the pathogenesis of pSS has offered many possible targets for intervention, systemic treatment options for pSS remain limited. Traditional DMARDs (including corticosteroids, hydroxychloroquine, methotrexate, azathioprine, mycophenolate and ciclosporin A), and anti-TNF therapy have either shown limited effects or high rates of adverse events, making them unsuitable for long-term treatment[36]. Several biologic DMARDs, including rituximab and abatacept, have shown promising results in pSS, but none have yet been approved[36,37]. Treatment of the majority of pSS patients is therefore still focused on symptom relieve, but this approach is often insufficient to reduce disabling symptoms of dryness, fatigue and pain.

The large variation in primary and secondary outcomes used in clinical trials in pSS makes it difficult to compare trials and draw conclusions regarding the efficacy of therapies in pSS. Until recently, few trials evaluated the effect of treatment on extraglandular symptoms[37]. In

2013, two complementary indices were developed: the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI)[3,38]. The ESSDAI is completed by physicians and scores systemic disease activity, whereas the ESSPRI is patient-reported and measures the main symptoms of pSS[39]. The ESSDAI and ESSPRI are now used in most trials in pSS and have shown adequate sensitivity to change[40]. Determination of the minimal clinical important improvement in ESSDAI (a decrease of ≥3 points from baseline) and ESSPRI (a decrease of ≥1 point or 15% from baseline) have made it possible to define response according to the ESSDAI and ESSPRI[41].

Abatacept is a fully human biological DMARD consisting of cytotoxic T-lymphocyte antigen 4 (CTLA-4) coupled to the Fc tail of IgG. CTLA-4 binds to the co-stimulatory molecules CD80 and CD86 on antigen presenting cells. By blocking the co-stimulatory signal provided by antigen presenting cells, abatacept inhibits activation of T-cells and T-cell dependent B-cell hyperactivity (figure 1). Abatacept can be administered as intravenous or subcutaneous injections and has shown beneficial effects and a good safety profile in rheumatoid arthritis and polyarticular juvenile idiopathic arthritis[42,43]. In systemic lupus erythematosus (SLE), clinical trials of abatacept have failed to achieve their primary outcome, but abatacept may be effective in treating arthritis and nephritis[44]. In our open label trial of intravenous abatacept in 15 pSS patients with short disease duration and active disease, improvements were seen in ESSDAI and ESSPRI, fatigue, and HR-QoL[45]. Another small open label study also showed beneficial effects of intravenous abatacept in pSS, although no validated clinical or patient reported outcome measurements were used[46]. In a recent open label trial, pSS patients were treated with intravenous abatacept for 24 months, after which they showed improvement of salivary flow and ESSDAI score[47]. Abatacept decreased the number and activation of circulating follicular T-helper (Tfh) cells[48], and attenuated B-cell activity, as reflected by decreased autoantibodies levels, circulating plasmablasts and levels of BTK in B-cells[49]. These results warrant further investigation of the efficacy and safety of abatacept in a randomised controlled trial (RCT).

Considering the prominent role of B-cell hyperactivity in the pathogenesis of pSS, the efficacy of targeting of CD20 expressing B-cells by rituximab has been studied in several open label trials and RCTs. Although most studies showed promising results, two larger RCTs did not reach their primary endpoint[50,51]. Consequently, there is no consensus regarding the efficacy of rituximab in pSS, and treatment with rituximab is currently reserved for patients with severe organ involvement. However, considering the beneficial effect on several clinical, biological and histological outcomes, and results from post-hoc analyses which have identified patients who may benefit from treatment[52], rituximab is still worth further investigation.

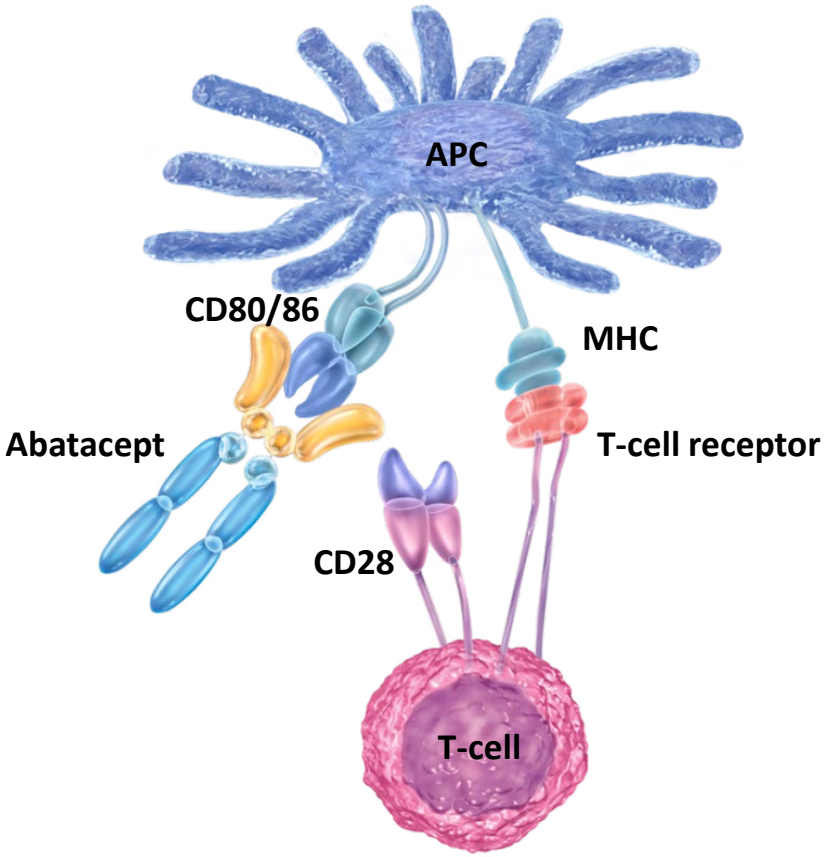

Figure 1: Abatacept mechanism of action.
Abatacept binds to CD80/86 on antigen presenting cells, thereby preventing binding of CD80/86 to CD28 on T-cells, which is a co-stimulatory signal needed for activation of the T-cell. APC=antigen presenting cell. MHC=major histocompatibility complex. Adapted and reproduced from training presentation with permission of Bristol-Myers Squibb.

GENERAL AIM AND OUTLINE

The general aim of this thesis was to improve the understanding and management of pSS, focusing on three topics. First, the prevalence and pathogenesis of vaginal sicca symptoms and sexual dysfunction in pSS were explored. Second, new tools to classify patients with pSS were evaluated: the new ACR-EULAR classification criteria and SGUS. Finally, the efficacy and safety of abatacept treatment and other systemic treatment options for pSS were assessed.

Part one of this thesis describes the impact of pSS on vaginal dryness and sexual dysfunction, and explores the pathogenesis of vaginal dryness in pSS. **Chapter 2** describes a case-control study in which the self-reported sexual function of 46 patients and 43 healthy controls was compared. Within the group of pSS patients, the relationship between sexual dysfunction and other psychosocial aspects of pSS was studied. **Chapter 3** describes a translational study exploring the pathogenesis of vaginal dryness in pSS, by quantifying and comparing immunological and histopathological markers in the cervix and vagina of 9 pSS patients and 8 controls. In **chapter 4**, the vaginal microbiome of the same group of pSS patients and controls is compared.

Part two of this thesis focuses on the classification and stratification of patients with suspected or confirmed pSS. In **chapter 5**, the validity of the recently developed ACR-EULAR classification criteria was evaluated in a cohort of patients clinically suspected with pSS. **Chapter 6** assesses whether addition of salivary gland ultrasound to the ACR-EULAR classification criteria influences the validity of these criteria. In **chapter 7**, SGUS is used to determine the clinical phenotype of patients who have been diagnosed with pSS and participate in a longitudinal registry.

Part three of this thesis discusses the efficacy and safety of systemic treatment options for pSS. **Chapter 8** presents the results of a randomised controlled trial of abatacept treatment in pSS, the Abatacept Sjögren Active Patients phase III (ASAPIII) trial. In **chapter 9,** the biological and clinical efficacy of rituximab is reviewed. **Chapter 10** discusses the safety profile of several systemic treatment options for pSS.

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PART I

Vaginal dryness and sexual dysfunction

CHAPTER 2

The impact of primary Sjögren's syndrome on female sexual function

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ABSTRACT

Objective. Prevalence of vaginal dryness and dyspareunia is high in women with primary SS (pSS). Our aim was to compare sexual function and sexual distress in women with pSS with healthy controls, as well as to assess parameters that are associated with sexual dysfunction and distress in pSS.

Methods. Forty-six women fulfilling the American-European Consensus Group criteria for pSS (mean age 46.3 years, S.D. 10.5) and 43 age-matched healthy controls were included. Participants completed self-administered questionnaires, namely the Female Sexual Function Index (FSFI), Female Sexual Distress Scale (FSDS), Multidimensional Fatigue Inventory (MFI), Hospital Anxiety and Depression Scale (HADS), Maudsley Marital Questionnaire (MMQ) and RAND 36-item Health Survey (RAND-36). In addition, the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI) and Patient Reported Index (ESSPRI) were recorded in patients.

Results. Women with pSS had impaired sexual function compared with healthy controls (median FSFI 20.6 vs. 30.3, $p<0.001$), as reflected by significantly lower scores in the domains of desire, arousal, orgasm, lubrication and pain. Furthermore, pSS patients experienced more sexual distress (median FSDS 7 vs. 4, $p<0.05$) and were sexually active less frequently than controls (76% vs. 93%, $p<0.05$). Sexual dysfunction correlated significantly with patient-reported symptoms of pSS (ESSPRI), symptoms of fatigue (MFI), depressive symptoms (HADS), relationship dissatisfaction (MMQ) and lower mental quality of life (RAND-36), but not with systemic disease activity (ESSDAI).

Conclusion. Women with pSS have impaired sexual function and more sexual distress compared with healthy controls. Sexual function and distress are influenced by vaginal dryness and patient-reported symptoms of pSS as well as psychosocial factors.

INTRODUCTION

Primary SS (pSS) is the second most common systemic autoimmune disease, with a female:male ratio of 9:1[1]. pSS is characterized by sicca symptoms of the eyes and mouth, together with a variety of extraglandular symptoms such as disabling fatigue and arthritis. Besides these well-known symptoms, women with pSS often experience vaginal dryness and dyspareunia[2–5]. Chronic dyspareunia can even be the presenting symptom of pSS[6].

Of all sicca symptoms in pSS, vaginal dryness has the greatest impact on quality of life[7]. The pathogenesis of vaginal dryness in pSS is not known, but a possible explanation is local inflammation of the vaginal mucosa[8].

Sexual health is considered an important aspect of physical and mental health and is associated with general well-being and satisfaction with life[9]. Previous studies reported female sexual dysfunction in 24-74% of patients with rheumatic disorders[10,11]. Sexual function is influenced by physical as well as psychological consequences of rheumatic diseases, such as pain, fatigue, stiffness, functional impairment, depression, anxiety, negative body image, reduced libido, hormonal imbalance and side effects from treatments[12]. In pSS, vaginal dryness and dyspareunia may provide an extra barrier to the enjoyment of sexual activity[13]. Recently Maddali Bongi et al.[14] found that 62% of patients with pSS rated sexual activity as important. However, 68% of the patients reported alterations in their sexual ability because of the symptoms of pSS, especially vulvar or vaginal dryness, dyspareunia and reduced sexual drive.

Data on sexual function in women with pSS are scarce. Previous studies have focused on the prevalence of vaginal sicca symptoms and dyspareunia or frequency of intercourse rather than on the whole concept of sexual function. Furthermore, the aetiology of sexual dysfunction in pSS is unclear. Therefore the aim of this study was to evaluate sexual dysfunction, sexual distress and vaginal complaints in women with pSS compared with healthy controls. In addition, it was assessed whether systemic disease activity, patient-reported symptoms and psychosocial consequences of pSS are associated with sexual dysfunction and distress.

PATIENTS AND METHODS

Between March and August 2013, 78 women with pSS were invited to join this study by an information letter. Of the 60 patients who were interested in joining the study and who were willing to receive questionnaires, 46 patients completed and returned the questionnaires, giving a final response rate of 59%. Patients who responded did not differ significantly from non-responders in age, disease duration, patient-reported symptoms or systemic disease

activity. Age-matched controls were recruited by an advertisement and by asking women in a general physician's office. In total, 120 healthy controls were interested in joining the study and received the information letter and questionnaires, of which 43 returned the completed questionnaires, giving a response rate of 36%. Participants were between 18 and 60 years of age. Patients fulfilled the American-European Consensus Group criteria for pSS[15]. Exclusion criteria were the presence of another rheumatic or systemic autoimmune disease or FM. The study was approved by the Medical Research Ethics Committee of the University Medical Center Groningen (METc2012.292). All participants provided written informed consent in compliance with the Declaration of Helsinki.

Assessment methods

Participants completed a number of self-administered questionnaires. Sexual function was measured by the 19-item Female Sexual Function Index (FSFI). The FSFI measures sexual function in six subdomains: desire, arousal, orgasm, lubrication, satisfaction and pain. Higher scores indicate better sexual function. The total score is calculated as the sum of the six domain scores and has a range of 2-36[16]. A cut-off score <26.55 has been proposed to indicate sexual dysfunction[17]. The FSFI differentiates between sexual intercourse and sexual activity, which includes masturbation. Question 15 asks how satisfied participants are with the sexual relationship with their partner. In retrospect, we added a not applicable option for participants without a partner who did not answer question 15[18]. Psychological distress caused by sexual dysfunction was measured with the 12-item Female Sexual Distress Scale (FSDS). Higher FSDS scores indicate more sexual distress[19]. The Dutch version of the FSFI and FSDS showed good psychometric qualities[20]. In addition, participants were asked whether they had experienced vaginal itching and vaginal infections in the past year, whether they were still menstruating and whether pSS patients had ever talked to their rheumatologist about sexual problems, and if not, for what reason.

Disease activity was measured with the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI) and Patient Reported Index (ESSPRI) in pSS patients[21,22]. The ESSDAI is a physician-administered systemic disease activity index that measures extraglandular symptoms of pSS. The ESSDAI was recorded during a routine visit of the patient to the rheumatologist or nurse practitioner in the inclusion period. The median time period between the visit to the outpatient clinic and returning the questionnaire was 28 days (interquartile range 7-48 days). The ESSPRI is a self-report questionnaire consisting of three numerical rating scales to assess symptoms of dryness, pain and fatigue, which represents the burden of disease. Together, the ESSDAI and ESSPRI give a clear picture of objective and subjective signs of disease activity[23].

The Multidimensional Fatigue Inventory (MFI) was used to measure five main dimensions of fatigue: general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity. Higher MFI scores indicate more fatigue[24]. Anxiety and depression were measured

with the Hospital Anxiety and Depression Scale (HADS), which has been developed specially for somatic populations. Higher HADS scores indicate more psychological symptoms[25,26]. Relationship satisfaction was measured with the general relationship satisfaction subscale of the Maudsley Marital Questionnaire (MMQ)[27]. The RAND 36-item Health Survey (RAND-36) was used to assess health-related quality of life. The RAND-36 includes eight domain scores, which can be converted to a physical and mental component score[28].

Statistical analysis

Sample size calculation was performed using data from a Dutch study concerning sexual function in women with SSc, an autoimmune disease, the symptoms of which partially overlap with pSS. Moreover, it is the only Dutch population with a systemic autoimmune disease for which FSFI scores are available. In this study, the healthy control group had a mean FSFI score of 27.6 (S.D. 6.2)[29]. A difference of 5 points in the FSFI score is considered clinically relevant. A sample size of 78 achieves 80% power to detect a difference of 5 points between the null hypothesis (mean of 27.6) and the alternative hypothesis (mean of 22.6) with an estimated S.D. of 7.5 and a significance level (a) of 0.05 using a two-sided Mann-Whitney U-test. To correct for missing data, 10% extra patients were included, yielding a final required sample size of 86 (43 pSS patients and 43 healthy controls).

Descriptive statistics were calculated for all variables. Independent samples t-test, Mann-Whitney U-test, chi-square test and Fisher's exact test were used as appropriate to compare differences between groups. A subanalysis was performed excluding participants who scored zero on certain FSFI questions because of sexual inactivity in the past 4 weeks, as advised by Meyer-Bahlburg et al.[18]. Furthermore, a subgroup analysis was performed according to menstrual status.

Spearman's correlation coefficients were used to evaluate the relationships between the FSFI score, FSDS score and other outcome measures. Patients and healthy controls who did not have intercourse were excluded from the FSFI correlations because the reasons for not having sexual intercourse may differ from the reasons for sexual dysfunction. Variables that correlated significantly with the FSFI total score were entered in a multivariable linear regression model. In case residuals were non-normally distributed, parameters were transformed (log, square root or logit) before being entered into the equation. Statistical analyses were executed using SPSS Statistics 20 (SPSS, Chicago, IL, USA).

RESULTS

All sociodemographic and disease characteristics are shown in table 1. No significant differences were found in age, menstrual status, relationship status or education level between pSS patients and healthy controls. Patients used NSAIDs more often and were in paid employment less frequently than controls.

Table 1. Sociodemographic and disease characteristics in patients with pSS and healthy controls

	pSS (n=46)	Controls (n=43)	P-value
Age, mean (S.D.), years	46.3 (10.5)	44.4 (11.3)	0.419
Disease duration, mean (S.D.), years	7 (4-14)	NA	
ESSDAI score (range 0-123), median (IQR)	5 (2-7)	NA	
ESSPRI total score (range 0-10), median (IQR)	6.9 (4.7-7.4)	NA	
ESSPRI subscale score (range 0-10), median (IQR)			
Dryness	6 (4-8)	NA	
Fatigue	8 (5-8)	NA	
Pain	7 (2-8)	NA	
Medication, n (%)			
NSAIDs	18 (39)	1 (2)	**0.000**
Antidepressants, anxiolytics	7 (15)	4 (10)	0.420
Antihypertensives	9 (20)	7 (16)	0.687
OCP or contraceptive injection	1 (2)	5 (12)	0.103
Corticosteroids	5 (11)	0	
HCQ	8 (17)	0	
Pilocarpine	3 (7)	0	
Postmenopausal status, n (%)	20 (44)	12 (28)	0.126
Relationship, n (%)	36 (78)	35 (81)	0.713
Education level, n (%)			0.166
Low (primary, 0-8 years)	3 (7)	1 (2)	
Medium (secondary, 9-16 years)	28 (62)	20 (47)	
High (higher vocational/university, ≥17 years)	14 (31)	22 (51)	
Paid employment, n (%)	22 (52)	38 (91)	**0.000**

Significant P-values are presented in bold. Missing values were 6% for employment status and <5% for all other parameters. ESSDAI: European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; ESSPRI: European League Against Rheumatism Sjögren's Syndrome Patient Reported Index; IQR: Interquartile range; OCP: oral contraceptive pill.

Sexual function and vaginal complaints

The FSFI total score and FSFI subscale scores for desire, arousal, orgasm, lubrication and pain were significantly lower in patients compared with healthy controls, indicating worse sexual function in pSS patients (table 2 and figure 1). These differences remained significant after

excluding participants who did not have intercourse or were not sexually active in the past 4 weeks (data not shown). Furthermore, patients with pSS had significantly more distress related to sexual dysfunction than sexually healthy controls. Fewer patients were sexually active in the past 4 weeks compared with controls (76% vs. 93%, p<0.05), whereas no significant difference was found for sexual intercourse in the past 4 weeks (72% vs. 81%, p> 0.05). After excluding participants who were inactive, more patients had impaired sexual function than healthy controls (56% vs. 27%, p<0.05). As shown in table 2, more patients with pSS used lubricants. There were no significant differences between the proportion of patients and healthy controls with complaints of vaginal itching or vaginal infections during the last year.

Subgroup analysis according to menstrual status revealed that the FSFI total score was significantly lower in premenopausal patients compared with premenopausal healthy controls (median 19.5 vs. 30.3, p<0.01), whereas the difference between postmenopausal patients and healthy controls was not statistically significant (median 21.7 vs. 28.7, P=0.24).

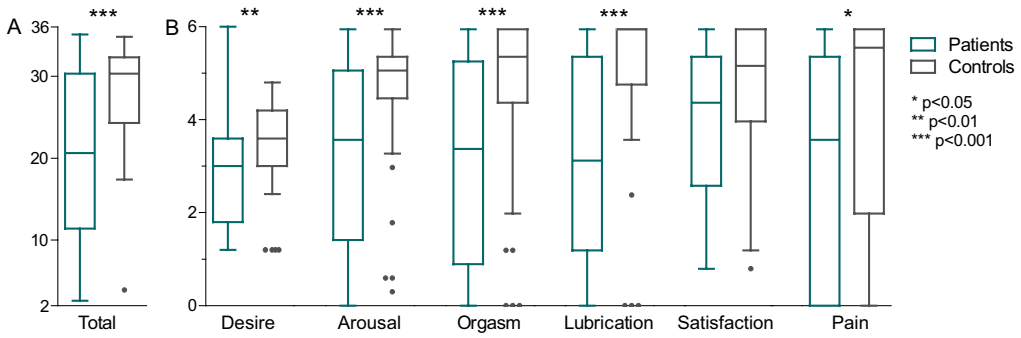

Figure 1. FSFI (A) total and (B) subscale scores in patients with pSS and healthy controls.
Box-and-whisker plots (Tukey): boxes indicate medians with IQRs, whiskers indicate 1.5 times the interquartile distances, • indicate outliers.

Psychological characteristics, symptoms of pSS and quality of life

Patients with pSS had higher scores in all five domains of the MFI as well as higher HADS depression and anxiety scores compared with controls, indicating more symptoms of fatigue, depression and anxiety in patients (table 2). Patients had higher MMQ scores than healthy controls, indicating that they were less satisfied with their relationship, although this difference was not statistically significant. The RAND-36 physical and mental component scores were lower in patients with pSS than healthy controls. In patients, subjective symptoms of pSS (ESSPRI) were positively correlated with the HADS anxiety score (r=0.355, p<0.05) and HADS depression score (r=0.410, p<0.01) and negatively correlated with the RAND mental component score (r=0.517, p<0.001).

Table 2. Sexual function and distress, vaginal symptoms, psychological symptoms, fatigue, relationship dissatisfaction and quality of life in patients and healthy controls

	pSS (n=46)	Controls (n=43)	P-value
FSFI total score (range 2-36)	20.6 (11.4-30.3)	30.3 (24.3-32.3)	**0.000**
FSFI subscale scores			
Desire (range 1.2-6)	3.0 (1.8-3.6)	3.6 (3.0-4.2)	**0.008**
Arousal (range 0-6)	3.6 (1.4-5.1)	5.1 (4.5-5.4)	**0.000**
Orgasm (range 0-6)	3.4 (0.9-5.3)	5.4 (4.4-6.0)	**0.001**
Lubrication (range 0-6)	3.2 (1.2-5.4)	6.0 (4.8-6.0)	**0.000**
Satisfaction (range 0.8-6)	4.4 (2.6-5.4)	5.2 (4.0-6.0)	0.052
Pain (range 0-6)	3.6 (0.0-5.4)	6.0 (2.8-6.0)	**0.010**
FSDS (range 0-44)	7 (1-23)	4 (0-8)	**0.023**
Use of lubricant, n (%)	16 (35)	6 (14)	**0.023**
Vaginal itching complaints, n (%)	21 (46)	12 (28)	0.083
Vaginal infection in last year, n (%)	14 (30)	10 (23)	0.446
MFI (range 4-20)			
General fatigue	16 (13.0-18.3)	10 (7.0-13.0)	**0.000**
Physical fatigue	15 (11.8-17.3)	6 (5.0-12.0)	**0.000**
Reduced activity	12 (7.0-15.0)	8 (5.0-10.0)	**0.000**
Reduced motivation	9 (6.8-12.0)	6 (5.0-10.0)	**0.014**
Mental fatigue	10 (6.0-14.5)	6 (4.0-12.0)	**0.016**
HADS (range 0-21)			
Anxiety	5 (3.0-9.0)	3 (1.0-6.0)	**0.044**
Depression	4 (1.8-6.0)	2 (0.0-3.0)	**0.000**
MMQ (range 0-80)[a]	10 (4.0-16.0)	5 (2.0-12.0)	0.077
RAND-36 (range 0-100)			
Physical component score	37.3 (29.6-47.5)	55.2 (49.4-58.0)	**0.000**
Mental component score	49.4 (36.9-51.4)	53.9 (47.7-56.8)	**0.002**

Values are presented as median (IQR) unless otherwise indicated. Significant P-values are presented in bold. Missing values were 7% for the FSFI total score and <5% for all other parameters. [a]The MMQ (marital subscale) was not filled out by 10 patients and 8 controls because they were not in a relationship. FSDS: Female Sexual Distress Scale; FSFI: Female Sexual Function Index; HADS: Hospital Anxiety and Depression Scale; MFI: Multidimensional Fatigue Inventory; MMQ: Maudsley Marital Questionnaire; RAND-36: RAND 36-item Health Survey.

Parameters related to sexual dysfunction in patients with pSS

Reduced sexual function, as indicated by a lower FSFI total score, was associated with more patient-reported symptoms of pSS (ESSPRI), reduced motivation and mental fatigue (MFI), depressive symptoms (HADS), relationship dissatisfaction (MMQ) and lower mental quality of life (RAND-36 mental component score), but was irrespective of disease duration and disease activity of pSS (ESSDAI; table 3). Only the HADS depression score was significantly related to sexual dysfunction in multivariable regression analysis (table 4).

More sexual distress, as indicated by a higher FSDS total score, was associated with more patient-reported symptoms of pSS (ESSPRI), all five domains of fatigue (MFI), symptoms of anxiety and depression (HADS), relationship dissatisfaction (MMQ) and lower mental quality of life (RAND-36 mental component score), but was irrespective of disease duration or extraglandular symptoms (ESSDAI; table 3). Relationship status, education level, paid employment, menstrual status and the presence of vaginal itching complaints or vaginal infections did not have a significant effect on sexual dysfunction or distress (data not shown).

Communication with rheumatologist

Thirty-one patients with pSS (67%) never talked about sexual complaints with their rheumatologist. Of these 31 patients, 18 patients (58%) had an FSFI score <26.55, implying that these patients did not talk about sexual complaints despite having sexual dysfunction. The main reasons why patients with low FSFI scores never talked about their sexual complaints with their rheumatologist were that the subject was never brought up by their rheumatologist (n=5), the complaints were not severe enough (n=3), the use of lubricants solved their problems (n=2) or the patient did not have a sexual relationship (n=2).

Table 3. Relation of sexual function and sexual distress with patient characteristics and clinical assessments in patients with pSS

	FSFI[a] (n=33)	P-value	FSDS (n=46)	P-value
Age	-0.349	0.050	0.219	0.148
Disease duration	-0.139	0.447	0.077	0.616
ESSDAI	0.065	0.723	-0.069	0.651
ESSPRI total	-0.378	**0.033**	0.504	**0.000**
Dryness	-0.129	0.480	0.239	0.114
Fatigue	-0.162	0.376	0.305	**0.041**
Pain	-0.273	0.130	0.365	**0.014**
MFI				
General fatigue	-0.223	0.219	0.344	**0.021**
Physical fatigue	-0.328	0.067	0.366	**0.014**
Reduced activity	-0.309	0.091	0.399	**0.007**
Reduced motivation	-0.444	**0.011**	0.545	**0.000**
Mental fatigue	-0.389	**0.028**	0.474	**0.001**
HADS				
Anxiety	-0.293	0.104	0.342	**0.021**
Depression	-0.555	**0.001**	0.411	**0.005**
MMQ	-0.526	**0.004**	0.340	**0.045**
RAND-36				
Physical component score	0.049	0.791	-0.066	0.666
Mental component score	0.365	**0.040**	-0.444	**0.002**

Significant p-values are presented in bold. [a]Patients who did not have sexual intercourse in the past four weeks were excluded (n=13). ESSDAI: European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; ESSPRI: European League Against Rheumatism Sjögren's Syndrome Patient Reported Index; FSDS: Female Sexual Distress Scale; FSFI: Female Sexual Function Index; HADS: Hospital Anxiety and Depression Scale; MFI: Multidimensional Fatigue Inventory; MMQ: Maudsley Marital Questionnaire; RAND-36: RAND 36-item Health Survey.

Table 4. Multivariable linear regression model of parameters associated with FSFI total score in patients with pSS (n=30)

	b	95% CI	P
ESSPRI total score[a]	4.867	9.418, 19.151	0.486
MFI reduced motivation	0.186	0.640, 1.011	0.645
MFI mental fatigue[b]	0.394	0.900, 0.111	0.120
HADS depression score[c]	21.377	36.062, 6.692	**0.006**
MMQ relationship satisfaction[a]	1.784	3.902, 0.333	0.094
RAND-36 mental component score	15.196	2.879, 33.272	0.095

Patients who had not had intercourse in the past 4 weeks and/or did not have a partner were excluded (n=16). Significant P-values are presented in bold. Adjusted R2=0.385. [a]Reverse and logarithmic transformation. [b]Logarithmic transformation. [c]Square root transformation. ESSPRI: European League Against Rheumatism Sjögren's Syndrome Patient Reported Index; FSFI: Female Sexual Function Index; HADS: Hospital Anxiety and Depression Scale; MMQ: Maudsley Marital Questionnaire; RAND-36: RAND 36-item Health Survey.

DISCUSSION

The present study demonstrated that women with pSS have significantly more sexual dysfunction in the domains of desire, arousal, orgasm, lubrication and pain compared with healthy controls. We found that 56% of the patients had sexual dysfunction, which is comparable to the proportion in a large SLE cohort[30]. Furthermore, patients experienced more sexual distress and a higher proportion of patients were sexually inactive. Of all domains of the FSFI, lubrication showed the greatest difference between groups, and women with pSS used lubricant more often than healthy controls, confirming that vaginal dryness is a frequent symptom in pSS[2–5]. Vaginal dryness, leading to dyspareunia, can play a major role in sexual dysfunction.

Besides vaginal dryness, our results showed that a variety of physical and psychological consequences of pSS are linked to sexual dysfunction. Sexual dysfunction in pSS was associated with more patient-reported symptoms of pSS as measured with the ESSPRI, fatigue, symptoms of depression, relationship dissatisfaction and lower mental quality of life, but not with systemic manifestations as measured by the ESSDAI. The association with patient-reported symptoms of pSS was even more clear for sexual distress. Apparently, subjective symptoms of pSS play a larger role in sexual dysfunction than objective signs of systemic involvement. However, since sexual dysfunction and distress are established by subjective measurements (FSFI and FSDS), one might expect that the association with patient-reported symptoms of pSS (ESSPRI) would be larger than the association with an objective index of disease activity (ESSDAI). The ESSPRI and ESSDAI are complementary indices that are weakly correlated with each other[23]. It would be interesting to evaluate the association between the ESSDAI score and objective measurements of vaginal inflammation and lubrication.

When performing multivariable regression analysis, only depression remained significantly correlated with the FSFI score, and therefore appears to be the most important predictor of sexual dysfunction. Depression is known to contribute to sexual dysfunction and might be a confounder in the reported association between subjective symptoms of pSS and sexual dysfunction[31]. In concordance with earlier studies, our results showed that patients with pSS have more depressive symptoms than healthy controls and that depression is associated with higher ESSPRI scores[32,33]. However, the results of self-report questionnaires evaluating depressive symptoms, such as the HADS, should be interpreted with caution in patients with pSS. The HADS includes questions about reduced motivation, which could be a symptom of depression, but is also related to fatigue. As our results confirm, patients with pSS often suffer from severe fatigue[34].

In the subgroup analysis of the FSFI stratified according to menopausal status, we found a significant difference in the FSFI total score between premenopausal patients and healthy controls. In postmenopausal patients, the influence of pSS might be (partly) concealed by other factors that influence sexual function, such as changing hormone levels. Nevertheless, we do see a trend towards a lower FSFI score in postmenopausal patients, which might become significant in an adequately powered sample.

Our study showed that the majority of patients with sexual dysfunction rarely talked about sexual complaints with their rheumatologist. The sexual health of patients with rheumatic diseases is often neglected, as both patients and physicians may find it difficult to address sexual complaints, partly because effective treatment options are not yet available. More knowledge about the pathogenesis and treatment of vaginal dryness and sexual dysfunction in pSS will make this subject easier to discuss. However, by simply acknowledging and discussing these complaints, rheumatologists can help patients cope with their sexual problems. If necessary, patients can be referred to a gynaecologist or sexologist. As for other sicca symptoms of pSS, patients can be offered local symptomatic treatment of vaginal dryness with lubricants, topical oestrogens and moisturizers. Treatment with biologics such as rituximab and abatacept have a beneficial effect on disease activity, fatigue and quality of life and thus may also improve sexual function[35–37]. Future trials on systemic treatment of pSS with biologic therapies or other DMARDs should include sexual function as an outcome measurement.

This study has some limitations. First, a selection bias cannot be excluded, although there were no differences in age or disease characteristics between responders and non-responders in the patient group. Unfortunately we do not have any information about the non-responders in the control group. Another limitation is that the FSFI was validated in a population with stable sexual relationships. In our study, patients without a relationship were also included because these patients can still be sexually active. However, excluding patients who were

sexually inactive did not change the results. Finally, the multivariable analysis of predictors of the FSFI score was intended to be exploratory and should be interpreted with caution due to the relatively small number of patients.

In conclusion, women with pSS experience significantly more sexual dysfunction and distress than healthy controls. Sexual function in pSS is influenced by physical barriers such as vaginal dryness, pain and fatigue, as well as psychological consequences of the disease. This study shows that sexual dysfunction should not be ignored in pSS patients. Asking about sexual complaints is important, since many patients will not bring up the subject themselves. Research is needed regarding the pathogenesis and development of a treatment for vaginal dryness and sexual dysfunction in pSS. Such knowledge will increase awareness among rheumatologists and supports research into tailored intervention strategies with a multi-disciplinary approach.

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CHAPTER 3

Vaginal dryness in primary Sjögren's syndrome: a histopathological case-control study

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ABSTRACT

Objectives. To study clinical, histopathological and immunological changes in the vagina and cervix of women with primary Sjögren syndrome (pSS), which may explain vaginal dryness.

Methods. We included 10 premenopausal female pSS patients with vaginal dryness, and 10 premenopausal controls undergoing a laparoscopic procedure. The Vaginal Health Index was recorded. Multiplex immunoassays and flow cytometry were performed on endocervical swab and cervicovaginal lavage samples to evaluate cellular and soluble immune markers. Mid-vaginal and endocervical biopsies were taken and stained for various leucocyte markers, caldesmon (smooth muscle cells), ERG (endothelial cells) and anti-podoplanin (lymphatic endothelium). The number of positive pixels/μm^2 was calculated.

Results. One patient was excluded because of chlamydia, and 2 controls because of endometriosis observed during their laparoscopy. Vaginal health was impaired in pSS. CD45+ cells were increased in vaginal biopsies of women with pSS compared to controls. Infiltrates were predominantly located in the peri-epithelial region, and mostly consisted of CD3+ lymphocytes. In the endocervix, CD45+ infiltrates were present in patients as well as in controls, but a higher number of B-lymphocytes was seen in pSS. Vascular smooth muscle cells were decreased in the vagina of pSS patients. No differences were found in leucocyte subsets in the vaginal and endocervical lumen. CXCL10 was increased in endocervical swab samples of pSS patients.

Conclusion. Women with pSS show impaired vaginal health and increased lymphocytic infiltration in the vagina compared to controls. Vaginal dryness in pSS might be caused by vascular dysfunction, possibly induced by interferon-mediated pathways.

INTRODUCTION

Primary Sjögren syndrome (pSS) is a systemic autoimmune disease with a heterogeneous presentation, including sicca symptoms, systemic symptoms such as fatigue, and extraglandular involvement[1]. A hallmark of pSS is lymphocytic infiltration of the salivary and lacrimal glands. Besides sicca symptoms of eyes and mouth, vaginal dryness is common in women with pSS, which causes dyspareunia and sexual dysfunction[2–6]. While usually vaginal dryness occurs after menopause, in pSS vaginal dryness often occurs at younger age[7–9]. Two studies evaluating vaginal health in pSS reported erythema of the vaginal epithelium[10,11], while others did not find any macroscopic changes of the vagina and cervix[4,8]. In a previous study, we did not observe changes in the vaginal microbiome in pSS[12].

The pathophysiology of vaginal dryness in pSS is still unknown. Normally, the vaginal surface is humidified and lubricated by transudate from the lamina propria, which contains rich venous and lymphatic networks, as well as by mucus produced by the endocervical glandular epithelium[13]. In premenopausal pSS patients with dyspareunia, lymphocytic infiltrates were found in the stroma underlying the vaginal epithelium[2,14]. Further, chronic cervicitis was observed in biopsies of 42% of pSS patients[11]. Local inflammation may influence production of transudate from blood vessels in the vagina, or compromise the function of the mucus-producing glandular epithelium of the endocervix.

In previous studies, few or no healthy controls were included, and no quantitative analyses were performed. As leucocytes are physiologically present in the vagina and cervix of healthy women[15–17], quantitative analysis and comparison with a control group are necessary to assess whether the lymphocytic infiltration observed in pSS is indeed pathological. Furthermore, changes in the vascularization of the vagina were not taken into account as a possible cause of vaginal dryness.

To identify appropriate treatment for vaginal dryness in pSS, the pathogenesis of this symptom needs to be elucidated. The objective of this study was therefore to assess clinical and histopathological changes in the vagina and cervix of women with pSS compared to controls, which may explain vaginal dryness. We also explored whether possible inflammatory changes in the vagina and cervix of pSS patients were reflected by changes in immune cells and effector molecules in the vaginal lumen.

PATIENTS AND METHODS

Study population

In a prospective exploratory case-control study, we included 10 women with pSS who fulfilled ACR-EULAR criteria and reported vaginal dryness. We also included 10 age-matched controls without systemic autoimmune diseases who were scheduled for a laparoscopic procedure. To eliminate the influence of physiological hormonal changes to the vaginal mucosa, only pre-menopausal patients and controls were included. Other inclusion criteria were age ≥18 and written informed consent. Exclusion criteria were pregnancy or breast-feeding, presence of inflammatory or infectious gynaecological disease, previous chemotherapy, current use of an intra-uterine contraceptive device, hormone replacement therapy or vaginal oestrogen supplementation, and use of systemic corticosteroids or DMARDs ≤6 months before inclusion. The study complies with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the University Medical Center Groningen (METC 2015/039).

Study procedures

Participants were instructed not to have sexual intercourse, or use tampons, lubricants or any other vaginal products within 72 hours before the study visit. On the day of examination, participants completed a questionnaire including the Female Sexual Function Index (FSFI) and questions about comorbidities, medication use, smoking status, vaginal symptoms, and presence of vaginal bacterial or fungal infections in the past year. In pSS patients, the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), and the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) were recorded. Blood samples were obtained.

Gynaecological examination was performed by an experienced gynaecologist. The 5 domains of the vaginal health index (VHI: elasticity, fluid secretion, pH, epithelial mucosa, moisture) were scored on a 1-5 scale, resulting in a total score of 5-25 (supplementary table 1)[18]. Cervicovaginal lavage (CVL) samples were collected by flushing 7 mL of phosphate-buffered saline (PBS) over the cervix and vagina, aspirating the PBS and then repeating the procedure[19]. Endocervical swab (ES) samples were collected by rotating eSwabs (Copan diagnostics, Murrieta, CA) in the endocervical canal. The eSwabs were put in 5 mL of PBS. CVL and ES samples were immediately put on ice.

Another eSwab, suspended in eSwab transport medium, was used for PCR to detect *Chlamydia trachomatis* and *Neisseria gonorrhoea*. A vaginal secretion sample was collected for fungal culture. ThinPrep Pap tests (Hologic, Marlborough, MA) were performed on cervical samples collected with a Cervex brush (Rovers Medical Devices, Oss, the Netherlands).

Finally, full-thickness mid-vaginal and endocervical punch biopsies were collected, after administration of local anaesthesia in pSS patients or general anaesthesia in controls. Vaginal and cervical biopsies were fixed in 4% paraformaldehyde and embedded in paraffin.

Evaluation of vaginal and endocervical biopsies

Vaginal and endocervical tissue sections were stained for H&E, periodic acid-Schiff diastase (PAS-D) and various leucocyte markers (CD45, CD3, CD4, CD8, CD20). Tissue sections were also stained for blood/lymphatic vessel-associated markers: avian V-ets erythroblastosis virus E26 oncogene homolog (ERG) which is a nuclear stain for endothelial cells, anti-podoplanin (clone D2-40) which stains lymphatic endothelium, and caldesmon which stains smooth muscle cells present in the tunica media of arterioles and larger venules. Endocervical tissue sections were additionally stained for CD138, as many plasma cells were seen in H&E stained tissue sections.

H&E and PAS-D stained sections were examined by a dedicated gynaecopathologist to check for gynaecological morbidity and fungal infections. Immunohistologically stained sections were quantitatively analysed by counting the number of diaminobenzinine-stained pixels/μm^2 of parenchyma, using the Positive Pixel Count algorithm (version 9.1) in Aperio ImageScope v12.1 (Aperio Technologies). For CD4, only strong positive pixels were counted, to exclude non-specific staining. The epithelial layer was excluded for analysis of endothelial markers and CD138, as no blood or lymphatic vessels are present in the epithelium, and CD138 is expressed by stratified squamous epithelium. To quantify vaginal atrophy, epithelial thickness and number of cell layers were counted at 40x magnification, in three areas of the biopsy in which the epithelium was thinnest and no dermal papillae were present. The mean epithelial thickness and number of cell layers were calculated.

Evaluation of cellular and soluble immune markers

Serum was frozen at -80°C. EDTA whole blood was lysed with ammonium chloride and centrifuged. The supernatant was discarded and cells were washed and suspended in FACS buffer in a concentration of 10^6 cells/ml. To collect endocervical material, the swabs containing ES samples were gently scraped on the edge of the Falcon tubes in which they were kept after collection. The ES and CVL samples were then resuspended and centrifuged, after which the supernatant was frozen at -80°C and cells were resuspended in FACS buffer at a concentration of 10^6 cells/ml.

Flow cytometry analysis of leucocyte subsets in cells from whole blood, ES and CVL was performed on the day of collection of the samples. Cells were washed and stained with antibodies directed against leucocyte markers (supplementary table 2), after which they were washed and resuspended in FACS buffer. Shortly before analysis, cells were stained with propidium iodide (eBioscience) and passed through a 35 µm nylon mesh. Antibody panel optimization and titrations were performed in cells from whole blood, and confirmed in ES and CVL cells. Fluorescence-minus-one controls were included to determine background fluorescence. Data were acquired using a LSRII flow cytometer (BD Biosciences). Data were analysed using FlowJo (Tree Star). The gating strategy is described in supplementary figure 1.

Serum samples and supernatants of the CVL and ES samples were thawed and analysed for levels of APRIL (a proliferation-inducing ligand), BAFF (B-cell activation factor), IFN-γ, RANK-L, TNF-α, CCL2, CCL4, CX3CL, CXCL9, CXCL10, CXCL11, CXCL13, IL-6, IL-7, IL-8, and IL-17A, using a human magnetic Luminex© premixed 16-plex assay (R&D Systems, Minneapolis, USA), according to the manufacturer's protocol. Data were acquired on a Luminex© 200 system.

Statistical analysis

Statistical analyses were executed using SPSS Statistics 23 (SPSS, Chicago, IL, USA). Mann-Whitney U-test, Chi-Square test or Fisher's exact test were used as appropriate to compare differences between groups. Spearman's correlation coefficients were used to evaluate correlations. P values of <0.05 were considered to indicate statistical significance.

RESULTS

Clinical characteristics

One pSS patient was excluded due to presence of Chlamydia trachomatis. Two controls were excluded due detection of endometriosis during laparoscopy, as the pathogenesis of endometriosis comprises immunological changes[20] and an association between endometriosis and pSS has been described[21,22]. Characteristics of remaining participants are shown in supplementary table 3. Median age was 36 (IQR 33-46) for pSS patients (n=9) and 41 (IQR 36-44) for controls (n=8). All pSS patients had a positive salivary gland biopsy (focus score ≥1), and 7 (78%) were anti-SSA antibody positive. Median ACR-EULAR score was 9 (ICR 5-9) and median ESSDAI 6 (ICR 3-9).

Gynaecological symptoms and examination

Compared to controls, patients with pSS showed lower FSFI scores (indicating sexual dysfunction), used lubricants more often, and had increased prevalence of superficial dyspareunia (table 1). The VHI score was significantly lower in pSS patients, indicating impaired vaginal health (table 1, figure 1). Of VHI subdomains, the mucosa score was significantly decreased in pSS, indicating frailty and a higher bleeding tendency of the epithelium. Upon inspection of the vulva, vagina and cervix, no major abnormalities were found. Some redness of the vulva was noted in 3 pSS patients. One patient with active cutaneous vasculitis on her legs showed petechiae on the labia majora. Superficial vulvar rhagades were seen in 3 patients and 1 control. Vaginal pH did not differ significantly between groups and none of the participants showed signs of vaginal atrophy.

Table 1. Patient reported and gynaecological outcomes

	pSS (n=9)	Controls (n=8)	P-value
Patient reported outcomes			
Sexual inactivity in past 4 weeks	3 (33)	2 (25)	1.000
FSFI (range 2-36)[a]	22.2 (21.0-28.7)	30.6 (29.6-34.5)	**0.026**
Desire (range 1.2-6)	3.3 (2.6-3.6)	3.6 (3.0-5.0)	0.310
Arousal (range 0-6)	4.5 (2.9-5.7)	5.4 (4.7-5.7)	0.394
Lubrication (range 0-6)	4.4 (1.9-5.2)	5.9 (5.4-6.0)	**0.004**
Orgasm (range 0-6)	5.2 (4.1-5.7)	6.0 (5.4-6.0)	0.093
Satisfaction (range 0.8-6)	5.2 (4.2-5.6)	5.2 (4.8-6.0)	0.485
Pain (range 0-6)	3.2 (1.6-4.5)	6.0 (5.1-6.0)	**0.009**
Vaginal dryness (NRS, range 0-10)	5.0 (5.9-7.0)	1.0 (0.0-1.8)	**0.001**
Use of lubricants	5 (56)	0	**0.029**
Dyspareunia	9 (100)	2 (25)	**0.002**
Deep, during intercourse	4 (44)	1 (13)	0.294
Superficial, during intercourse	7 (78)	0 (0)	**0.002**
After intercourse	4 (44)	1 (13)	0.294
Vaginal or vulvar symptoms in past 2 weeks	8 (89)	3 (38)	0.050
Vaginal itching	3 (33)	1 (13)	0.576
Burning sensation vagina/vulva	4 (44)	1 (13)	0.294
Reeking vaginal discharge	4 (44)	1 (13)	0.294
Abnormal vaginal discharge	3 (33)	1 (13)	0.576
Vaginal infections in past year	2 (22)	4 (50)	0.335
Gynaecological examination			
VHI (range 5-25)	19.0 (16.5-21.5)	23.0 (20.3-24.5)	**0.015**
Elasticity (range 1-5)	4.0 (3.5-4.5)	4.0 (4.0-5.0)	0.321
Fluid secretion (range 1-5)	4.0 (3.0-5.0)	5.0 (4.3-5.0)	0.074
Moisture (range 1-5)	4.0 (3.0-5.0)	5.0 (4.3-5.0)	0.139
pH (range 1-5)	5.0 (4.0-5.0)	4.5 (4.0-5.0)	0.673
Mucosa (range 1-5)	3.0 (3.0-3.0)	5.0 (3.5-5.0)	**0.008**
Vaginal pH	4.4 (4.1-4.9)	4.6 (4.4-4.7)	0.606

Data are presented as median (IQR), or n (%), unless stated otherwise. [a]For FSFI analysis, patients who were not sexually active in the past 4 weeks were excluded (3 pSS patients and 2 controls). FSFI: Female Sexual Function Index; NRS: Numeric Rating Scale.

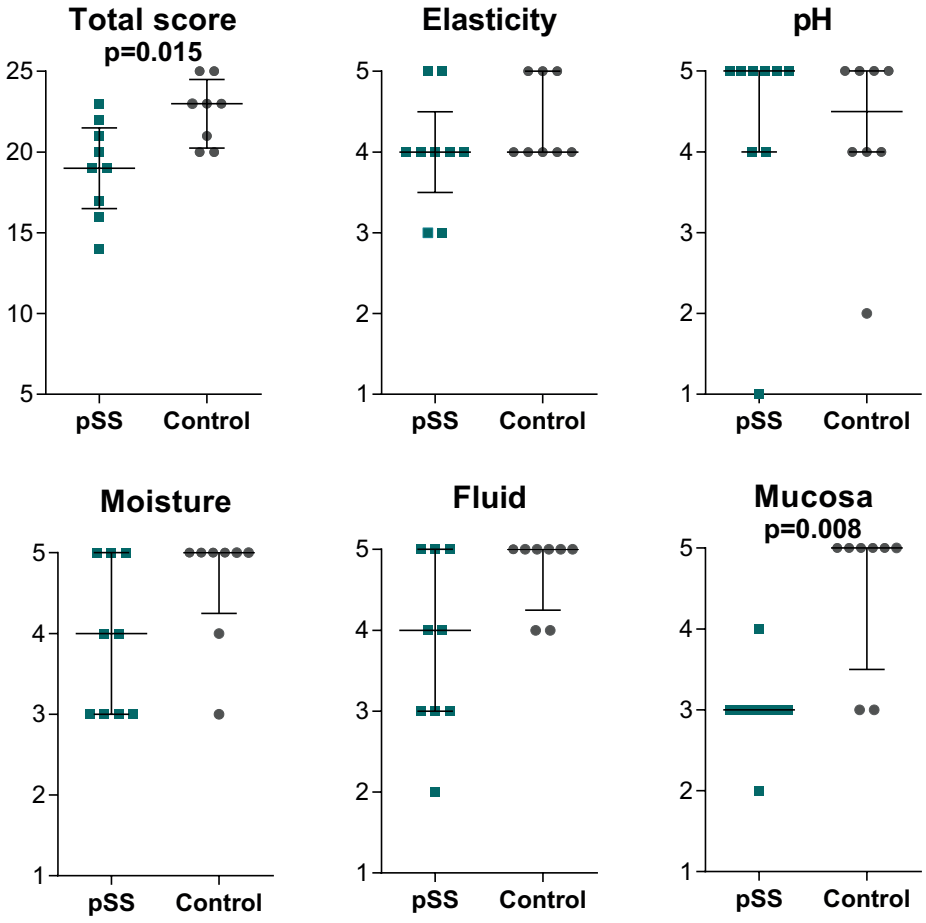

Figure 1. Vaginal health index in patients and controls.
Low scores correspond to low vaginal health.

Histological findings

No major abnormalities or fungal infections were found in vaginal or cervical H&E and PAS-D stained tissue sections. One vaginal biopsy from a control was excluded from further analysis, as it was very superficial, consisting of 98% epithelium. Three pSS patients and two controls were excluded from analysis of endocervical biopsies, because only ectocervical tissue or mucus was collected due to difficulties reaching the endocervical tissue through the external cervical ostium.

No significant differences were found in the number of cell-layers (patients: median 25, IQR 21-33; controls: median 25, IQR 20-26) or thickness (patients: median 251 µm, IQR 197-271; controls: median 243, IQR 142-252) of the vaginal epithelium.

Lymphocytic infiltration in vagina and endocervix

Compared to controls, vaginal tissue from pSS patients contained significantly higher numbers of CD45$^+$ cells (table 2, figure 2). Lymphocytic infiltrates in pSS patients were mainly located in the lamina propria just below the epithelium (peri-epithelial layer), with a peri-epithelial localization and aggregates in dermal papillae (figures 3 and 4). Of all leucocyte subsets, only CD3$^+$ lymphocytes were significantly increased in the vagina. In endocervical tissue sections, there was no significant difference in total numbers of CD45$^+$ cells, albeit that the number of CD20$^+$ B-lymphocytes was significantly higher in pSS patients (table 2, figure 2). Lymphocytic infiltration in the endocervix was also mostly located in the peri-epithelial layer (figures 3 and 4).

Endothelial changes in vagina and cervix

To explore whether blood vessels and lymphatic vessels in vagina and endocervix are affected in pSS, we stained for endothelial markers (supplementary figure 2). The number of caldesmon$^+$ cells was significantly lower in vaginal biopsies of women with pSS, indicating a decrease in vascular smooth muscle cells (table 2, figure 2). There seemed to be a tendency towards an increase in number of lymphatic endothelial cells (D2-40) in pSS. No significant differences were found in other endothelial markers in the vagina or endocervix.

Table 2. Quantitative analysis of leucocyte and endothelial markers in the vagina and endocervix

	Vagina			Endocervix		
	pSS (n=9)	**Control (n=7)**	**P-value**	**pSS (n=6)**	**Control (n=6)**	**P-value**
CD45	0.34 (0.26-0.53)	0.26 (0.12-0.27)	**0.012**	1.12 (0.45-1.82)	0.60 (0.32-2.97)	1.000
CD3	0.49 (0.28-0.56)	0.19 (0.12-0.27)	**0.008**	0.66 (0.38-1.28)	0.44 (0.20-1.57)	0.485
CD4	0.23 (0.14-0.34)	0.13 (0.12-0.32)	0.470	0.66 (0.25-1.21)	0.34 (0.24-1.25)	1.000
CD8	0.48 (0.32-0.99)	0.34 (0.22-0.51)	0.210	1.00 (0.73-1.49)	0.64 (0.28-2.05)	0.485
CD20	0.22 (0.17-0.47)	0.20 (0.14-0.40)	0.837	0.53 (0.44-2.45)	0.32 (0.25-0.55)	**0.041**
ERG	0.23 (0.17-0.26)	0.26 (0.18-0.28)	0.470	0.50 (0.41-0.78)	0.67 (0.23-0.87)	0.818
Caldesmon	0.06 (0.03-0.07)	0.11 (0.07-0.21)	**0.031**	0.15 (0.06-0.57)	0.14 (0.05-0.30)	0.818
D2-40	0.11 (0.06-0.26)	0.06 (0.04-0.09)	0.210	0.30 (0.12-0.41)	0.20 (0.09-0.27)	0.240
CD138[a]	ND	ND	ND	1.03 (0.17-2.01)	0.22 (0.11-2.87)	0.792

Values are median (IQR) number of positive pixels/μm^2. [a]CD138 was analysed in 6 patients and 5 controls, as one control did not show representative endocervical tissue in the CD138 stained tissue section. ERG: avian V-ets erythroblastosis virus E26 oncogene homolog; D2-40: anti-podoplanin (clone D2-40); ND: Not done.

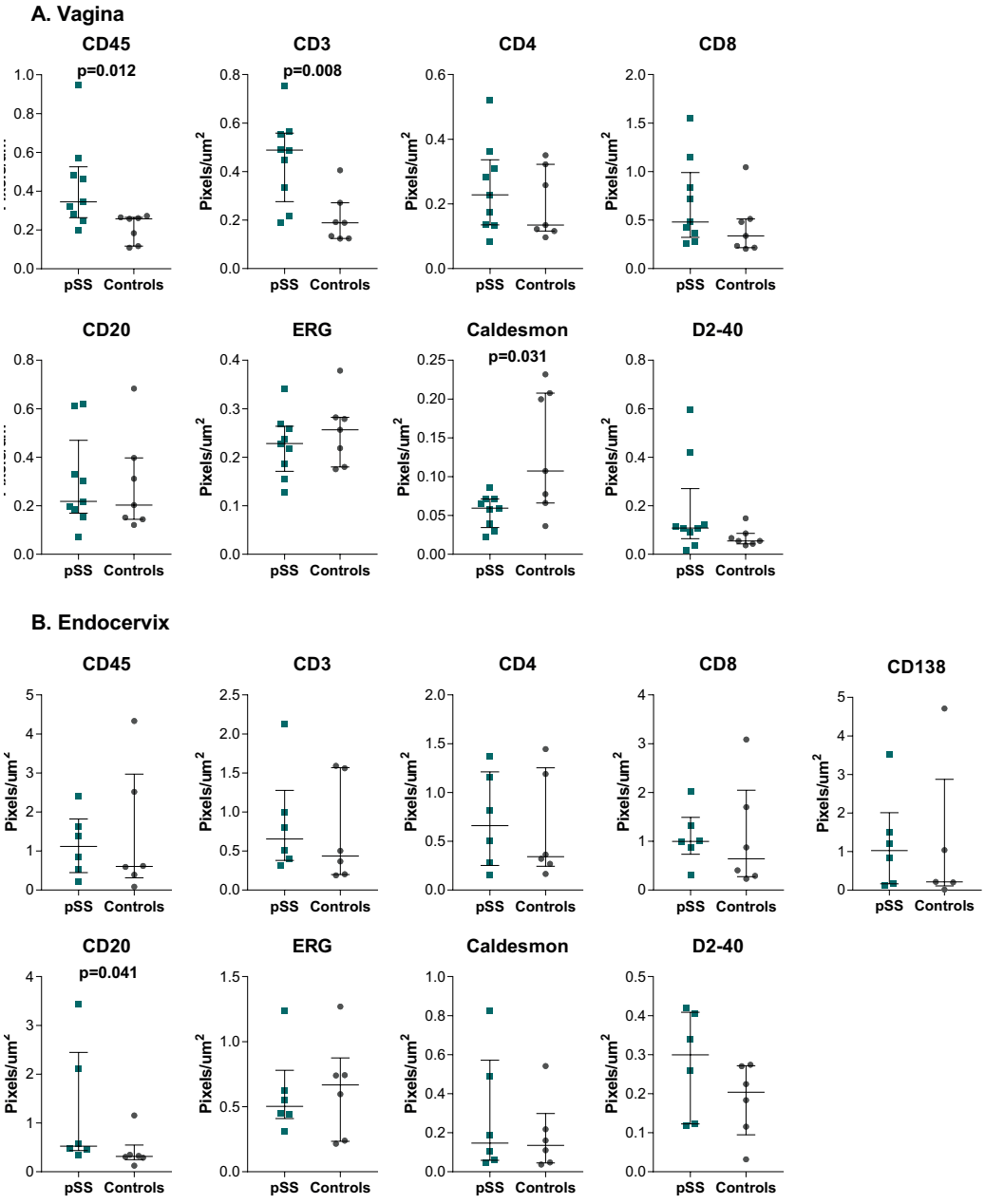

Figure 2. Quantification of leucocyte subsets and markers for blood and lymphatic vessels.
Markers for leucocyte subsets, blood and lymphatic vessels are expressed as number of positive pixels/µm^2, in vaginal (A) and endocervical (B) tissue in patients with primary Sjögren's syndrome and controls. ERG: avian V-ets erythroblastosis virus E26 oncogene homolog. D2-40: anti-podoplanin (clone D2-40).

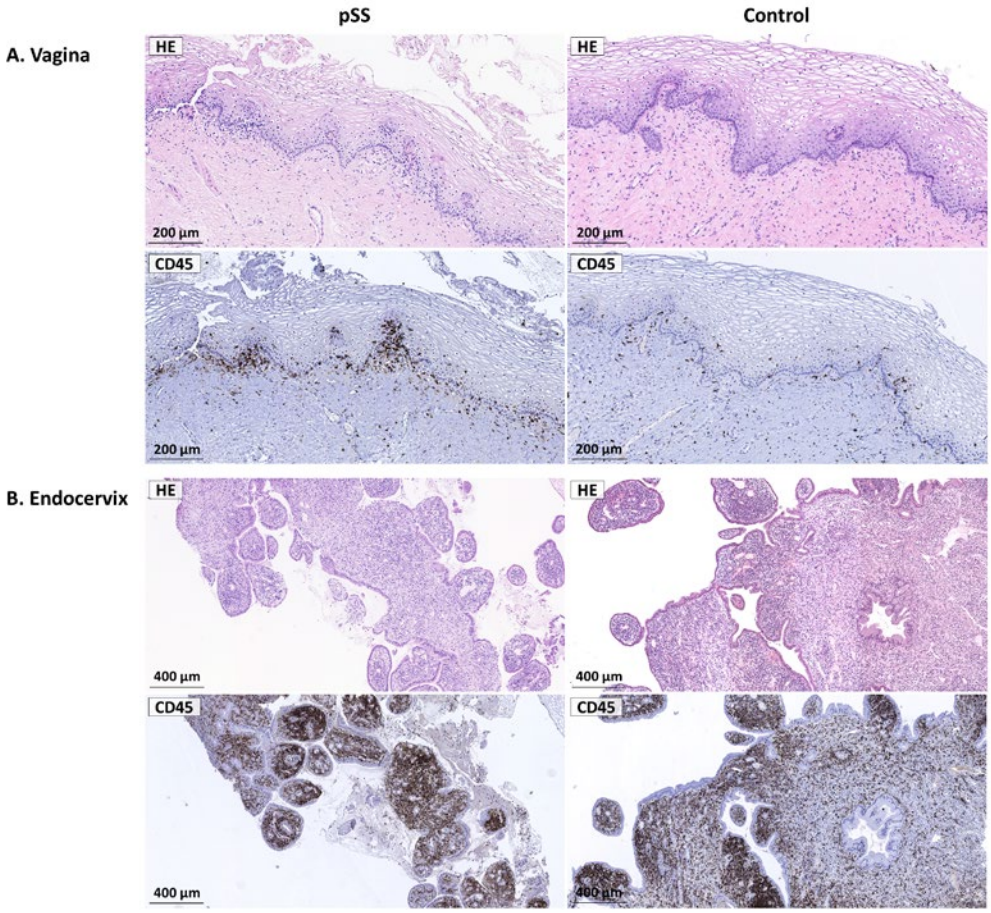

Figure 3. Hematoxylin and eosin (HE) and CD45 stains.
Examples are shown of the vaginal and endocervical tissue of a pSS patient and a control.

Immune markers in blood, CVL and ES

Next, we explored whether the histological changes in the vagina and endocervix are reflected by cellular and soluble immune markers in the lumen. No differences were found in the proportion of leucocyte subsets in CVL or ES (supplementary table 4).

A significantly higher level of CXCL10 was found in ES samples of patients with pSS (supplementary table 5). No other significant differences in chemokine or cytokine levels of patients and controls were found in ES or CVL samples. In serum, CXCL10 and CXCL11 were significantly increased in pSS patients. Within the group of pSS patients, a strong correlation was seen between CXCL10 in ES and CXCL10 in serum (ρ=0.717, p=0.03), and between CXCL10 in serum and number of CD45+ positive cells in the vagina (ρ=0.667, p=0.05). Levels of IFN-γ, IL-17A, CCL4, CX3CL, and CXCL9 were below detection limits in serum, CVL as well as ES in most patients.

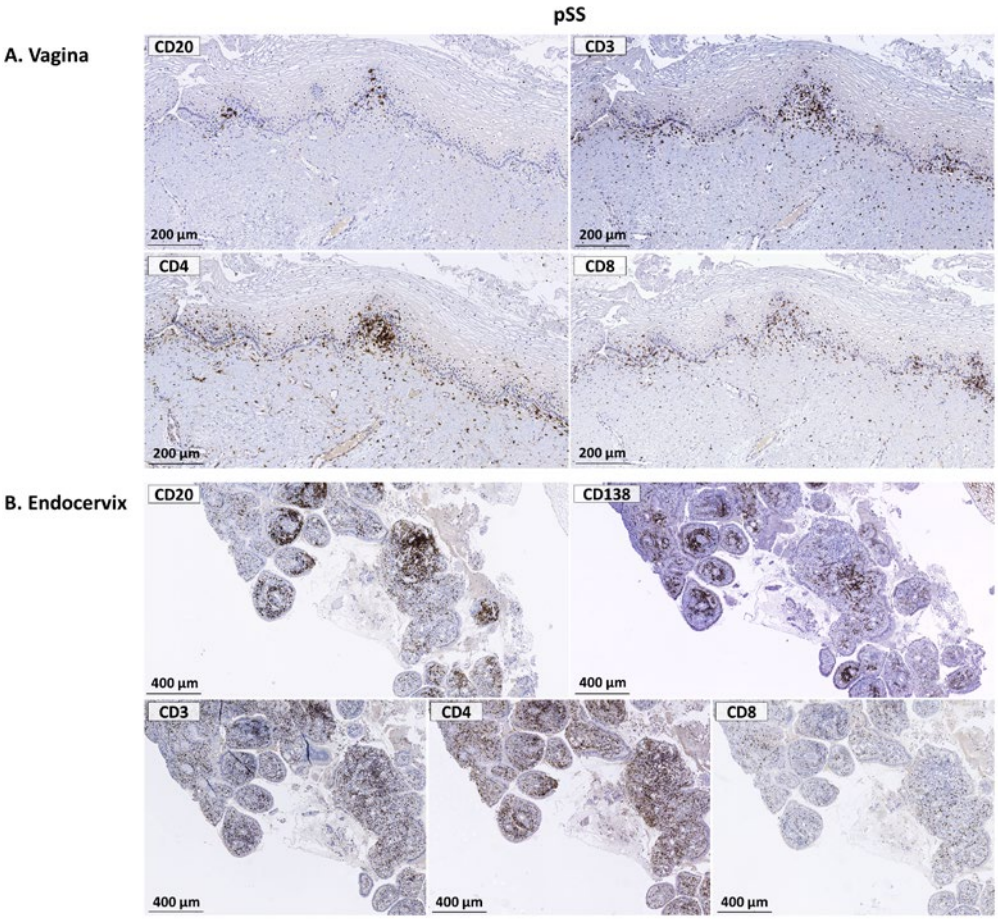

Figure 4. Lymphocyte subsets in the vagina and endocervix.
An example is shown of the vaginal and endocervical tissue of the same pSS patient as shown in figure 3.

DISCUSSION

Women with pSS and vaginal sicca symptoms often experience sexual dysfunction and dyspareunia. We observed that women with pSS have impaired vaginal health and increased bleeding tendency of the vaginal epithelium. Furthermore, we found a peri-epithelial infiltration and decreased number of vascular smooth muscle cells in the vaginal wall of pSS patients, which likely contribute to vaginal dryness. In contrast to post-menopausal women, the vaginal dryness in women with pSS cannot be explained by atrophic vaginitis, as no signs of atrophy or increased pH were found.

Our study provides the first in-depth, quantitative evaluation of immunological and histopathological markers in the cervicovaginal mucosa of a well-defined group of pSS

patients, compared to healthy controls. By including only pre-menopausal patients, matching patients for age and screening for infections we minimized the influence of confounders. We found higher numbers of infiltrating CD45+ cells in vaginal biopsies of pSS patients, with a peri-epithelial localization and aggregates in dermal papillae. This difference in CD45+ cells seems to be largely due to CD3+ T-cells. Although both CD4+ and CD8+ T-cells were present in peri-epithelial infiltrates, neither were significantly overrepresented in pSS patients. The exact phenotype of the infiltrating CD3+ T-cells in the vagina of pSS patients remains to be established. In the endocervix, CD45+ infiltrates were present in patients as well as controls, but with a higher number of B-lymphocytes in pSS patients. The vaginal and endocervical epithelium remained intact in pSS. Lymphocytes did not seem to migrate through the epithelial layer, as no differences were found in the composition of leucocyte subsets in the vaginal and endocervical lumen using flow cytometry.

Our findings are in line with previous observations, showing the presence of inflammatory infiltrates in the vagina and cervix of women with pSS in H&E stained sections[2,11,14]. Why lymphocytes migrate to these sites is not yet known, but likely CXCL10 is involved. This IFN-induced chemokine plays a dominant role in pSS pathogenesis, and increased levels are reported in saliva, tear fluid, serum, and now also in ES samples[23,24]. The origin of CXCL10 in the ES samples is not known yet. Given the correlation with serum levels, a part of CXCL10 in the ES samples may be derived from serum by transudation, but it might also be produced locally. Salivary gland ductal epithelial cells produce CXCL10, which subsequently results in formation of periductal infiltrates[25]. Likewise, vaginal and endocervical epithelial cells might produce this chemokine, explaining the characteristic peri-epithelial vaginal infiltrate in the lamina propria.

The formation of transudate from the lamina propria, which is rich in capillaries and post-capillary venules, is important for humidification of the vagina. The lymphocytic infiltrate may either damage capillaries/post-capillary venules at these sites or otherwise interfere with generation of the transudate. Importantly, we observed that numbers of vascular smooth muscle cells are significantly decreased in the vagina of pSS patients. Whether this decrease reflects destruction of vascular smooth muscle cells, or a decrease in total number of arterioles, remains to be elucidated. Either way, a decrease in smooth muscle cells may disturb the production of transudate, considering the important role of smooth muscle cells in the regulation of the blood flow in the vaginal vascular network during sexual arousal[26].

Although the reason for the decrease in smooth muscle cells is not clear, there are several studies showing that blood vessel homeostasis is disturbed in pSS. Numbers of circulating endothelial precursor cells are increased in pSS, indicating endothelial damage[27]. Second, circulating angiogenic T-cells are expanded, which contribute to endothelial repair but may also have cytotoxic and pro-inflammatory effects[28]. Third, soluble ICAM1 and soluble VCAM1

are elevated in serum of pSS patients, which are associated with endothelial cell activation and dysfunction[29]. Finally, functional impairment of the arterial wall and vascular smooth muscle cells has been described in pSS[29,30]. Taken together, we hypothesize that vaginal dryness is impaired in pSS patients as result of vascular dysfunction. Endothelial damage may also explain the increased bleeding tendency of the vaginal epithelium in pSS patients. The development of vascular dysfunction might be mediated by the IFN pathway, similar to SLE, in which IFN alters the balance between endothelial cell apoptosis and vascular repair mediated by endothelial cell progenitors and myeloid angiogenic cells[31,32].

This study focussed on the vaginal and cervical epithelium, as these are the main sources of vaginal lubrication. Whether the vestibular glands (Bartholin's and Skene's glands) are affected by pSS remains unknown. However, Bartholin's glands only provide a small contribution to lubrication of the vestibule of the vagina of healthy individuals[33] and whether the para-urethral glands (Skene's glands) contribute to lubrication of the vulva is still under debate[34]. Skene's glands most likely only produce some fluid during orgasm, if ever.

Limitations of our study are the small sample size and subjective measurement of vaginal dryness. Furthermore, as we did not include pSS patients without vaginal dryness, or non-pSS controls with vaginal dryness, it still has to be evaluated whether the cervicovaginal changes that we found in women with pSS are the cause or a consequence of vaginal dryness, and whether they are specific for pSS patients. Future studies should objectively quantify vaginal lubrication in a larger group of patients, and evaluate the relationship of vaginal dryness with our findings. Lastly, although we aimed to include all patients during the follicular phase of the menstrual cycle, two controls were included in the luteal phase, as their laparoscopic procedures could not be planned in the follicular phase. Menstrual cycle phase might influence soluble immune markers in the vagina and cervix, but probably does not influence cellular markers[15,19,35].

In conclusion, our study shows that women with pSS and vaginal dryness have sexual dysfunction, impaired vaginal health and increased lymphocytic infiltration in the vaginal lamina propria. We postulate that vaginal dryness in women with pSS is caused by vascular dysfunction, possibly induced by interferon-mediated pathways.

COMPETING INTERESTS

The authors of this article declare no conflicts of interests.

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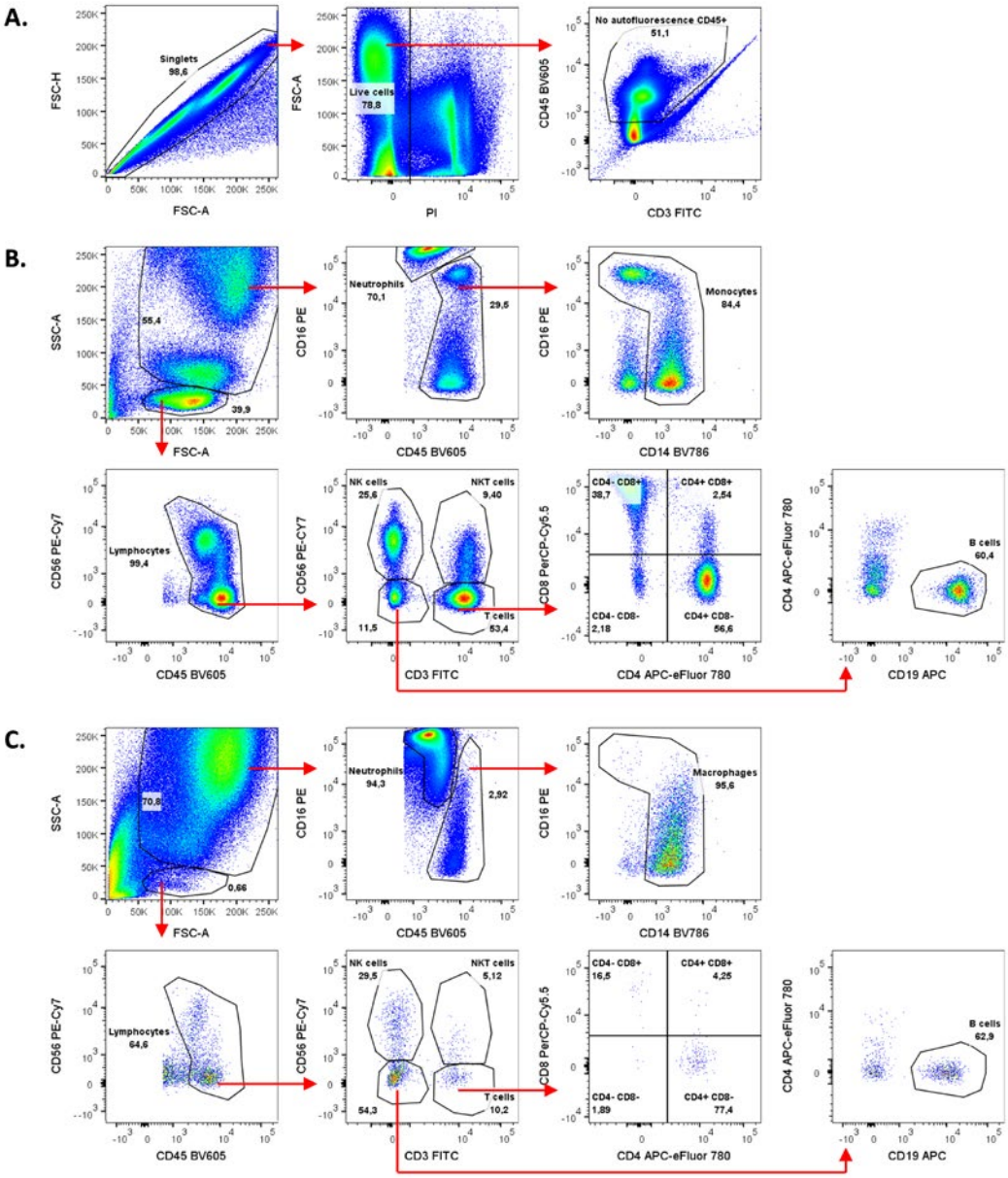
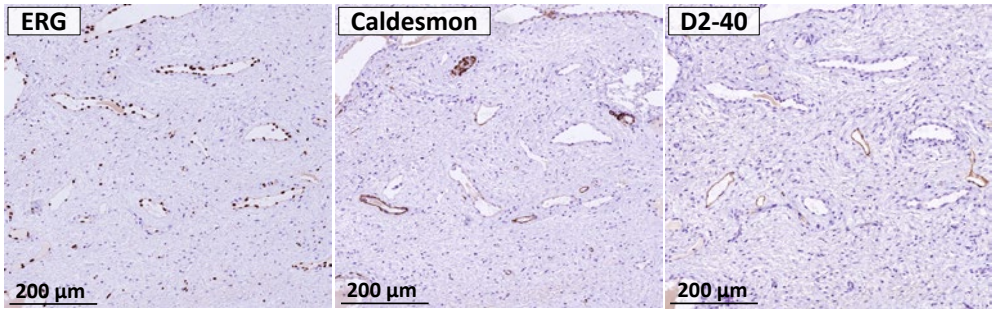


Supplementary figure 1. Example of gating strategy.
Singlets and live cells were selected, after which autofluorescent cells, which were present in some ES samples, and CD45- cells were excluded by plotting CD3-FITC to CD45-BV605 (A). Subsequently, leucocyte subsets were selected, as shown in the gating strategy for whole blood (B) and cervicovaginal and endocervical swab samples (C). Gating strategies in CVL and ES samples were based on whole blood samples of the same participants, with the exception of the neutrophil gate, as CD16 expression is lower in neutrophils from CVL and ES compared to whole bood[36].



Supplementary figure 2. Endothelial markers in the vagina of a pSS patient.
ERG: avian V-ets erythroblastosis virus E26 oncogene homolog; D2-40: anti-podoplanin (clone D2-40).

Supplementary table 1. Vaginal health index

Score	Elasticity	Fluid	pH	Mucosa	Moisture
1	None	None	≥6.1	Petechiae before contact	None, inflamed
2	Poor	Scant, thin, yellow	5.6-6.0	Bleeds with light contact	None, not inflamed
3	Fair	Superficial, thin, white	5.1-5.5	Bleeds with scraping	Minimal
4	Good	Moderate, thin, white	4.7-5.0	Not friable, thin	Moderate
5	Excellent	Normal, white	≤4.6	Not friable, normal	Normal

Vaginal health index as described by Bachmann[18].

Supplementary table 2. Antibodies used for flow cytometry

Antigen	Fluorochrome	Clone	Company
CD45	BV605	Hi30	BD Biosciences
CD3	FITC	Sk7	BD Biosciences
CD8	PerCP-Cy5.5	Sk1	BD Biosciences
CD4	APC-eFluor780	Okt4	eBioscience
CD19	APC	Hib19	BD Biosciences
CD14	BV786	M5e2	BD Biosciences
CD16	PE	3G8	BD Biosciences
CD56	PE-Cy7	HCD56	Biolegend

Supplementary table 3. Clinical and disease characteristics of patients and controls

	pSS (n=9)	Controls (n=8)	P-value
Age	36 (33-46)	41 (36-44)	0.609
Smoking status			0.793
Current	0 (0)	1 (13)	
Past smoker	3 (33)	3 (38)	
Use of oral contraceptives	6 (67)	3 (38)	0.347
Menstrual cycle day[a]	10 (8-13)	13 (7-23)	0.536
Presence of cervical ectopy	4 (44)	5 (63)	0.637
Pap score>1[b]	1 (11)	0 (0)	1.000
Positive fungal culture[c]	2 (22)	1 (13)	1.000
Anti-SSA positive	7 (78)		
Positive salivary gland biopsy	9 (100)		
ACR-EULAR score	8 (5-9)		
Time since diagnosis, years	3 (2-10)		
Time since onset of symptoms, years	9 (7-20)		
ESSDAI	6 (3-9)		
ESSPRI	5 (4-7)		
Previous use of DMARDs	6 (67)		
Corticosteroids	2 (22)		
Hydroxychloroquine	2 (22)		
Abatacept	4 (44)		
Rituximab	1 (11)		
Indication for laparoscopy			
Bilateral oophorectomy due to BRCA mutation		6 (75)	
Tubal ligation reversal		1 (13)	
Removal of benign ovarian cyst		1 (13)	

Data are presented as median (IQR), or n (%). [a]Excluding 1 patient and 1 control who used oral contraceptives continuously. [b]One pSS patient had a Pap score of 2. [c]Low density growth of fungi was found in 2 patients (Candida albicans), and 1 control (Saccharomyces cerevisiae). None of the participants showed presence of fungi in the PAS-D stained vaginal or endocervical tissue. ESSDAI: EULAR Sjögren's Syndrome Disease activity Index; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index.

Supplementary table 4. Leucocyte subsets in the vaginal and endocervical lumen and whole blood of patients and controls

	pSS	Control	P-value
CVL	**N=8**	**N=6**	
Monocytes	1.18 (0.97-1.47)	1.72 (0.69-2.40)	0.414
Neutrophils	98.46 (98.32-98.88)	97.97 (96.12-99.04)	0.414
Lymphocytes	0.48 (0.16-0.59)	0.51 (0.38-1.70)	0.414
NK-cells	36.20 (27.35-36.58)	32.28 (12.67-46.02)	0.491
NKT-cells	5.28 (2.05-12.19)	13.64 (6.72-19.03)	0.081
B-cells	11.56 (4.48-19.45)	11.08 (8.24-14.07)	0.852
CD3+ T-cells	17.89 (10.61-22.92)	15.78 (7.86-27.91)	0.950
CD4+ T-cells	8.94 (4.38-13.58)	6.82 (4.13-16.49)	0.573
CD8+ T-cells	5.62 (2.98-7.35)	4.11 (2.19-7.08)	0.573
CD4+CD8+ T-cells	0.16 (0.00-0.77)	0.02 (0.00-0.26)	0.491
CD4-CD8- T-cells	0.90 (0.47-1.12)	0.52 (0.34-3.35)	0.755
ES	**N=8**	**N=7**	
Monocytes	2.750 (1.589-4.254)	4.128 (2.062-8.908)	0.336
Neutrophils	95.76 (93.43-97.70)	95.84 (93.43-97.70)	0.336
Lymphocytes	0.88 (0.70-2.44)	2.45 (0.62-3.43)	0.463
NK-cells	17.63(7.98-53.95)	22.53 (15.99-27.33)	0.779
NKT-cells	7.57 (2.36-12.29)	13.58 (5.12-25.59)	0.121
B-cells	15.49 (10.14-29.10)	5.58 (4.78-20.35)	0.336
CD3+ T-cells	20.26 (12.71-38.40)	22.13 (10.25-38.71)	0.955
CD4+ T-cells	12.38 (6.46-23.03)	10.38 (7.93-25.08)	0.955
CD8+ T-cells	5.09 (2.28-12.77)	7.95 (1.69-10.11)	0.867
CD4+CD8+ T-cells	0.24 (0.02-0.96)	0.27 (0.09-0.44)	1.000
CD4-CD8- T-cells	0.92 (0.36-2.70)	0.81 (0.67-1.36)	1.000
Whole blood	**N=9**	**N=7**	
Monocytes	8.11 (5.70-8.88)	6.09 (5.95-9.25)	0.837
Neutrophils	66.40 (58.88-74.82	69.66(67.31-71.69)	0.918
Lymphocytes	21.58 (17.62-33.86)	24.84 (24.28-25.94)	0.758
NK-cells	6.72 (6.26-10.04)	14.11 (8.04-24.40)	0.252
NKT-cells	2.81 (0.88-4.06)	5.00 (1.55-9.40)	0.299
B-cells	16.53 (9.04-22.92)	8.83 (6.92-12.14)	0.071
CD3+ T-cells	66.71 (56.13-73.70)	63.15 (62.01-72.51)	1.000
CD4+ T-cells	40.63 (33.00-51.25)	44.88 (40.17-48.20)	0.536
CD8+ T-cells	20.55 (15.45-20.77)	16.56 (14.37-27.12)	0.408
CD4+CD8+ T-cells	0.29 (0.19-0.80)	0.61 (0.21-1.36)	0.837
CD4-CD8- T-cells	3.37 (1.85-4.10)	1.61 (0.99-3.00)	0.071

Values are median (IQR) percentages. Monocytes, neutrophils and lymphocytes are expressed as percentage of the total number of CD45+ leucocytes. Lymphocyte subsets are expressed as percentage of the total number of lymphocytes. One patient and two controls were excluded from flow cytometric analysis of CVL, and one patient and one control were excluded from analysis of ES, as their samples contained few viable leucocytes. One control was excluded from flow cytometric analysis of whole blood due to technical difficulties during the measurements. CVL: Cervicovaginal lavage; ES: Endocervical swab.

Supplementary table 5. Cytokine and chemokine levels in patients and controls

	pSS (n=9)	Control (n=8)	P-value
CVL			
APRIL	0.00 (0.00-144.51)	5.68 (0.00-58.34	0.888
BAFF	33.46 (10.36-122.63)	22.09 (17.30-123.23)	1.000
RANK-L	0.00 (0.00-1.67)	0.64 (0.00-9.44)	0.236
TNF-α	0.00 (0.00-4.21)	0.00 (0.00-1.60)	0.888
CCL2	0.00 (0.00-22.29)	0.00 (0.00-151.43)	0.743
CXCL10	39.74 (8.13-111.10)	18.30 (12.80-40.76)	0.541
CXCL11	23.77 (7.28-38.98)	29.63 (16.72-51.82)	0.423
CXCL13	0.00 (0.00-0.00)	0.00 (0.00-10.35)	0.743
IL-6	11.41 (3.04-213.83)	15.99 (3.52-31.39)	0.673
IL-7	1.05 (0.76-1.36)	1.05 (0.83-1.90)	0.541
IL-8	1593 (456-3385)	2064 (1140-5169)	0.481
ES			
APRIL	129.88 (33.59 – 355.03)	99.41 (67.18-180.40)	0.815
BAFF	38.53 (15.56-84.37)	23.64 (11.30-36.49)	0.423
RANK-L	0.63 (0.32-11.77)	7.17 (1.70-48.63)	0.277
TNF-α	1.66 (0.00-5.24)	0.54 (0.00-1.60)	0.321
CCL2	53.36 (0.00-78.32)	34.88 (7.32-108.14)	1.000
CXCL10	37.12 (19.40-66.08)	12.58 (5.89-31.11)	**0.046**
CXCL11	31.97 (9.65-47.15)	27.29 (20.8332.85)	0.606
CXCL13	0.00 (0.00-19.48)	0.00 (0.00-0.00)	0.277
IL-6	78.12 (26.94-223.22)	42.32 (6.98-173.80)	0.606
IL-7	1.36 (1.05-1.98)	1.82 (1.09-2.46)	0.673
IL-8	2355 (334-3387)	1807 (1087-2398)	0.743
Serum			
APRIL	2180 (1842-3047)	2198 (1788-2511)	0.815
BAFF	1279 (984-1373)	1049 (948-1155)	0.167
RANK-L	0.00 (0.00-0.00)	0.00 (0.00-10.04)	0.200
TNF-α	0.00 (0.00-0.21)	0.00 (0.00-0.00)	0.743
CCL2	206 (147-297)	236 (203-268)	0.423
CXCL10	51.65 (33.88-77.98)	22.71 (19.77-28.81)	**0.008**
CXCL11	37.82 (26.12-77.92)	7.28 (4.91-11.71)	**0.001**
CXCL13	43.87 (21.62-136.52)	17.29 (1.31-24.20)	0.074
IL-6	1.98 (1.85-2.30)	1.85 (1.76-2.21)	0.481
IL-7	5.30 (3.61-9.98)	6.34 (4.32-8.81)	0.888
IL-8	2.72 (1.62-4.28)	2.89 (2.38-3.45)	0.888

Values are median (IQR) levels of cytokines and chemokines in pg/ml. Values of 0.00 represent levels below detection limits. Levels of interferon-γ, IL17A, CCL4, CX3CL, and CXCL9 were below detection limits in serum, CVL as well as ES in most patients, and difference between groups was not tested. CVL: Cervicovaginal lavage; ES: Endocervical swab.

CHAPTER 4

Normal vaginal microbiome in women with primary Sjögren's syndrome-associated vaginal dryness

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INTRODUCTION

Dryness of epithelial surfaces is characteristic for patients with primary Sjögren's syndrome (pSS). Vaginal dryness is frequently reported by pSS-women and is associated with sexual dysfunction[1,2]. Recently we showed that dysbiosis of the oral microbiome is largely similar between oral dryness patients with and without pSS when compared with healthy controls[3,4]. The objective of our current study was to assess whether the vaginal microbiome of women with pSS-associated vaginal dryness differs from controls.

METHODS

This study was approved by the medical ethical committee of the University Medical Center Groningen, Groningen, the Netherlands (METc 2015/039). All participants completed written informed consent according to the declaration of Helsinki.

Patients and controls

In a case-control design, we compared the vaginal microbiome of ten premenopausal pSS-women with that of ten age-matched premenopausal women without pSS, who underwent general anesthesia for a laparoscopic procedure. Exclusion criteria were genital inflammatory or infectious comorbidity, endometriosis and use of disease modifying antirheumatic drugs, corticosteroids, vaginal estrogens or an intrauterine contraceptive device. All pSS-patients fulfilled the 2016 ACR/EULAR classification criteria. All participants completed a questionnaire on vaginal symptoms. Patient-reported vaginal dryness was scored using a numeric rating scale (NRS, range 0-10). Vaginal health was assessed with the vaginal health index (VHI)[5]. The VHI was scored by two gynaecologists (MM and KT). The VHI was first described by Bachmann et al. in 1995 and was developed at the Robert Wood Johnson Medical School (Brunswick, NJ, USA) to assess female urogenital health in a clinically objective manner[6].

Sample collection

From each participant, a gynecologist collected a cervicovaginal lavage (CVL) and an endocervical swab (ES). Cervicovaginal lavages were collected with 10mL sterile phosphate buffered saline (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Endocervical swab samples were collected with flocked swabs (Eswab, COPAN, Brescia, Italy). Samples were centrifuged at 900 g. The pellet and supernatant of the samples were stored separately at -80°C.

DNA isolation, 16S rRNA gene sequencing and taxonomy assignment

DNA isolation was performed on the supernatant of the CVL and ES samples with a DNeasy UltraClean Microbial kit (QIAGEN Benelux B.V., Venlo, The Netherlands). The V3-V4 region of

the 16S rRNA gene was amplified by PCR using modified 341F and 806R primers, as described before[7]. Subsequently, paired-end sequencing was performed on a Illumina MiSeq platform. PANDAseq was used to discard reads with a quality score <0.9[8]. Samples were rarefied to 25,000 reads per sample. Taxonomy assignment was performed with the ARB software environment (release 5.5) with SILVA125 as reference database[9,10]. The relative abundance of bacterial species was determined by the proportion of reads per species relative to the total number of reads per sample. Species with an overall mean relative abundance <0.01% were removed.

Statistical analysis

QIIME v1.9.1 was used to assess alpha- and beta-diversity[11]. Alpha-diversity was measured by the number of observed species and Shannon index. Beta-diversity was assessed by Bray-Curtis distance. *Adonis* function from the R-*vegan* package was used to estimate the explained variance (R^2-value) and significance (p-value) of phenotype data on the variation in microbiota composition between samples using 999 permutations[12]. Comparative statistics and clustering analyses were performed in R v3.3.1. A p-value <0.05 and a Benjamini-Hochberg false discovery rate corrected (FDR) p-value (indicated as q-value) <0.10 were used as significance cut-offs.

RESULTS AND DISCUSSION

After inclusion, one pSS-patient was diagnosed with Chlamydia in the ES and two control women with endometriosis at laparoscopy. These women were excluded, resulting in 9 pSS-women and 8 controls for further analyses (table 1).

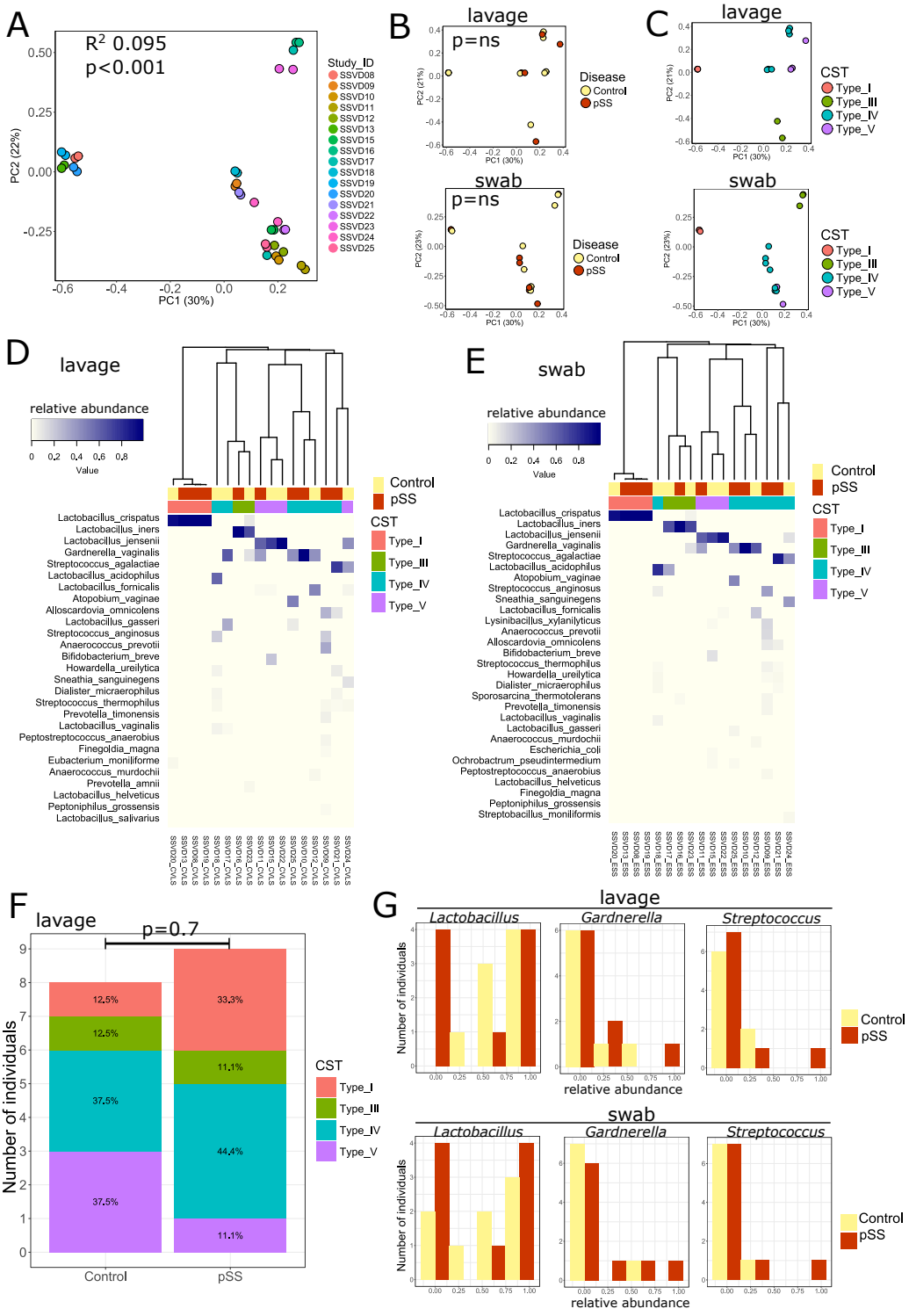
As expected, scores for vaginal dryness, dyspareunia and use of lubricants were higher in pSS-women[2]. Furthermore, pSS-women scored significantly lower on the total VHI-score[5]. Vaginal pH-values were normal in pSS-patients. Microbiota composition of CVL and ES samples were highly similar within individuals, with 95% being explained by individuality (*adonis*, p<0.001; figure 1A). Disease (pSS vs. control) did not affect overall vaginal microbiota composition in both CVL and ES samples (*adonis*, p>0.05; figure 1B). Despite the small sample size, we were able to identify in both groups (pSS and controls), four of the five vaginal community state types (CSTs) previously described (figures 1C-E)[13]. Distribution of CSTs and distribution of the three most prevalent genera (i.e., *Lactobacillus*, *Gardnerella* and *Streptococcus*) showed similar patterns in pSS-women and controls (figures 1F,G). Also, the mean relative abundance of these three genera did not differ between pSS-women and controls (p>0.05). Patient-reported vaginal dryness severity (NRS-score) did not correlate with the relative abundance of the three most prevalent genera (Spearman, p>0.05). The small number of pSS-patients did not allow us to analyse associations between vaginal microbiota and disease activity.

Table 1: Study population characteristics

Characteristic	pSS n=9	Control n=8	P[a]
Age, mean (sd)	38 (9)	40 (4)	0.6
SSA positive, n (%)	7 (78)	na	
SSB positive, n (%)	6 (67)	na	
Disease duration in years, mean (sd)	8 (7)	NA	
Smoking, n (%)	3 (33)	4 (50)	0.8
Pack years, mean (sd)	0.7 (2)	0.7 (1)	0.4
Numeric Rating Scale on dryness (0-10):			
Eyes, mean (sd)	7 (1)	2 (2)	**0.001**
Mouth, mean (sd)	7 (1)	1 (2)	**<0.001**
Vagina, mean (sd)	6 (2)	1 (2)	**0.002**
Use of lubricants, n (%)	5 (56)	0 (0)	**0.05**
Dyspareunia, n (%)	9 (100)	2 (25)	**0.01**
Vaginal Health Index total score, mean (sd)	19 (3)	23 (2)	**0.02**
pH posterior fornix, mean (sd)	4.6 (0.7)	4.7 (0.5)	0.6
Current medication			
Oral contraceptives, n (%)	6 (67)	3 (38)	0.5
Current NSAIDs, n (%)	2 (22)	0 (0)	0.5
ESSDAI - total, mean (sd)	6 (3)	NA	
ESSPRI - dryness, mean (sd)	6 (1)	NA	
ESSPRI - fatigue, mean (sd)	6 (3)	NA	
ESSPRI - pain, mean (sd)	3 (3)	NA	
ESSPRI - total, mean (sd)	5 (2)	NA	
Reason for laparoscopic procedure in controls			
BRCA1 or BRCA2 mutation, n	NA	6	
Refertilisation, n	NA	2	
Mucous cyst of the adnex, n	NA	1	

[a]Chi-square test and Wilcoxon rank sum test were used for categorical and numerical data, respectively. pSS: primary Sjögren's syndrome; sd: standard deviation; na: not assessed; NA: not applicable; NSAIDs: non-steroidal anti-inflammatory drugs; ESSDAI: EULAR Sjögren's syndrome disease activity index; ESSPRI: EULAR Sjögren's syndrome patient reported index.

Figure 1. (next page) Vaginal microbiota composition in premenopausal women with pSS and controls.
(A) Principal coordinate analysis of CVL and ES samples shows high similarity within individuals (overlapping dots are separated slightly for enhanced clarity). (B) No clustering of pSS-women or control women is observed based on vaginal microbiota composition in CVL (lavage) or ES (swab) samples. (C) CVL and ES samples show evident clustering based on the four community state types (CSTs). (D and E) CST-I, dominated by Lactobacillus crispatus, CST-III, dominated by Lactobacillus iners, CST-IV, a heterogeneous non-lactobacilli dominated type and CST-V, which is dominated by Lactobacillus jensenii were identified using Bray-Curtis distance clustering, based on the relative abundance of bacterial species with a relative abundance >0.1%. (F) Distribution of CSTs did not differ between pSS-women and controls (Fisher's exact test). (G) Histograms of the three most abundant genera show similar patterns in pSS-women and controls. CST: community state type; CVL:, cervicovaginal lavage; ES: endocervical swab; pSS: primary Sjögren's syndrome.


A
R² 0.095
p<0.001
PC1 (30%)
PC2 (22%)
Study_ID
SSVD08
SSVD09
SSVD10
SSVD11
SSVD12
SSVD13
SSVD15
SSVD16
SSVD17
SSVD18
SSVD19
SSVD20
SSVD21
SSVD22
SSVD23
SSVD24
SSVD25
B
lavage
p=ns
swab
p=ns
Disease
Control
pSS
C
lavage
swab
CST
Type_I
Type_III
Type_IV
Type_V
D
lavage
relative abundance
Value
Lactobacillus_crispatus
Lactobacillus_iners
Lactobacillus_jensenii
Gardnerella_vaginalis
Streptococcus_agalactiae
Lactobacillus_acidophilus
Lactobacillus_fornicalis
Atopobium_vaginae
Alloscardovia_omnicolens
Lactobacillus_gasseri
Streptococcus_anginosus
Anaerococcus_prevotii
Bifidobacterium_breve
Howardella_ureilytica
Sneathia_sanguinegens
Dialister_micraerophilus
Streptococcus_thermophilus
Prevotella_timonensis
Lactobacillus_vaginalis
Peptostreptococcus_anaerobius
Finegoldia_magna
Eubacterium_moniliforme
Anaerococcus_murdochii
Prevotella_amnii
Lactobacillus_helveticus
Peptoniphilus_grossensis
Lactobacillus_salivarius
E
swab
relative abundance
Value
Lactobacillus_crispatus
Lactobacillus_iners
Lactobacillus_jensenii
Gardnerella_vaginalis
Streptococcus_agalactiae
Lactobacillus_acidophilus
Atopobium_vaginae
Streptococcus_anginosus
Sneathia_sanguinegens
Lactobacillus_fornicalis
Lysinibacillus_xylanilyticus
Anaerococcus_prevotii
Alloscardovia_omnicolens
Bifidobacterium_breve
Streptococcus_thermophilus
Howardella_ureilytica
Dialister_micraerophilus
Sporosarcina_thermotolerans
Prevotella_timonensis
Lactobacillus_vaginalis
Lactobacillus_gasseri
Anaerococcus_murdochii
Escherichia_coli
Ochrobactrum_pseudintermedium
Peptostreptococcus_anaerobius
Lactobacillus_helveticus
Finegoldia_magna
Peptoniphilus_grossensis
Streptobacillus_moniliformis
Control
pSS
CST
Type_I
Type_III
Type_IV
Type_V
F
lavage
p=0.7
Number of individuals
12.5%
12.5%
37.5%
37.5%
33.3%
11.1%
44.4%
11.1%
Control
pSS
CST
Type_I
Type_III
Type_IV
Type_V
G
lavage
Lactobacillus
Gardnerella
Streptococcus
Number of individuals
relative abundance
Control
pSS
swab
Lactobacillus
Gardnerella
Streptococcus
Number of individuals
relative abundance
Control
pSS

Our results indicate that the vaginal microbiome in pSS-women with vaginal dryness is similar to that of controls, which contrasts the observed difference in vaginal microbiota composition between postmenopausal women with and without vaginal dryness[14]. The different outcomes may be explained by different underlying causes of vaginal dryness (i.e., pSS in premenopausal versus loss of estrogen in postmenopausal women)[14]. Under the influence of estrogen, glycogen is deposited in the epithelium of the vagina[15]. Lactobacilli use the breakdown products of glycogen to produce lactic acid, which contributes to the low vaginal pH, and thereby inhibits the growth of other bacteria[15].

Apparently, the unique vaginal microbiome – dominated by acid producing lactobacilli – is less dependent on dryness than the oral microbiome. Oral dryness is associated with higher *Lactobacillus* relative abundance, which contributes to oral diseases (i.e., dental caries and Candida infection). In the vagina, lactobacilli represent a healthy microbiome and are essential for the low vaginal pH[15]. Our study suggests that pSS-associated vaginal dryness in premenopausal women does not negatively influence homeostasis of the vaginal ecosystem.

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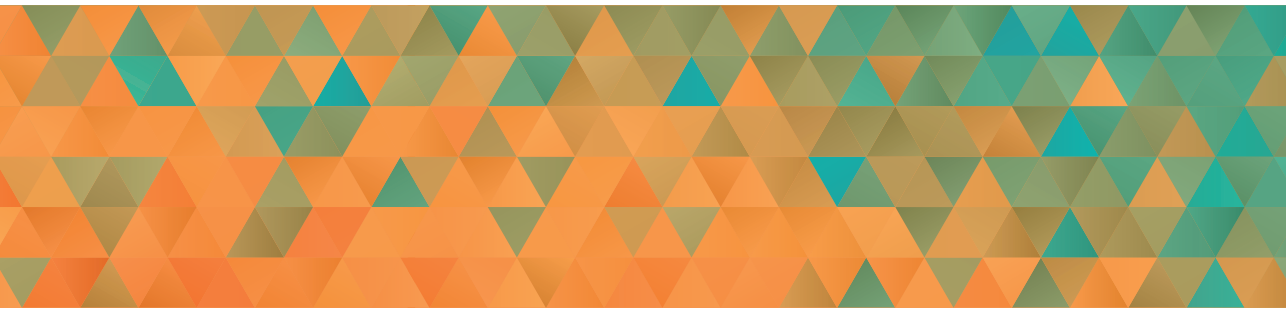

PART II

Classification and stratification

CHAPTER 5

Validation of the ACR-EULAR criteria for primary Sjögren's syndrome in a Dutch prospective diagnostic cohort

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ABSTRACT

Objective. To validate the ACR-EULAR classification criteria for primary Sjögren's syndrome (pSS), and compare them to the AECG and ACR criteria in a Dutch prospective diagnostic cohort.

Methods. Consecutive patients (n=129) referred for suspicion of pSS underwent a multidisciplinary evaluation, including a labial and/or parotid gland biopsy. Patients with an incomplete work-up (n=8) or associated systemic auto-immune disease (n=7) were excluded. ACR-EULAR classification was compared to expert classification, AECG and ACR classification. Additionally, the accuracy of individual ACR-EULAR items to discriminate pSS from non-pSS was evaluated. The validity of criteria sets was described separately using parotid or labial gland biopsy results for classification.

Results. Of the 114 evaluated patients, the expert panel classified 34 (30%) as pSS and 80 (70%) as non-pSS. Using labial gland biopsy results, ACR-EULAR classification showed 87% absolute agreement (κ=0.73) with expert classification, with a sensitivity of 97% and specificity of 83%. Using the parotid gland biopsy results, the ACR-EULAR criteria performed excellent as well. Focus score, anti-SSA titer and ocular staining score showed good to excellent accuracy, whereas unstimulated whole saliva (UWS) and Schirmer's test had poor accuracy. The ACR-EULAR and AECG criteria had equal validity. Compared to ACR classification, ACR-EULAR classification showed higher sensitivity but lower specificity.

Conclusions. The ACR-EULAR criteria showed good agreement with expert classification, but some patients may be misclassified as pSS. UWS and Schirmer's test showed poor discriminative value. The ACR-EULAR criteria performed equally to the AECG criteria, and had higher sensitivity but lower specificity than the ACR criteria.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease, characterized by lymphocytic infiltration of the exocrine glands, resulting in dryness symptoms[1]. Patients present with a spectrum of signs and symptoms, evolving over time, making clinical diagnosis and classification challenging.

Currently, multiple criteria sets are in use for classification of pSS (supplementary table 1). Most researchers and clinicians utilize the 2002 American-European Consensus Group (AECG) criteria, which include items evaluating the presence of sicca symptoms of the eye and mouth, functional impairment of the exocrine glands, presence of anti-SSA/SSB antibodies and a focus score of ≥1 in the salivary gland biopsy[2]. However, questions were raised about the inclusion of sicca symptoms in the AECG criteria. Therefore, in 2012, Shiboski et al. proposed the American College of Rheumatology (ACR) criteria for pSS. The ACR criteria include only objective tests and were designed to be used as entry criteria for clinical trials, in order to ease comparison of results between trials[3]. The ACR criteria require presence of two out of the following three items: focus score of ≥1, positive serology, and ocular staining score (OSS) ≥3. Positive serology was defined as presence of anti-SSA/SSB antibodies or rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Agreement between the AECG and ACR criteria was 78% and 81% in two prospective diagnostic cohorts[4,5].

Although widely used, the AECG and ACR criteria sets have not been endorsed by both the ACR and the European League Against Rheumatism (EULAR). To be able to compare different study populations in trials and cohorts, international consensus regarding the classification of pSS is crucial. Therefore, the International Sjögren's Syndrome Criteria Working Group developed the 2016 ACR-EULAR criteria for pSS using methodology endorsed by both the ACR and EULAR[6,7].

The ACR-EULAR criteria combine features of the AECG and ACR criteria (supplementary table 1). Instead of including sicca symptoms as an item, the ACR-EULAR criteria added the presence of sicca symptoms or a EULAR Sjögren's syndrome disease activity index (ESSDAI) of ≥1 as an entry criterion. In the ACR-EULAR criteria, positive serology is solely based on the presence of anti-SSA antibodies, while anti-SSB, ANA and RF positivity were not adopted. The OSS score was added to the ACR-EULAR criteria with a cut-off of ≥5, instead of ≥3 as used for the ACR criteria, and the van Bijsterveld score with a cut-off of ≥4 was allowed as an alternative. Sialography and scintigraphy were not included in the ACR-EULAR criteria and some updates were made in the exclusion criteria for classification as pSS.

Before the ACR-EULAR classification criteria can be implemented reliably, it is important to validate these criteria in external, prospective cohorts with complete data on all ACR-EULAR items. Recently, the ACR-EULAR criteria were validated in a cohort of Japanese patients[8].

However, this study had several limitations. The analysis was performed in a retrospective cohort with incomplete data. In a part of the patients, unstimulated whole saliva (UWS) was replaced by tests assessing stimulated whole saliva (SWS). OSS was not available, and replaced by the van Bijsterveld score, making the comparison with the ACR criteria less reliable. Moreover, clinical diagnosis was used as gold standard instead of expert classification based on anonymised case vignettes. Considering these limitations, and taking into account that the Japanese population may not show the same characteristics as Caucasian populations, further validation of the ACR-EULAR criteria is needed.

The primary objective of our study is therefore to validate the ACR-EULAR criteria for pSS using classification according to expert opinion as the gold standard, in a Dutch prospective diagnostic cohort in a daily clinical practice setting. In addition, the performance of the individual components of the ACR-EULAR criteria was assessed, and the ACR-EULAR criteria were compared to the AECG and ACR criteria.

METHODS

Study population

The study population consisted of consecutive patients, aged ≥18 years, who were referred to the Sjögren Expertise Center of the University Medical Center Groningen (UMCG), a tertiary referral center, for suspicion of pSS between December 2013 and August 2016. Informed consent was obtained from all patients according to the Declaration of Helsinki. Patients with incomplete diagnostic work-up making it impossible to apply the AECG, ACR and ACR-EULAR criteria were excluded, as well as patients who were diagnosed with an associated systemic auto-immune disease (e.g. rheumatoid arthritis, systemic lupus erythematosus), as determined by the expert panel. The study was approved by the Medical Research Ethics Committee of the UMCG (METc2013.066).

Diagnostic evaluation

Patients were evaluated by a team of clinical experts, consisting of rheumatologists, oral and maxillofacial surgeons, pathologists and one ophthalmologist, all very experienced in diagnosing pSS. The multidisciplinary work-up included evaluation of all items of the three criteria sets[2,3,6,7]. The rheumatologist performed a clinical history and physical examination, recorded the presence of signs and symptoms of pSS, and the ESSDAI score[9]. Laboratory tests included evaluation of complete blood count, erythrocyte sedimentation rate, C-reactive protein, ANA, anti-SSA and anti-SSB antibodies, RF, IgG, complement C3 and C4, cryoglobulinemia and hepatitis C serology. When indicated, additional examinations such as X-rays, pulmonary function tests, thoracic high resolution computed tomography or nailfold capillaroscopy were performed to facilitate clinical diagnosis.

Evaluation by the oral and maxillofacial surgeon included determination of sicca symptoms, a physical evaluation of the oro-facial and neck area, and analysis of UWS and SWS. A labial and/or parotid gland biopsy was taken by the same oral and maxillofacial surgeon[10]. Salivary gland sialography or scintigraphy were not performed. Salivary gland biopsies were evaluated by a head and neck pathologist and trained resident for focus score (foci/4 mm^2)[11], presence of germinal centers, lymphoepithelial lesions and IgA, IgG and IgM plasma cell ratio.

Ophthalmological evaluation included determination of sicca symptoms, Schirmer's test, tear break-up time and OSS. OSS was defined using slit-lamp evaluation of lissamine green (LG) staining of the temporal and medial conjunctiva and fluorescein staining of the cornea[12].

Case ascertainment

All patients were classified as pSS or non-SS according to the ACR-EULAR, AECG and ACR criteria[2,3,6,7]. Fulfillment of the classification criteria was determined separately using the labial or parotid gland biopsy outcome for classification. Patients who did not undergo both biopsies, making it impossible to determine classification when either the labial or parotid gland biopsy results were taken into account, were excluded from that part of the analysis. Although the AECG criteria exclude patients with lymphoma, we classified patients with mucosa-associated lymphoid tissue (MALT) lymphoma who fulfilled the AECG criteria as pSS, as pSS can result in the development of MALT lymphoma[13].

The clinical diagnosis made by the treating rheumatologist was recorded. For expert classification, all cases were described in an anonymised clinical vignette, including the outcomes of all tests described above, which were reviewed by an expert panel (HB, AJS, EB) and scored as pSS or non-pSS. HB reviewed all vignettes, while AJS and EB each reviewed half of the vignettes. The experts were blinded to the clinical diagnosis and classification by the other experts. In case of discordance between the classifications by the experts, the vignette was discussed in a consensus meeting with all three experts to reach expert classification.

Statistical analysis

Statistical analyses were executed using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA). Descriptive sociodemographic and disease characteristics were described as mean ± SD, median (interquartile range) or number (%) as appropriate. The agreement between the clinical diagnosis and expert classification and between the three criteria sets was evaluated with percentage of absolute agreement and Cohen's kappa coefficient. The performance of the ACR-EULAR score and individual ACR-EULAR items to predict expert classification was evaluated with the area under the ROC curve (AUC), which was interpreted as no discrimination (0–0.5), poor (0.5–0.7), fair (0.7–0.8), good (0.8–0.9) or excellent (0.9–1.0) accuracy[14]. The agreement of the three criteria sets with expert classification was evaluated with the percentage of absolute agreement, Cohen's kappa coefficient (κ), sensitivity and

specificity. κ was interpreted as poor (0.0–0.2), fair (0.2–0.4), moderate (0.4–0.6), good (0.6–0.8) or excellent (0.8–1.0) agreement[15].

RESULTS

Of the 129 consecutive patients who gave informed consent, 15 were excluded from evaluation in this study because of incomplete data or associated auto-immune diseases (figure 1). All remaining patients (n=114) underwent a salivary gland biopsy. Of most patients (n=100), biopsies of both glands were obtained, whereas 5 patients underwent only a labial gland biopsy and 9 patients underwent only a parotid gland biopsy.

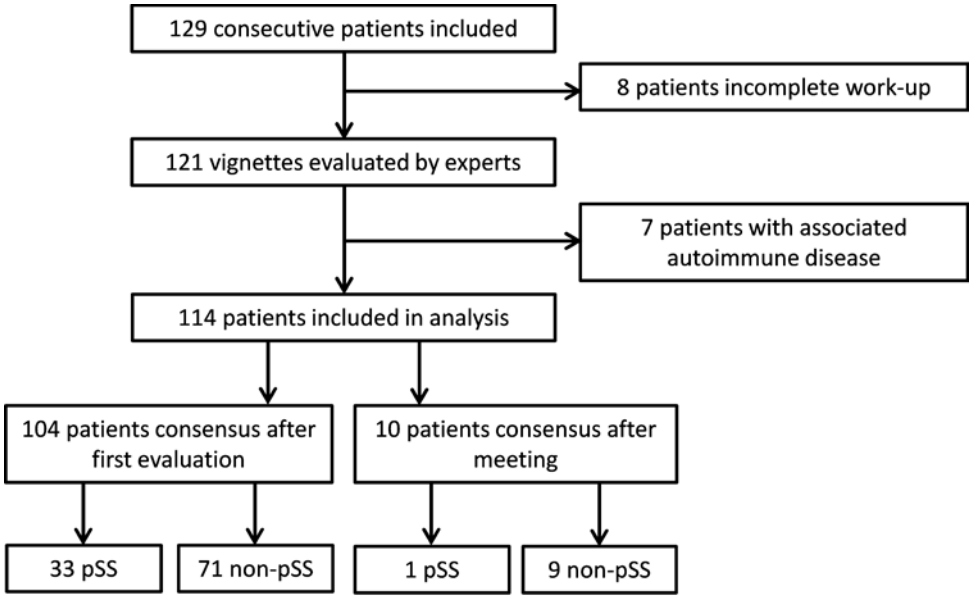

Figure 1: Flowchart of inclusion and expert panel evaluation.
pSS: primary Sjögren's syndrome.

Expert classification

After the first evaluation of the case vignettes, the expert panel agreed on the classification as pSS or non-pSS in 104 patients. For the remaining 10 patients, expert classification was reached during the consensus meeting. Of the 34 patients classified as pSS by the expert panel, the mean age was 52.3±15.3 years and 32 (94%) patients were female. Of the 80 patients classified as non-pSS, the mean age was 50.2±12.6 years and 69 (86%) patients were female.

The expert classification showed 89% agreement with the clinical diagnosis made by the treating rheumatologist (κ=0.77). Eleven patients were clinically diagnosed with pSS by the

treating physician, but classified as non-pSS by the experts, and one patient was clinically not diagnosed with pSS, but classified as pSS by the experts.

Comparison of criteria to expert classification

Taking the labial gland biopsies into account for classification, the ACR-EULAR score had an AUC of 0.94 (95% CI 0.88-1.00) to discriminate pSS from non-pSS. The ACR-EULAR criteria and AECG criteria both showed an absolute agreement of 87% (κ=0.73) with expert classification, with 97% sensitivity and 83% specificity (table 1). The ACR criteria showed an absolute agreement of 91% (κ=0.79) with expert classification, with 91% sensitivity and 91% specificity.

Taking the parotid gland biopsies into account for classification, the ACR-EULAR score had an AUC of 0.97 (95% CI 0.92-1.00) to discriminate pSS from non-pSS. The ACR-EULAR criteria and AECG criteria both showed an absolute agreement of 92% (κ=0.82) with expert classification, with 91% sensitivity and 92% specificity (table 1). The ACR criteria showed an absolute agreement of 93% (κ=0.83) with expert classification, with 85% sensitivity and 96% specificity.

Table 1. Comparison of ACR-EULAR, AECG and ACR classification with expert classification

Criteria including labial gland biopsy			**Expert classification**	
			SS	Non-SS
ACR-EULAR[a]			n=34	n=76
	SS	n=46	33	**13**
	Non-SS	n=64	**1**	63
AECG[a]			n=34	n=76
	SS	n=46	33	**13**
	Non-SS	n=64	**1**	63
ACR[a]			n=33	n=77
	SS	n=37	30	**7**
	Non-SS	n=73	**3**	70
Criteria including parotid gland biopsy			**Expert classification**	
			SS	Non-SS
ACR-EULAR[b]			n=34	n=78
	SS	n=37	31	**6**
	Non-SS	n=75	**3**	72
AECG[b]			n=34	n=78
	SS	n=37	31	**6**
	Non-SS	n=75	**3**	72
ACR[c]			n=34	n=79
	SS	n=32	29	**3**
	Non-SS	n=81	**5**	76

Discrepant cases are bold. Due to missing or inconclusive labial gland biopsies, [a]4 patients were excluded from the comparison of ACR-EULAR, AECG and ACR classification vs. expert classification. Due to missing or inconclusive parotid gland biopsies, [b]2 patients were excluded from the comparison of ACR-EULAR and AECG classification vs. expert classification and [c]1 patient was excluded from the comparison of ACR classification vs. expert classification.

Description of patients with discrepant ACR-EULAR and expert classification

Characteristics of patients with concordance or discrepancy between the expert and ACR-EULAR classification are shown in figure 2 and figure 3, taking into account the labial or parotid gland biopsy for classification, respectively. Patients who were classified as non-pSS by the experts but pSS by the ACR-EULAR criteria showed low biological activity, and most of them had ACR-EULAR scores between 4 and 6. Interestingly, the Schirmer's test was often positive, while the OSS was mostly negative in this group of patients. Patients who were classified as pSS by the experts but non-pSS by the ACR-EULAR criteria, when taking into account the labial (n=1) or parotid gland biopsy (n=3) for classification, were not included in the figures. However, a detailed list of discrepant cases is provided in supplementary table 2. Of these 17 discrepant cases, 9 were also classified differently by the two experts during the first round of evaluation. For these patients, expert classification was reached during the consensus meeting.

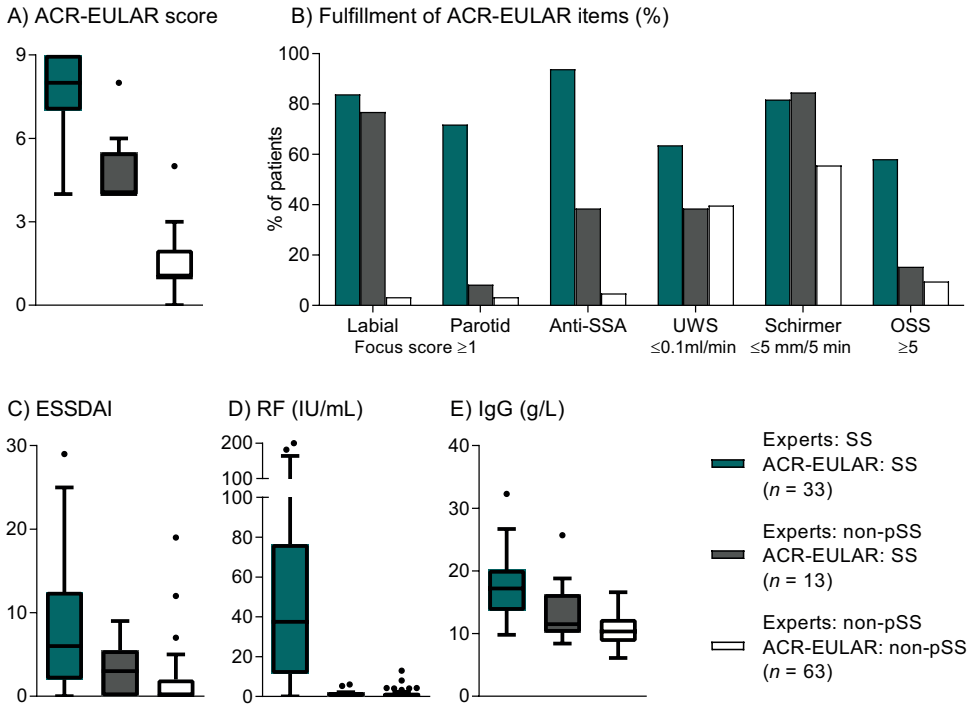

Figure 2: Characteristics of groups including the labial gland biopsy results.
Comparison of patients who are classified as SS or non-pSS by the experts and ACR-EULAR criteria including the labial gland biopsy results. OSS: ocular staining score; UWS: unstimulated whole saliva.

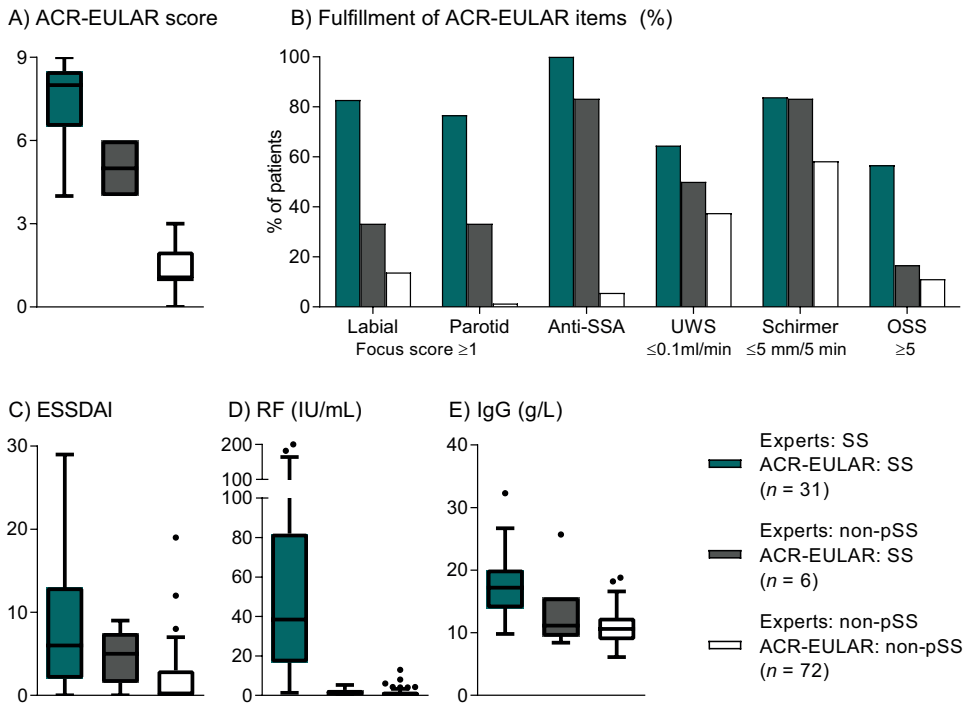

Figure 3: Characteristics of groups including the parotid gland biopsy results.
Comparison of patients who are classified as SS or non-pSS by the experts and ACR-EULAR criteria including the parotid gland biopsy results. OSS: ocular staining score; UWS: unstimulated whole saliva.

Performance of individual ACR-EULAR items

In this prospective cohort, 33 (97%) pSS patients and 78 (98%) non-pSS patients reported sicca symptoms. The ESSDAI was ≥1 in 31 (91%) pSS patients and 40 (51%) non-pSS patients. Only one non-pSS patient did not fulfill the entry criteria of the ACR-EULAR criteria, as she had neither sicca complaints nor an ESSDAI≥1. None of the patients was solely SSB positive. Focus score and anti-SSA titer showed excellent accuracy and OSS showed good accuracy to discriminate pSS from non-pSS. UWS and Schirmer's test showed poor accuracy to discriminate pSS from non-pSS (figure 4).

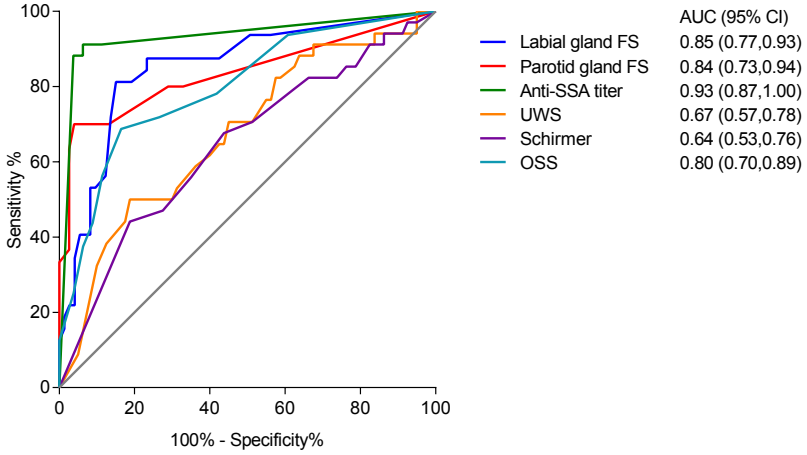

Figure 4: ROC curves of diagnostic tests, using expert classification as gold standard.
FS: focus score (foci/4 mm²); OSS: ocular staining score; ROC: receiver operating characteristic; UWS: unstimulated whole saliva.

Comparison of ACR-EULAR with AECG and ACR classification

Taking the labial gland biopsies into account, the ACR-EULAR criteria showed an absolute agreement of 98% (κ=0.96) with the AECG criteria and 91% (κ=0.81) with the ACR criteria (table 2). Taking the parotid gland biopsies into account, the ACR-EULAR criteria showed an absolute agreement of 98% (κ=0.96) with the AECG criteria and 95% (κ=0.90) with the ACR criteria.

While ACR-EULAR classification was very similar to AECG classification, ACR classification was stricter, as some patients were classified as pSS by the ACR-EULAR criteria but as non-pSS by the ACR criteria. These patients had either a positive biopsy or positive serology, in combination with a positive UWS and/or Schirmer's test, but a negative OSS.

Table 2. Comparison of ACR-EULAR with AECG and ACR classification

Criteria including labial gland biopsy			**ACR-EULAR**	
			SS	Non-SS
AECG[a]	SS	n=46	n=46 45	n=64 **1**
	Non-SS	n=64	**1**	63
ACR[b]	SS	n=37	n=45 36	n=64 **1**
	Non-SS	n=72	**9**	63
Criteria including parotid gland biopsy			**ACR-EULAR**	
			SS	Non-SS
AECG[c]	SS	n=37	n=37 36	n=75 **1**
	Non-SS	n=75	**1**	74
ACR[d]	SS	n=32	n=37 32	n=74 **0**
	Non-SS	n=79	**5**	74

Discrepant cases are bold. Due to missing or inconclusive labial gland biopsies, [a]4 patients were excluded from the comparison of ACR-EULAR vs. AECG classification and [b]5 patients were excluded from the comparison of ACR-EULAR vs. ACR classification. Due to missing or inconclusive parotid gland biopsies, [c]2 patients were excluded from the comparison of ACR-EULAR vs. AECG classification and [d]3 patients were excluded from the comparison of ACR-EULAR vs. ACR classification.

DISCUSSION

This study evaluated the validity of the 2016 ACR-EULAR criteria for pSS, in comparison to the AECG and ACR criteria, in an external, prospective diagnostic cohort in a daily clinical practice setting. All ACR-EULAR items were evaluated, including labial and/or parotid gland biopsies in all patients. In our multidisciplinary setting, the ACR-EULAR score showed excellent accuracy with expert classification as gold standard.

In accordance to the original validation cohort[6], the ACR-EULAR criteria showed very high sensitivity when labial gland biopsies are used. We found a specificity of 83%, which is lower than the specificity of 95% reported by Shiboski et al[7]. Recently, an even lower specificity of 76.7% was found in a retrospective cohort of Japanese patients[8]. Taken together, these results suggest that some non-pSS sicca patients may be misclassified as pSS by the ACR-EULAR criteria. This occurs mostly in patients who have an ACR-EULAR score of 4 to 6, based on either presence of SSA antibodies or focus score ≥1, combined with a decreased Schirmer's test and/or UWS. In approximately half of the patients with discrepancy between the expert and ACR-EULAR classification, the experts also disagreed on the classification after the first round of evaluation of the vignettes. This illustrates that a subset of patients suspected for pSS is difficult to diagnose. Using the cut-off of ≥4 for the ACR-EULAR score does ensure high sensitivity of the ACR-EULAR criteria, but for the clinical diagnosis, other clinical parameters have to be taken into account too, including more detailed histopathological characteristics (i.e., presence of germinal centers, lymphoepithelial lesions and plasma cell shift), the presence of comorbidities which may also partly explain the symptoms (i.e., presence of diabetes, autoimmune thyroiditis, fibromyalgia) and the use of medication which may cause sicca symptoms (i.e., beta blockers, antidepressants).

In our cohort, in most patients labial and parotid gland biopsies were taken simultaneously, which gave us the unique opportunity to evaluate the performance of the ACR-EULAR criteria when including labial as well as parotid gland biopsies. We found that the ACR-EULAR criteria also have excellent accuracy when using parotid gland biopsies, with good sensitivity and specificity. Interestingly, the sensitivity of the ACR-EULAR criteria is higher when using labial gland biopsies, while the specificity is higher when using parotid gland biopsies. A detailed comparison between the labial and parotid gland biopsy from a histopathological point of view falls beyond the scope of this article and will be discussed separately (manuscript in preparation).

In the analysis of the performance of individual ACR-EULAR items, the salivary gland focus score, anti-SSA and OSS showed good or excellent discriminative value. The accuracy of Schirmer's test and UWS was poor as they were positive in many non-pSS patients as well. In line with our findings, Shiboski et al. reported limited validity of these tests in the SICCA cohort, using a latent class model[3]. In contrast, Vitali et al. did find acceptable validity of Schirmer's

test and UWS, but the study population was different[16]. Vitali et al. included selected patients, pre-defined as patients with pSS, secondary Sjögren's syndrome or controls based on clinical judgment, whereas our cohort and the SICCA cohort included consecutive patients, resulting in a population representative of daily clinical practice.

The poor performance of Schirmer's test and UWS in our cohort might be explained by non-pSS patients with exocrine gland dysfunction due to other causes, as Schirmer's test and UWS are not able to discriminate between different causes of sicca symptoms[17,18]. The OSS shows good performance in our cohort, and we strongly recommend including evaluation of the OSS in the diagnostic work-up of Sjögren's syndrome. However, the OSS needs to be performed by a trained ophthalmologist, which is not always available. Therefore, the inclusion of Schirmer's test and UWS in the ACR-EULAR criteria has increased the feasibility of the criteria. To further improve the ACR-EULAR criteria, additional studies should evaluate whether other diagnostic tests such as salivary gland ultrasonography could complement the ACR-EULAR criteria[19].

As expected, the ACR-EULAR classification was very similar to AECG classification, and showed equal validity in our cohort. However, the ACR-EULAR criteria have several advantages over the AECG criteria in current daily practice. For example, the sensitivity of the AECG criteria would have been lower if the three pSS patients with MALT lymphoma in our cohort had been characterized as non-pSS, according to the exclusion criteria (data not shown). Lymphoma is no longer included in the exclusion criteria of the ACR-EULAR, and other exclusion criteria have also been adjusted. Additionally, sialography and scintigraphy have been excluded from the ACR-EULAR criteria as they are no longer commonly used for the evaluation of pSS. Sialography is a painful, time-consuming procedure and is contraindicated in patients with severe salivary gland dysfunction. Scintigraphy exposes patients to radiation, has limited specificity and is not widely available[20].

Compared to the ACR criteria, the ACR-EULAR criteria show slightly lower absolute agreement with expert consensus and lower specificity. On the other hand, the ACR-EULAR criteria show higher sensitivity, similar to recent findings in Japanese patients[8]. Furthermore, the ACR-EULAR criteria are more feasible than the ACR criteria in daily clinical practice, as it is often not necessary to perform a salivary gland biopsy or ocular staining score to reach the cut-off of ≥4 for classification as pSS. To avoid inclusion of patients who are misclassified as pSS in therapeutic trials, we do recommend performing a full diagnostic work-up[19].

An important strength of this study is the use of expert classification as gold standard. The AECG criteria are commonly used in our hospital, as shown by an agreement of 94% between the AECG criteria and the clinical diagnosis of the treating physician (data not shown). As the ACR-EULAR and AECG criteria show high agreement, the validity of the ACR-EULAR

classification would be overestimated when using clinical diagnosis by the treating physician as gold standard. Our expert panel consisted of three rheumatologists with broad experience in diagnosing pSS patients. Agreement between the treating physician and the expert panel was high, but the experts were stricter than the treating physician. A possible limitation is that the expert panel consisted only of physicians working in our expertise center. For some of the cases, one of the evaluating experts was therefore also the treating physician of the patient. We cannot exclude the possibility that despite anonymisation, some cases may have been recognized by the experts, but the influence of this potential source of bias is limited as all cases were evaluated by at least 2 experts. We did not include sialography and scintigraphy in our diagnostic work-up, which might have influenced our results regarding the AECG classification. However, as sialography and scintigraphy are not commonly performed anymore to diagnose pSS, we believe our results are representative of how the AECG criteria are most often applied.

In conclusion, the ACR-EULAR criteria showed excellent diagnostic accuracy in our prospective cohort. The ACR-EULAR criteria also have excellent accuracy when using parotid gland biopsies, with good sensitivity and specificity. The validity of Schirmer's test and UWS, as well as addition of new items should be further evaluated. Based on our results, we strongly recommend performing OSS to evaluate ocular signs of pSS. The ACR-EULAR criteria showed validity equal to the AECG criteria, and compared to the ACR criteria, high sensitivity but lower specificity. The ACR-EULAR criteria have important advantages compared to other criteria sets, and have been endorsed by both the ACR and EULAR, allowing for international consensus regarding the classification of pSS.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Supplementary table 1. Comparison of criteria sets

Items	ACR-EULAR	AECG	ACR
ESSDAI ≥1	+ (entry criterium)	-	-
Sicca symptoms	+ (entry criterium)	+	-
Salivary gland biopsy, focus score ≥1	+ (3 points)	+	+
Serology			
SSA antibodies	+ (3 points)	+	+
SSB antibodies	-	+	+
Antinuclear antibodies	-	-	+
Rheumatoid factor	-	-	+
Oral signs			
UWS ≤0.1ml/min	+ (1 point)	+	-
Sialography	-	+	-
Scintigraphy	-	+	-
Ocular signs			
Schirmer's test ≤5	+ (1 point)	+	-
Ocular staining	OSS ≥5 or vBv ≥4 (1 point)	vBv ≥4	OSS ≥3
Exclusion criteria			
Past head and neck radiation	+	+	+
AIDS	+	+	+
Sarcoidosis	+	+	+
Amyloidosis	+	-	+
Graft versus host disease	+	+	+
Pre-existing lymphoma	-	+	-
Current use of anticholinergic drugs	-[a]	+	-
Hepatitis C infection	+ (confirmed by PCR)	+	+
IgG4-related disease	+	-	+

[a]Patients using anticholinergic drugs should be evaluated for signs of oral and ocular dryness after a sufficient interval without using these drugs. ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; OSS: Ocular Staining Score; UWS: Unstimulated Whole Saliva; vBv: van Bijsterveld score.

Supplementary table 2. Detailed characteristics of patients with discrepancy between ACR-EULAR and expert classification

	Expert classification	ACR- EULAR Labial	ACR-EULAR Parotid	Sex	Age, years	Labial FS	Parotid FS	Anti-SSA titer	UWS, mlmin	Schirmer, mm/5min	OSS	RF[a], IU/ml	IgG[b], g/l	ESSDAI
1	SS	Non-SS	Non-SS	F	24	0.7	**1.5**	0	0.42	6	0	1.2	8.6	18
2	SS	SS	Non-SS	F	53	**1.1**	0.0	0	**0.02**	**0**	NR	2.8	13.1	2
3	SS	SS	Non-SS	F	67	**2.0**	0.0	0	0.18	10	**5**	0	20.2	9
4	Non-SS	SS	Non-SS	F	44	**1.2**	0.9	0	0.22	**2**	1	1.3	14.4	3
5	Non-SS	SS	Non-SS	F	52	**2.0**	0.6	0	0.32	**5**	**7**	0.9	10.6	0
6	Non-SS	SS	Non-SS	F	44	**1.3**	0.0	0	0.32	**3**	1	2.1	9.6	2
7	Non-SS	SS	Non-SS	F	53	**2.6**	0.0	0	0.60	**3**	3	0.0	11.9	3
8	Non-SS	SS	Non-SS	F	63	**1.8**	0.5	0	0.37	**4**	3	0.0	11.5	0
9	Non-SS	SS	Non-SS	F	57	**1.0**	0.4	0	0.25	**4**	2	0.0	18.8	5
10	Non-SS	SS	Non-SS	F	60	**1.8**	0.0	0	**0.00**	6	0	6.0	18.2	5
11	Non-SS	SS	NA	F	70	**2.8**	NA	0	**0.00**	**5**	1	1.0	9.7	0
12	Non-SS	SS	SS	F	46	**3.4**	**1.7**	**17**	0.32	35	0	0.6	8.4	6
13	Non-SS	SS	SS	F	51	**1.3**	0.0	**208**	**0.08**	**1**	0	0.0	10.8	0
14	Non-SS	SS	SS	F	57	0.0	0.0	**233**	**0.01**	**4**	0	1.4	11.5	2
15	Non-SS	SS	SS	F	47	0.4	0.6	**240**	0.19	**5**	2	1.9	12.3	7
16	Non-SS	SS	SS	F	61	0.0	0.0	**39**	**0.01**	**0**	**8**	5.3	25.7	9
17	Non-SS	Non-SS	SS	F	68	0.0	**1.0**	0	0.14	**2**	4	0.6	9.7	4

Scores that are positive according to the ACR-EULAR items are shown in bold. [a]Normal ≤5 IU/ml. [b]Normal ≤16 g/l. FS: focus score (foci/4 mm^2); NR: not reported.

CHAPTER 6

Incorporation of salivary gland ultrasonography into the American College of Rheumatology/European League Against Rheumatism criteria for primary Sjögren's syndrome

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ABSTRACT

Objective. To assess whether addition of salivary gland ultrasound (SGUS) or replacement of current criteria items by SGUS influences the performance of the ACR-EULAR criteria for primary Sjögren's syndrome (pSS).

Methods. Included were consecutive patients with complete data on all ACR-EULAR items (n=243), who underwent SGUS in our pSS expertise centre. Clinical diagnosis by the treating physician was used as gold standard. Separate analyses were performed for patients who underwent labial or parotid gland biopsies. The average score for hypoechogenic areas in one parotid and one submandibular gland was determined (range 0-3). Next, performance of the ACR-EULAR criteria was evaluated after addition of SGUS or replacement of current items by SGUS.

Results. Receiver operating characteristic analysis showed an optimal cut-off value of ≥1.5 for SGUS. The optimal weight for SGUS positivity was 1. Cut-off for ACR-EULAR fulfilment remained ≥4. In patients who underwent a labial gland biopsy (n=124), the original criteria showed an AUC of 0.965, sensitivity of 95.9% and specificity of 92.2%. After addition of SGUS, AUC was 0.966, with a sensitivity of 97.3% and specificity of 90.2%. In patients who underwent a parotid gland biopsy (n=198), similar results were found. Sensitivity of the criteria decreased substantially when SGUS replaced salivary gland biopsy or anti-SSA antibodies, while performance remained equal when SGUS replaced OSS, Schirmer's test or UWS.

Conclusion. Validity of the ACR–EULAR criteria remains high after incorporation of SGUS. With SGUS, clinicians are offered a larger array of tests to evaluate fulfilment of the ACR-EULAR criteria.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a common systemic autoimmune disease affecting the exocrine glands, manifesting as keratoconjunctivitis sicca (dry eyes) and xerostomia (dry mouth)[1,2]. Patients also often experience fatigue and several extra-glandular manifestations[2].

Several classification criteria sets for pSS have been developed during the past years. Of these, the 2002 American European Consensus Group (AECG) criteria have most often been used in daily clinical practice for many years[3–5]. Cornerstones of this criteria set are a focus score ≥1 in a salivary gland biopsy and presence of anti-SSA/anti-SSB antibodies[3]. These criteria take both subjective sicca complaints and objective measures for the ocular and oral complaints into account, whereby equal weights are assigned to the oral and ocular components[3]. However, the AECG criteria have not been endorsed by the American College of Rheumatology (ACR) and European league against Rheumatism (EULAR)[4,5]. In an effort to reach international consensus regarding classification criteria for pSS, recently the 2016 ACR-EULAR criteria were developed, consisting of items from the 2002 AECG and the 2012 ACR criteria[3–6]. Both EULAR and ACR have endorsed the ACR-EULAR criteria, and the criteria have been validated in multiple external cohorts[7–9]. The ACR-EULAR criteria show high sensitivity and specificity, regardless of the type of biopsy (parotid or labial) taken to assess the salivary gland focus score[8].

Upon a closer look at the ACR-EULAR criteria, a few key points become evident. First, salivary gland histopathology and presence of anti-SSA antibodies deservedly remain cornerstones in the classification of pSS. Second, tear gland involvement is measured using a functional test (Schirmer's test) and by imaging of structural damage of the ocular surface (Ocular Staining Score, OSS), while salivary gland involvement is only evaluated using a functional test (unstimulated whole saliva flow, UWS). Removal of sialography and scintigraphy from the criteria is an advantage of the ACR-EULAR criteria, considering the invasiveness and limited validity of these procedures[4,10–12]. However, the ACR-EULAR criteria now lack a test which measures structural salivary gland damage.

Currently, B-mode salivary gland ultrasonography (SGUS) is increasingly applied to assess structural changes of the salivary glands in pSS. SGUS is non-invasive, non-irradiating, inexpensive, relatively easy to perform in an outpatient setting and can be repeated for follow-up. Previous studies have demonstrated that SGUS has good accuracy to differentiate pSS from non-pSS[9,13–17]. Many scoring systems are applied for SGUS, but recent analyses showed that limiting scoring to hypoechogenic areas in both the submandibular and parotid gland on one side suffices for accurate differentiation between pSS and non-pSS[18]. Scoring of hypoechogenic areas showed good intra- and interobserver reliability[19,20]. This reduction of the scoring system further increases the feasibility of the technique for common application in a diagnostic setting.

In clinical cohort studies, addition of SGUS to the AECG and ACR criteria has been shown to increase the sensitivity of these criteria with a minor decrease in their specificity[10,21]. Unfortunately, SGUS was not tested as a new diagnostic technique in the cohorts in which the ACR-EULAR criteria were developed and validated, and not considered to be included in the criteria. Therefore, our primary objective was to assess whether presence of hypoechogenic areas on SGUS as a criteria item influences the performance of the ACR-EULAR criteria. The second objective was to evaluate the performance of the ACR-EULAR criteria when replacing current items with SGUS. Both objectives were evaluated in a large cohort of patients clinically suspected of pSS.

PATIENTS AND METHODS

Study population

The study population for this cohort study consisted of all eligible consecutive patients, who underwent an SGUS examination between October 2014 and July 2017. SGUS was performed as a routine diagnostic imaging technique in new patients clinically suspected of pSS as well as during baseline visits of pSS patients included in the Abatacept Sjögren Active Patients phase III (ASAPIII) trial (NCT02067910) or the REgistry of Sjögren syndrome in Umcg – LongiTudinal (RESULT) observational cohort study.

Exclusion criteria were age <18 years, presence of an associated systemic auto-immune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus) or, current use of biological disease modifying anti-rheumatic drugs (bDMARDs). Patients lacking a clinical diagnosis and patients with an incomplete diagnostic work-up according to the ACR-EULAR criteria were also excluded. The clinical diagnosis by experienced treating physicians was used as gold standard in all analyses. In case diagnosis was not clear-cut, consensus was achieved by consulting at least one other experienced physician.

This study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Medical Ethics Committee of the UMCG; METc 2018/309 and waived the requirement of written informed consent.

Salivary gland ultrasound

SGUS was performed using the MyLabSeven scanner (Esaote, Genova, Italy), equipped with a high resolution linear probe (4-13 MHz). All SGUS images were scored by A.J.S., K.D. or J.F.N, who previously showed good inter-observer agreement when scoring hypoechogenic areas[19]. Median intra-class correlation coefficients were 0.74 for parotid glands and 0.71 for submandibular glands. The presence of hypoechogenic areas was scored as follows: 0 for no hypoechogenic areas, 1 for a few scattered areas, 2 for several areas, and 3 for numerous

hypoechogenic areas[17]. The average score for presence of hypoechogenic areas, ranging from 0-3, in the submandibular and parotid gland on the right side was determined, which was previously shown to accurately differentiate between pSS and non-pSS[18]. If the right parotid or submandibular gland could not be scored (e.g., because of previous removal of that gland), scores of the left side were used. Receiver operating characteristic (ROC) analysis was used to determine the optimal cut-off value for SGUS to identify patients who were clinically diagnosed with pSS by the treating physicians, by choosing the cut-off for which the sum of sensitivity and specificity was the highest.

Classification according to the original ACR-EULAR criteria

All included patients had been subjected to a complete multidisciplinary work-up according to the ACR-EULAR criteria[4,5], including a labial gland biopsy, parotid gland biopsy or both. Separate analyses were performed, in which classification according to the ACR-EULAR criteria was determined using the outcomes of either labial or parotid gland biopsies. Patients who underwent both a labial and parotid gland biopsy were included in both analyses, with either the results of their labial or parotid gland biopsy being used to determine ACR-EULAR classification.

Incorporation of salivary gland ultrasound into the ACR-EULAR criteria

SGUS positivity was added as an item to the ACR-EULAR criteria. To keep the original criteria applicable, the weight of the original criteria items was kept as they were, i.e. 3 points for presence of anti-SSA antibodies and a focus score ≥1; and 1 point for an abnormal UWS, Schirmer's test and OSS score[4,5]. To select the optimal weight of SGUS, separate analyses of the performance of the modified ACR-EULAR criteria were performed, assigning a weight of either 1, 2 or 3 points for a positive SGUS.

Replacement of current ACR-EULAR criteria items by ultrasound

Next, five additional criteria sets were developed in which SGUS replaced one of the current items. The weight of the original items was again kept equal to the original criteria, and the optimal weight of the SGUS item was determined by doing separate analyses using a weight of 1, 2 or 3 points for a positive SGUS.

Statistical analysis

Statistical analyses were executed using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA). ROC analysis was performed to determine the accuracy of the original ACR-EULAR score, the ACR-EULAR score with addition of SGUS, and the ACR-EULAR score with SGUS as replacement of original items to predict the clinical diagnosis. Area under the curve (AUC) was interpreted as no discrimination (0-0.5) or poor (0.5-0.7), fair (0.7-0.8), good (0.8-0.9) or excellent (0.9-1.0) accuracy[22]. Optimal cut-off values of the different ACR-EULAR scores were determined, by choosing the cut-off for which the sum of sensitivity and specificity was the highest. Patients

were then classified according to this cut-off for the original and modified criteria sets. Finally, absolute agreement, sensitivity and specificity of the original and modified ACR-EULAR criteria sets, with clinical diagnosis as gold standard, were determined and compared.

RESULTS

SGUS was performed in 363 patients. Of these, 243 patients were eligible for inclusion (figure 1). Of the 342 included patients, 45 patients underwent only a labial biopsy, 119 patients underwent only a parotid gland biopsy, and 79 patients underwent both a labial and parotid gland biopsy. Including the patients who underwent both biopsies, 124 patients underwent a labial biopsy, and 198 patients underwent a parotid gland biopsy.

Characteristics of pSS and non-pSS patients are shown in table 1. All included patients fulfilled the entry criteria of the ACR-EULAR criteria. The characteristics of the patients who underwent a labial gland biopsy were similar to those of the patients who underwent a parotid gland biopsy (data not shown). Median time between SGUS and salivary biopsies was 7 months for labial gland biopsies and 6 months for parotid biopsies.

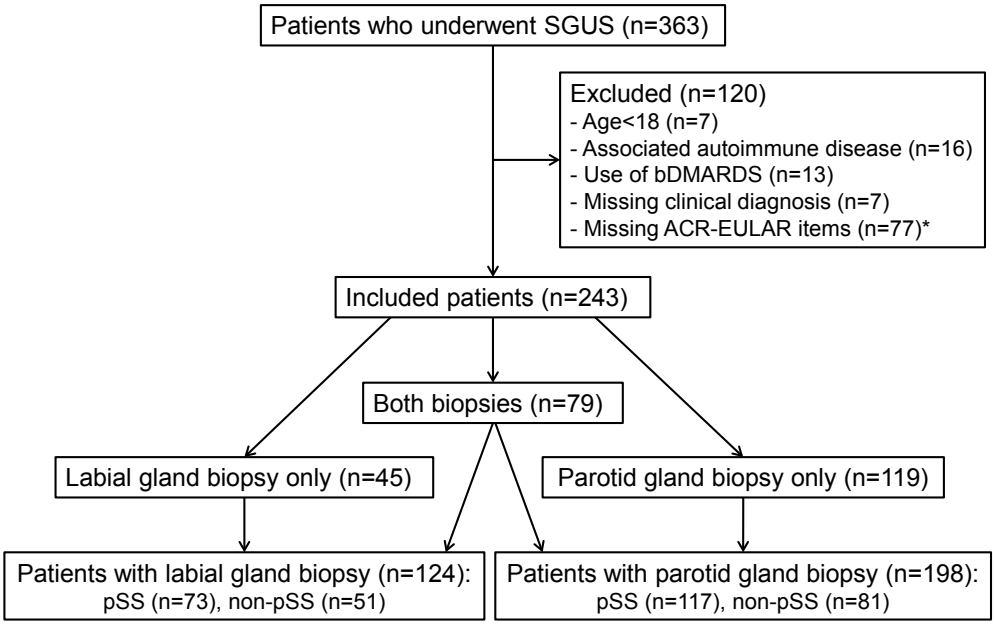

Figure 1. Flow chart of patient inclusion.
* Missing items were: salivary gland biopsy (n=72); Schirmer's test (n=4); ocular staining score (n=2) and unstimulated whole saliva flow (n=2). In the majority of these patients, either the patients could be classified as primary Sjögren's syndrome (pSS) without the need of a positive salivary gland biopsy, or a positive biopsy would not have resulted in a clinical diagnosis of pSS. ACR: American college of rheumatology; bMARDs: biological disease modifying anti-rheumatic drugs; EULAR: European league against rheumatism; SGUS: salivary gland ultrasound.

Table 1. Characteristics of study population

Characteristics	pSS (n=147)	Non-pSS (n=96)
Age, mean ± SD years	53 ± 14	52 ± 14
Female	131 (89)	81 (84)
SGUS score, median [interquartile range]	2.0 [1.0;2.5]	0.5 [0.5;1.0]
SGUS score ≥1.5	106 (72)	10 (10)
FS≥1 in labial gland biopsy[a]	64 (88)	5 (10)
FS≥1 in parotid gland biopsy[b]	89 (76)	2 (2)
Anti-SSA+	125 (85)	9 (9)
Ocular Staining Score ≥5	70 (48)	11 (12)
Schirmer ≤5 mm/5 min	113 (77)	56 (58)
Unstimulated whole saliva ≤0.1 ml/min	105 (71)	42 (44)

Values are the number (%) unless indicated otherwise. [a]n=124 (73 pSS, 51 non-pSS. [b]n=198 (117 pSS and 81 non-pSS).
pSS: primary Sjögren's syndrome; SGUS: salivary gland ultrasonography; FS: focus score (foci/4mm^2).

Performance of SGUS

The accuracy of SGUS to predict clinical diagnosis was good, with an AUC of 0.860 (95% CI 0.821-0.900), and an optimal cut-off value of ≥1.5. SGUS was therefore considered positive when the average score for presence of hypoechogenic areas in one parotid and one submandibular gland was ≥1.5. Based on this cut-off point, SGUS was positive in 106 pSS and 6 non-pSS patients and negative in 41 pSS and 90 non-pSS patients. Absolute agreement with clinical diagnosis was 80.7%, sensitivity was 72.1% and specificity was 93.8%.

Performance of ACR-EULAR criteria with addition of SGUS

Supplementary table 1A-B shows the performance of the ACR-EULAR criteria, when SGUS was added to the criteria, using a weight of 1, 2 or 3 for a positive SGUS. The performance of the ACR-EULAR criteria including SGUS was highest when a positive SGUS was assigned a weight of 1 point. The optimal cut-off point of the original ACR-EULAR score to discriminate between pSS and non-pSS was confirmed to be ≥4. After the addition of SGUS to the ACR-EULAR criteria with a weight of 1 point, the optimal cut-off point of the modified ACR-EULAR score to discriminate between pSS and non-pSS remained ≥4 (supplementary table 1A-B). Based on these results, in the following analyses a cut-off of ≥4 was used for the original and modified ACR-EULAR score. A positive SGUS results in an increase of 1 point in the modified ACR-EULAR score (table 2A-B).

Table 2. Original and modified ACR-EULAR criteria incorporating salivary gland ultrasound

Item	Weight
A. Original ACR-EULAR criteria	
Focal lymphocytic sialadenitis and FS≥1	3 points
Anti-SSA/Ro positive	3 points
OSS ≥5 in at least 1 eye	1 point
Schirmer's test ≤5 mm/5 minutes in at least 1 eye	1 point
UWS flow rate ≤0.1 ml/minute	1 point
B. Modified ACR-EULAR criteria: addition of ultrasound	
Focal lymphocytic sialadenitis and FS≥1	3 points
Anti-SSA/Ro positive	3 points
OSS ≥5 in at least 1 eye	1 point
Schirmer's test ≤5 mm/5 minutes in at least 1 eye	1 point
UWS flow rate ≤0.1 ml/minute	1 point
Average SGUS score for hypoechogenic areas ≥1.5	1 point

For both sets, patients with a score of ≥4 are classified as pSS. ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; FS: focus score (foci/4mm^2); pSS: primary Sjögren's syndrome; OSS: ocular staining score; UWS: unstimulated whole saliva flow; SGUS: salivary gland ultrasound.

In patients who underwent a labial gland biopsy (n=124), the original ACR-EULAR criteria showed an AUC of 0.965 (95% CI 0.932 – 0.997) to predict clinical diagnosis (figure 2). Absolute agreement with clinical diagnosis was 94.4%, sensitivity was 95.9%, and specificity was 92.2%. After addition of SGUS, the modified ACR-EULAR criteria showed an AUC of 0.966 (95% CI 0.934 – 0.998), absolute agreement remained the same, sensitivity slightly increased to 97.3% and specificity slightly decreased to 88.2%.

The same analyses were performed in patients who underwent a parotid gland biopsy (n=198), and similar results were found (figure 2). In this group, the original criteria showed an AUC of 0.954 (95% CI 0.925 – 0.984) to predict clinical diagnosis. Absolute agreement with clinical diagnosis was 92.9%, sensitivity was 91.4%, and specificity was 95.1%. After addition of SGUS, the modified ACR-EULAR criteria showed an AUC of 0.964 (95% CI 0.939 – 0.989), absolute agreement remained the same, sensitivity slightly increased to 92.3%, and specificity slightly decreased to 93.8%.

To summarize, addition of SGUS to the ACR-EULAR criteria resulted in negligible changes in the performance of the criteria, and did not change its optimal cut-off point.

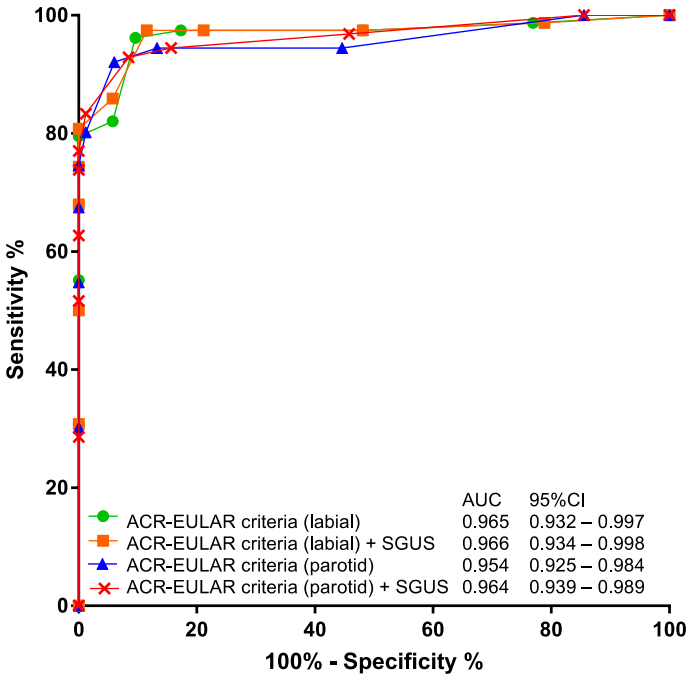

Figure 2. Receiver operating characteristics curves of the original American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria and adjusted criteria with addition of salivary gland ultrasonography (SGUS).
AUC: area under the curve; 95% CI: 95% confidence interval.

Performance of ACR-EULAR criteria with replacement of items by SGUS

For the following analysis, five modified sets of criteria were used in which one of the original items was replaced with SGUS. When SGUS replaced current criteria items in patients who underwent a labial gland biopsy (n=124), the optimal weight for SGUS was again 1 point, regardless of which original criteria items was replaced by SGUS (supplementary table 2). The optimal cut-off point to discriminate between pSS and non-pSS remained ≥4.

When SGUS replaced the labial gland biopsy or anti-SSA antibodies, there was a considerable decrease in accuracy and sensitivity, while there was only a slight decrease in specificity compared to the original criteria (table 3A, figure 3A). On the other hand, when SGUS replaced the OSS, Schirmer's test or UWS, no major changes in accuracy, sensitivity and specificity occurred.

The same analyses were performed in patients who underwent a parotid gland biopsy (n=198). When SGUS replaced the OSS, Schirmer's test or UWS, the optimal weight for SGUS was again 1 point (supplementary table 3), with only minor changes in sensitivity and

specificity and even an increase in accuracy (table 3B, figure 3B). When SGUS replaced the parotid gland biopsy or anti-SSA antibodies, optimal weight for SGUS would be 3 points. However, regardless of whether SGUS was assigned 1, 2 or 3 points in these analyses, accuracy of the ACR-EULAR criteria drops substantially (supplementary table 3).

To summarize, SGUS can replace the OSS, Schirmer's test or UWS in the classification of pSS without major changes in the performance of the criteria. The salivary gland biopsy or the measurement of anti-SSA antibodies on the other hand, cannot be completely replaced by SGUS, since this led to a considerable decrease in the performance of the criteria.

Table 3. Performance of the original and modified ACR-EULAR criteria sets with SGUS replacing current items

	AUC	95% CI	Agreement	Sensitivity	Specificity
A. Patients with labial gland biopsy (n=124)					
Original ACR-EULAR criteria	0.965	0.932-0.997	94.4%	95.9%	92.2%
SGUS replacing labial gland biopsy	0.903	0.849-0.957	87.9%	82.2%	94.1%
SGUS replacing anti-SSA antibodies	0.943	0.902-0.985	89.5%	86.3%	94.1%
SGUS replacing OSS	0.964	0.931-0.996	93.5%	95.9%	88.2%
SGUS replacing Schirmer's test	0.969	0.938-1.000	93.5%	94.5%	92.2%
SGUS replacing UWS	0.967	0.937-0.998	93.5%	97.3%	88.2%
B. Patients with parotid gland biopsy (n=198)					
Original ACR-EULAR criteria	0.954	0.925-0.984	92.9%	91.4%	95.1%
SGUS replacing parotid gland biopsy	0.925	0.887-0.962	88.4%	83.8%	95.1%
SGUS replacing anti-SSA antibodies	0.918	0.879-0.956	86.9%	79.5%	97.5%
SGUS replacing OSS	0.964	0.938-0.990	93.4%	92.3%	95.1%
SGUS replacing Schirmer's test	0.964	0.939-0.989	89.9%	84.6%	97.5%
SGUS replacing UWS	0.969	0.946-0.992	92.9%	90.6%	96.3%

In all criteria sets a weight of 1 point for SGUS and cut-off value of ≥4 for fulfilment of the criteria was used. ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; OSS: Ocular Staining Score; UWS: unstimulated whole saliva flow; SGUS: salivary gland ultrasound.

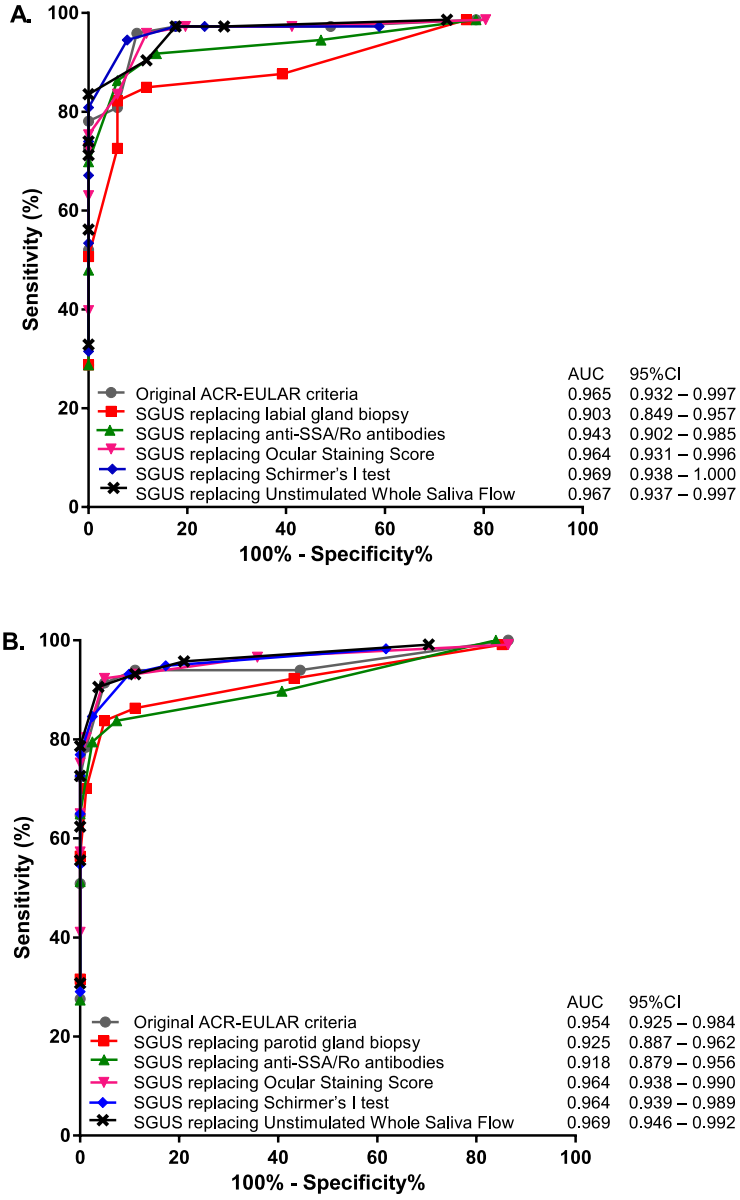

Figure 3. Receiver operating characteristic curves of the original American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) criteria and adjusted criteria with replacement of original items by salivary gland ultrasonography (SGUS).

A. ACR-EULAR criteria including labial gland biopsy outcome. B. ACR-EULAR criteria including parotid gland biopsy outcome. AUC: area under the curve.

DISCUSSION

In this large clinical cohort study, we aimed to investigate the performance of the ACR-EULAR criteria when a positive SGUS was added to the criteria. The performance of the ACR-EULAR criteria was best when SGUS was assigned a weight of 1 point.

In the first part of this study, it was shown that addition of SGUS to the ACR-EULAR criteria only marginally increased sensitivity and marginally decreased specificity, while overall accuracy remained the same. Although addition of SGUS did not improve the accuracy of the ACR-EULAR criteria in our cohort, it improves their feasibility in clinical practice, by allowing rheumatologists to choose from a larger array of tests.

Previously, two other studies incorporated SGUS in the ACR-EULAR classification criteria[23,24]. In the study by Le Goff et al., in which the AECG and ACR-EULAR classification criteria were compared, the addition of SGUS to the ACR-EULAR criteria was also investigated[23]. The authors, however, arbitrarily assigned a weight of 1 point to a positive SGUS and used the same cut-off value as the original ACR-EULAR criteria (i.e. ≥4). In our study, it was confirmed with a meticulous statistical analysis that the optimal weight to assign to SGUS was indeed 1 point and that the optimal cut-off value to classify a patient as having pSS remained ≥4. In the study by Le Goff et al.[23], similar results were found regarding the performance of the ACR-EULAR criteria after addition of SGUS i.e. sensitivity was slightly increased and specificity slightly decreased.

In the study by Takagi et al., the weight of the original criteria items was also kept[24]. In contrast to our study, 3 points were assigned to SGUS positivity, and the optimal cut-off point to discriminate between SS and non-SS increased to ≥5. The combined ACR-EULAR and SGUS scoring system showed an improved accuracy compared to the original criteria. Unfortunately, a fair comparison between the study of Takagi et al. and ours cannot be made, since the methodology of their study differed greatly from ours[24]. Importantly, complete data regarding the ACR-EULAR items was only available in a small subset of the included patients (62 out of 213 patients), Saxon's test, which measures stimulated whole saliva, was used instead of UWS and patients with secondary SS were not excluded. Furthermore, a different, more complicated SGUS score was used.

In the second part of this study, the performance of the ACR-EULAR criteria was evaluated when SGUS replaced current classification items. We found that SGUS could replace the OSS, Schirmer's test or UWS in the classification of pSS, without decreasing the accuracy of the ACR-EULAR criteria. However, when SGUS replaced the salivary gland biopsy in the classification of pSS or the measurement of anti-SSA antibodies, the performance of the criteria significantly decreased.

In a previous study, we showed that the combination of a positive SGUS and presence of SSA antibodies had a positive predictive value of 97% for classification as pSS, according to the ACR-EULAR criteria[14]. Based on these results, Mossel et al. suggested that for classification purposes, the first step of a classification work-up could be SGUS and determination of anti-SSA positivity. When both are positive, patients can already be classified as pSS. The current study confirms these results, as the combination of anti-SSA positivity and SGUS positivity is indeed enough for fulfilment of the adjusted ACR-EULAR criteria. As the next step in the work-up for classification, we recommend a salivary gland biopsy, since the sensitivity of the ACR-EULAR criteria decreased substantially when the salivary gland biopsy was completely replaced by SGUS. When it comes to clinically diagnosing a patient with pSS, on the other hand, we prefer a full work-up, including an SGUS and as many items of the ACR-EULAR criteria as possible, to allow a clinician to decide on the best possible treatment for that particular patient.

When SGUS is added to the ACR-EULAR criteria, the cut-off of 4 points can be fulfilled solely based on the Schirmer's test, OSS, UWS and SGUS. In our database, this combination only occurred in one patient, who was clinically diagnosed as non-SS. Therefore, we cannot draw a definite conclusion about the validity of the ACR-EULAR criteria in this specific subgroup. Based on our expert opinion, we would recommend only classifying such a patient as pSS if also a positive biopsy or anti-SSA antibodies is present, until there is more data available regarding this subgroup.

In this study, we used a simplified SGUS scoring system, similar to the ones used by other groups[15,21,23]. However, the lack of a consensus scoring system complicates the incorporation of SGUS into the ACR-EULAR criteria. Jousse-Joulin et al. recently published an atlas with consensual definitions of SGUS abnormalities[20]. The next step will be to agree on a consensus scoring system with a validated cut-off. As soon as a validated cut-off is set, SGUS hopefully will be incorporated into the ACR-EULAR criteria. Addition of SGUS, as a measure for structural damage of the salivary glands, would balance the ACR-EULAR criteria by including two items to measure tear as well as salivary gland involvement.

A strong point of our study is the use of a large cohort of patients from daily clinical practice, including pSS as well as non-pSS sicca patients, with complete data for all ACR-EULAR items. Furthermore, analyses were performed separately for patients who underwent a labial and/or a parotid gland biopsy, which makes our data relevant to all diagnostic centers, regardless of the type of biopsy performed. A potential limitation of the study is the use of clinical diagnosis performed by expert clinicians working in a tertiary referral centre for pSS, instead of expert consensus, as gold standard. However, using expert consensus as gold standard would also have introduced bias, depending on the familiarity of the experts with SGUS in pSS.

In conclusion, the validity of the ACR-EULAR criteria remains high after incorporation of SGUS. SGUS is non-invasive, non-irradiating, inexpensive, and relatively easy to perform in an outpatient setting, and could replace OSS, Schirmer's test or UWS in centers with less access to these tests. Incorporation of SGUS into the ACR-EULAR criteria improves their feasibility in clinical practice, by allowing rheumatologists to choose from a larger array of tests. The modified criteria enable a step-wise approach for classification, starting with determination of SSA-antibodies and SGUS, which decreases the number of invasive salivary gland biopsies needed for classification.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interests.

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Supplementary table 1. Optimal weight of SGUS in the ACR-EULAR classification criteria and optimal cut-off of the modified ACR-EULAR score

Weight SGUS	AUC	95% CI	Cut-off point	Sensitivity	Specificity
A. Modified ACR-EULAR criteria incl. labial gland biopsy					
			3	97.3%	78.4%
1 point	**0.966**	**0.934-0.998**	**4**	**97.3%**	**88.2%**
			5	84.9%	94.1%
			3	97.3%	78.4%
2 points	0.965	0.932-0.997	4	97.3%	84.3%
			5	86.3%	92.2%
			3	97.3%	76.5%
3 points	0.962	0.929-0.995	4	97.3%	84.3%
			5	86.3%	88.2%
B. Modified ACR-EULAR criteria incl. parotid gland biopsy					
			3	94.0%	86.4%
1 point	**0.964**	**0.939-0.989**	**4**	**92.3%**	**93.8%**
			5	82.1%	98.8%
			3	96.6%	85.2%
2 points	0.967	0.943-0.991	4	92.3%	91.4%
			5	82.9%	97.5%
			3	96.6%	85.2%
3 points	0.965	0.941-0.990	4	94.9%	90.1%
			5	82.9%	95.1%

Analysis of the optimal weight of SGUS and optimal cut-off of the modified ACR-EULAR score when SGUS is added to the ACR-criteria, based on the best combination of sensitivity and specificity. The optimal weight and cut-off point are highlighted in bold. SGUS: salivary gland ultrasound.

Supplementary Table 2. Optimal weight and cut-off after replacement of items with SGUS in patients with labial gland biopsies

Weight SGUS	AUC	95% CI	Cut-off point	Sensitivity	Specificity
A. SGUS replacing anti-Ro/SSA antibodies					
			3	91.8%	86.3%
1 point	**0.943**	**0.902 – 0.985**	**4**	**86.3%**	**94.1%**
			5	69.9%	100%
			3	93.2%	86.3%
2 points	0.941	0.898 – 0.983	4	87.7%	90.2%
			5	71.2%	98.0%
			3	93.2%	84.3%
3 points	0.936	0.892 – 0.980	4	89.0%	90.2%
			5	72.6%	94.1%
B. SGUS replacing labial gland biopsy					
			3	84.9%	88.2%
1 point	**0.903**	**0.849 – 0.957**	**4**	**82.2%**	**94.1%**
			5	72,6%	94.1%
			3	87.7%	88.3%
2 points	0.911	0.860 – 0.963	4	82.2%	90.2%
			5	72.6%	94.1%
			3	89.0%	84.3%
3 points	0.910	0.858 – 0.962	4	84.9%	90.2%
			5	72.6%	90.2%
C. SGUS replacing Ocular Staining Score					
			3	97.3%	80.4%
1 point	**0.964**	**0.931 – 0.996**	**4**	**95.9%**	**88.2%**
			5	83.6%	94.1%
			3	97.3%	78.4%
2 points	0.963	0.931 – 0.996	4	95.9%	86.3%
			5	86.3%	92.2%
			3	97.3%	76.5%
3 points	0.961	0.927 – 0.994	4	95.9%	84.3%
			5	86.3%	90.2%
D. SGUS replacing Schirmer's I Test					
			3	97.3%	82.4%
1 point	**0.969**	**0.938 – 1.000**	**4**	**94.5%**	**92.2%**
			5	80.8%	100%
			3	97.3%	78.4%
2 points	**0.969**	**0.937 – 1.000**	**4**	**94.5%**	**92.2%**
			5	84.9%	98.0%
			3	97.3%	76.5%
3 points	0.965	0.932 – 0.998	4	94.5%	88.2%
			5	84.9%	98.0%
E. SGUS replacing Unstimulated Whole Saliva Flow					
			3	97.3%	78.4%
1 point	**0.967**	**0.937 – 0.997**	**4**	**97.3%**	**88.2%**
			5	84.9%	94.1%
			3	97.3%	78.4%
2 points	0.964	0.933 – 0.995	4	90.4%	86.3%
			5	84.9%	98.0%
			3	97.3%	76.5%
3 points	0.961	0.929 – 0.993	4	90.4%	84.3%
			5	84.9%	96.1%

Analysis of the optimal weight of SGUS and optimal cut-off of the modified ACR-EULAR score when current items are replaced by SGUS in patients who underwent labial biopsies, based on the best combination of sensitivity and specificity. The optimal weight and cut-off point are highlighted in bold. SGUS: salivary gland ultrasound.

Supplementary Table 3. Optimal weight and cut-off after replacement of items with SGUS in patients with parotid gland biopsies

Weight SGUS	AUC	95% CI	Cut-off point	Sensitivity	Specificity
A. SGUS replacing anti-Ro/SSA antibodies					
			3	83.8%	92.6%
1 point	0.918	0.879 – 0.956	4	79.5%	97.5%
			5	65.0%	100%
			3	**87.2%**	**91.4%**
2 points	**0.922**	**0.884 – 0.960**	4	81.2%	95.1%
			5	71.8%	98.8%
			3	**87.2%**	**91.4%**
3 points	**0.921**	**0.883 – 0.959**	4	84.6%	93.8%
			5	73.5%	96.3%
B. SGUS replacing parotid gland biopsy					
			3	86.3%	88.9%
1 poin	0.925	0.887 – 0.962	4	83.8%	95.1%
			5	70.1%	98.8%
			3	91.5%	87.7%
2 points	0.935	0.900 – 0.960	4	84.6%	92.6%
			5	71.8%	97.5%
			3	92.3%	87.7%
3 points	**0.935**	**0.900 – 0.970**	**4**	**89.7%**	**91.4%**
			5	72.6%	95.1%
C. SGUS replacing Ocular Staining Score					
			3	93.2%	88.9%
1 point	**0.964**	**0.938 – 0.990**	**4**	**92.3%**	**95.1%**
			5	80.3%	98.8%
			3	95.7%	86.4%
2 points	0.966	0.941 – 0.991	4	92.3%	92.6%
			5	82.9%	98.8%
			3	95.7%	86.4%
3 points	0.964	0.939 – 0.990	4	94.9%	90.1%
			5	82.9%	96.3%
D. SGUS replacing Schirmer's I Test					
			3	**93.2%**	**90.1%**
1 point	**0.964**	**0.939 – 0.989**	4	84.6%	97.5%
			5	76.9%	100%
			3	94.0%	86.4%
2 points	0.966	0.943 – 0.990	4	84.6%	96.3%
			5	79.5%	100%
			3	95.7%	86.4%
3 points	0.965	0.940 – 0.989	4	85.5%	92.6%
			5	79.5%	98.8%
E. SGUS replacing Unstimulated Whole Saliva Flow					
			3	93.2%	88.9%
1 point	**0.969**	**0.946 – 0.992**	**4**	**90.6%**	**96.3%**
			5	78.6%	100%
			3	96.6%	85.2%
2 points	0.967	0.943 – 0.991	4	92.3%	91.4%
			5	82.9%	97.5%
			3	95.7%	86.4%
3 points	0.969	0.947 – 0.992	4	92.3%	92.6%
			5	79.5%	97.5%

Analysis of the optimal weight of SGUS and optimal cut-off of the modified ACR-EULAR score when current items are replaced by SGUS in patients who underwent parotid gland biopsies, based on the best combination of sensitivity and specificity. The optimal weight and cut-off point are highlighted in bold. SGUS: salivary gland ultrasound.

CHAPTER 7

Clinical phenotyping of primary Sjögren's patients using salivary gland ultrasonography – data from the REgistry of Sjögren syndrome in Umcg LongiTudinal (RESULT) cohort

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ABSTRACT

Objective. To investigate salivary gland ultrasound (SGUS) abnormalities in relation to patient characteristics, disease activity and disease damage in patients with primary Sjögren's syndrome (pSS).

Methods. Consecutive outpatients included in our REgistry of Sjögren Syndrome LongiTudinal (RESULT) cohort were selected. Included pSS patients were classified according to the ACR-EULAR criteria and underwent full ultrasonographic examination (Hocevar score 0-48) at baseline. Total SGUS scores of ≥15 were considered positive. Patient characteristics, disease activity and disease damage were compared between the different SGUS groups.

Results. In total, 172/186 pSS patients were eligible, of whom 136 (79%) were SGUS positive. SGUS positive patients had significantly longer disease duration, higher ESSDAI, higher SSDDI, more often a positive parotid gland biopsy, anti-SSA/SSB antibodies, abnormal unstimulated whole saliva (UWS) and ocular staining score (OSS), and higher levels of IgG and rheumatoid factor compared with SGUS negative patients. Regarding patient-reported outcome measurements (PROs), SGUS positive patients scored significantly lower on ESSPRI fatigue and pain, and more often found their disease state acceptable compared with SGUS negative patients.

SGUS total score showed significant associations with various clinical and serological parameters, and with PROs. Highest associations were found for UWS (ρ=-0.551) and OSS (ρ=0.532).

Conclusion. SGUS positive patients show a distinct clinical phenotype compared with SGUS negative patients in all aspects of the disease: clinical, functional, serological and PROs. SGUS could be a helpful tool in selecting patients for clinical trials and estimating treatment need.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a common systemic auto-immune disease[1]. Women are affected nine times more often than men[2]. pSS is a highly heterogeneous disease, which is reflected by the many different manifestations patients can have. Common symptoms, such as extreme fatigue and sicca symptoms have a major impact on the quality of life[1,3]. This heterogeneity already emerges during the diagnostic work-up of pSS, i.e., not every pSS patient has auto-antibodies or a focus score positive salivary gland biopsy, which suggests that there are different subgroups of patients. It would be of great value to be able to identify individual patients at high risk for a severe disease outcome. Prospective cohort studies are gaining more and more importance in this quest[4]. Since treatment options for pSS patients are eagerly awaited, but unfortunately still very limited, the search for proper selection methods for clinical trials is currently ongoing.

Regarding the care for (suspected) pSS patients, there is a unique collaboration between different departments at the University Medical Center Groningen (UMCG). The REgistry of Sjögren syndrome in Umcg – LongiTudinal (RESULT) cohort has been set up to identify biomarkers and clinical parameters that determine and predict the longitudinal course of pSS. Observational studies, like RESULT, are important as they provide information on long-term outcome of pSS, which reflects daily clinical practice.

Salivary gland ultrasonography (SGUS) is increasingly gaining acceptance as an imaging tool of the salivary glands in pSS. Nowadays, ultrasound is widely accessible in outpatient rheumatology clinics. SGUS is non-invasive and non-irradiating, which makes it patient-friendly and an ideal imaging modality for repeated use[5–7].

Previously, we have studied the validity of SGUS and found that a positive ultrasound, based on the total Hocevar score[6], predicts classification according to the American College of Rheumatology – European League Against Rheumatism (ACR-EULAR) criteria[8]. Subsequently we found that measuring only hypoechogenic areas in one parotid and one submandibular gland is sufficient to predict ACR-EULAR classification, which increases the feasibility of SGUS[9]. Although a simpler scoring system suffices for classification purposes, it is not yet known whether SGUS abnormalities can also be used for patient stratification, long-term follow-up or even as selection method for clinical trials. Therefore, a full SGUS evaluation according to the Hocevar score is performed in each patient included in the RESULT cohort.

The aim of this study was to investigate SGUS abnormalities in relation to patient characteristics, disease activity and disease damage in patients with pSS.

MATERIALS AND METHODS

REgistry of Sjögren syndrome in Umcg – LongiTudinal (RESULT) cohort

The observational RESULT cohort combines up-to-date quality of care with gathering long-term prospective follow-up data in a large cohort of patients. For participation in RESULT, we consider all consecutive patients with probable or confirmed pSS who visit the outpatient clinic of the Department of Rheumatology and Clinical Immunology in the UMCG, a tertiary referral expertise center. Inclusion in RESULT is ongoing and duration of follow-up will be 10 years.

The present cross-sectional analysis included the baseline visit of all patients who were included in the RESULT cohort between January 2016 and December 2018. Patients with missing ultrasonographic examination as well as patients who did not fulfill the ACR-EULAR criteria for pSS (i.e. probable pSS patients) were excluded[10,11].

Assessments

Imaging, clinical, functional, histopathological, serological parameters and patients-reported outcome measurements (PROs) were obtained according to a fixed protocol.

Salivary gland ultrasound

B-mode SGUS was performed using the MyLabSeven scanner (Esaote, Genova, Italy), equipped with a high-resolution linear probe (4-13 MHz). All ultrasonographic images were scored real-time by trained readers (AJS, KD, JFN, EM, RW). Test-retest reliability in our center was demonstrated previously[12]. The scoring system by Hocevar et al.[6] was applied (range 0-48), including the components parenchymal echogenicity, homogeneity, presence of hypoechogenic areas, hyperechogenic reflections and clearness of the salivary gland border. Total SGUS score of ≥15 was considered positive[8].

Other assessments

Demographic characteristics, EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI)[13], 28 joint disease activity score (DAS-28) (ESR and CRP)[14,15], number of tenderpoints, physician global disease activity (physician GDA), Sjögren's Syndrome Disease Damage Index (SSDDI)[16], unstimulated whole saliva flow (UWS)[17], Schirmer's test and ocular staining score (OSS)[18] were determined. Two methods were applied for Schirmer's test and OSS, i.e. when dividing in normal/abnormal the worst eye was selected and when applied as a continuous variable the mean of both eyes was used. A salivary gland biopsy was not mandatory for participation in RESULT and therefore, parotid and labial salivary gland focus score were recorded if available[19–21].

Serological parameters were determined, including presence of anti-SSA/SSB antibodies, immunoglobulin G (IgG) level, rheumatoid factor (RF) level, complement C3 and C4 levels and leukocyte count.

Patients completed a questionnaire, which included EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) dryness, fatigue and pain[22], patient acceptable symptom state (PASS), patient GDA and the 5-level EuroQoL five dimensions health status questionnaire (EQ-5D-5L)[23].

Statistics

Statistical analyses were performed using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA). Descriptive parameters were expressed as number (%) of patients for categorical data and mean (SD) or median (IQR) for continuous data.

Patient characteristics, disease activity and damage were compared between SGUS negative (score <15) and positive (score ≥15) patients. Subsequently, based on the median score of the SGUS positive group, SGUS positive patients were arbitrarily divided into two equal groups; patients with scores ≥15, but <27 were defined as medium-positive and patients with scores ≥27 were defined as high-positive.

Fisher's exact test or Chi square were used as appropriate to evaluate differences in categorical variables between the ultrasound groups. Independent Samples T-test or Mann-Whitney U test were used as appropriate to evaluate differences in continuous variables between the ultrasound groups. ESSDAI subdomains were summarized descriptively.

The association between SGUS total score and continuous variables was analyzed using Spearman correlation coefficient (ρ), and interpreted as poor association (0.0-0.2), fair (0.2-0.4), moderate (0.4-0.6), good (0.6-0.8) or excellent (0.8-1.0)[24]. All parameters were also evaluated using univariate logistic regression analysis with SGUS outcome (positive vs. negative) as dependent variable. In case of residuals with non-Gaussian distribution, variables were transformed (log or square root), before being entered into the model. The explained variance was evaluated using Nagelkerke R^2. P-values ≤0.05 were considered statistically significant.

All analyses were repeated when only taking the average score for 'hypoechogenic areas' in the right parotid and submandibular gland into account[9], instead of the total SGUS score as described by Hocevar et al.[6] For this score, a cut-off value of ≥1.5 was considered positive[25].

RESULTS

Between January 2016 and December 2018, 186 patients were included in RESULT. Fourteen patients were excluded for the present analysis due to a missing (n=3) or incomplete (n=5) ultrasonographic examination, or because they did not fulfill the ACR-EULAR criteria (n=6). Of the eligible patients (n=172), mean age was 53 years (SD 13.9), 156 patients (91%) were female, 136 patients (79%) were SGUS positive (i.e. SGUS score ≥15)[8] and median time since diagnosis was 8 years (table 1).

Table 1. Patients characteristics and comparison of SGUS negative and positive patients

Characteristic	Total group (n=172)	SGUS ≤14 (n=36)	SGUS ≥15 (n=136)	P value
General characteristics				
Age, years	52.9 (13.9)	56.0 (14.0)	52.0 (13.8)	0.13
Females	156 (90.7%)	31 (86.1%)	125 (91.9%)	0.29
Disease duration, years	8.0 (4.0-13.0)	5.0 (3.0-8.8)	8.5 (5.0-13.8)	**0.003**
Symptom duration, year[c]	15.0 (9.0-21.0)	11.0 (6.0-19.0)	15.0 (10.0-22.0)	0.06
BMI (kg/m^2)[a]	24.9 (4.2)	24.6 (3.6)	24.8 (4.3)	0.79
Clinical parameters				
ESSDAI total score[a]	4.0 (2.0-8.0)	2.0 (0.0-6.5)	4.0 (2.0-8.0)	**0.028**
ESSDAI categories[a]				**0.024**
ESSDAI=0	25 (14.6%)	10 (27.8%)	15 (11.1%)	
ESSDAI=1-4	75 (43.9%)	16 (44.4%)	59 (43.7%)	
ESSDAI ≥5	71 (41.5%)	10 (27.8%)	61 (45.2%)	
DAS28-ESR[b]	3.2 (1.0)	2.9 (0.8)	3.3 (1.0)	**0.027**
DAS28-CRP[b]	2.3 (1.9-2.6)	2.3 (1.9-2.5)	2.3 (1.8-2.7)	0.74
Tenderpoints[b]	1.5 (0.0-8.0)	2.0 (0.0-12.0	1.0 (0.0-8.0)	0.34
Physician GDA[c]	2.0 (1.0-3.0)	2.0 (1.0-3.0)	3.0 (1.0-4.0)	**0.026**
SSDDI total score[c]	2.0 (1.0-3.0)	1.5 (1.0-2.0)	2.0 (1.0-3.0)	**0.018**
UWS ≤0.1 mL/min[b]	111 (68.5%)	16 (45.7%)	95 (74.8%)	**0.001**
UWS flow, mL/min[b]	0.05 (0.01-0.13)	0.12 (0.03-0.27)	0.03 (0.00-0.11)	**<0.001**
Parotid gland biopsy, FS ≥1[e]	85 (81.0%)	12 (50.0%)	73 (90.1%)	**<0.001**
Labial gland biopsy, FS ≥1[f]	47 (81.0%)	11 (68.8%)	36 (85.7%)	0.14
Schirmer's test ≤5mm/5min[b]	121 (74.7%)	25 (69.4%)	96 (76.2%)	0.41
Schirmer ODS, mm/5min[b]	4.0 (0.9-10.0)	5.5 (2.6-11.1)	3.5 (0.0-9.6)	**0.020**
OSS ≥5[a]	58 (34.1%)	3 (8.3%)	55 (41.0%)	**<0.001**
OSS ODS[a]	2.5 (0.9-5.0)	0.5 (0.0-2.0)	3.5 (1.0-5.0)	**<0.001**
Serological parameters				
Anti-SSA antibodies[a]	154 (90.1%)	27 (75.0%)	127 (94.1%)	**0.001**
Anti-SSB antibodies[a]	92 (53.8%)	9 (25.0%)	83 (61.5%)	**<0.001**
IgG level >16.0 g/mL[a]	81 (47.4%)	5 (13.9%)	76 (56.3%)	**<0.001**
IgG level, g/mL[a]	15.5 (11.2-20.3)	11.2 (9.3-13.0)	16.9 (12.1-21.8)	**<0.001**
RF level >5.0 IU/mL[a]	115 (67.3%)	12 (33.3%)	103 (76.3%)	**<0.001**
RF level, IU/mL[a]	15.0 (2.6-42.0)	2.1 (0.6-10.6)	21.0 (5.2-51.0)	**<0.001**
Complement C3 level (g/L)[a]	1.12 (0.23)	1.20 (0.24)	1.10 (0.22)	**0.012**
Complement C4 level (g/L)[a]	0.19 (0.15-0.24)	0.20 (0.18-0.24)	0.18 (0.14-0.24)	**0.015**
Leucocyte count 10^9/L[a]	5.4 (1.9)	6.3 (2.0)	5.2 (1.8)	**0.002**
Patient-reported outcome measurements				
ESSPRI total score[a]	6.0 (4.3-7.0)	6.7 (5.0-7.7)	5.7 (4.3-7.0)	**0.016**
ESSPRI dryness[a]	6.0 (5.0-8.0)	6.0 (4.0-8.0)	7.0 (5.0-8.0)	0.26
ESSPRI fatigue[a]	7.0 (5.0-8.0)	8.0 (5.0-8.0)	7.0 (4.3-8.0)	**0.024**
ESSPRI pain[a]	5.0 (2.0-7.0)	7.0 (5.0-8.0)	4.5 (2.0-7.0)	**<0.001**
Patient GDA[b]	6.0 (4.0-8.0)	7.0 (4.3-8.0)	6.0 (4.0-8.0)	0.15
EQ-5D-5L[d]	0.77 (0.14)	0.73 (0.17)	0.80 (0.12)	0.23
PASS, acceptable[b]	117 (71.8%)	21 (58.3%)	96 (75.6%)	**0.042**

Data are expressed as number of patients (%), mean (SD) or median (IQR). [a]<5% missing data; [b]5-10% missing data; [c]10-15% missing data; [d]22% missing data. Data available for [e]61% and [f]34% of patients. Schirmer's test ≤5mm/min and OSS ≥5 were considered positive if criteria were met in at least one eye. For Schirmer ODS and OSS ODS, the mean score of both eyes was calculated. BMI: body mass index; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; EQ-5D-5L: 5-level EuroQoL five dimensions health status questionnaire; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; FS: focus score; GDA: global disease activity; OSS: ocular staining score; PASS: patient acceptable symptom state; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

Comparison of SGUS negative and positive patients

In figure 1, a heat map of the characteristics of the individual pSS patients is shown. The patient order has been determined based upon total SGUS score.

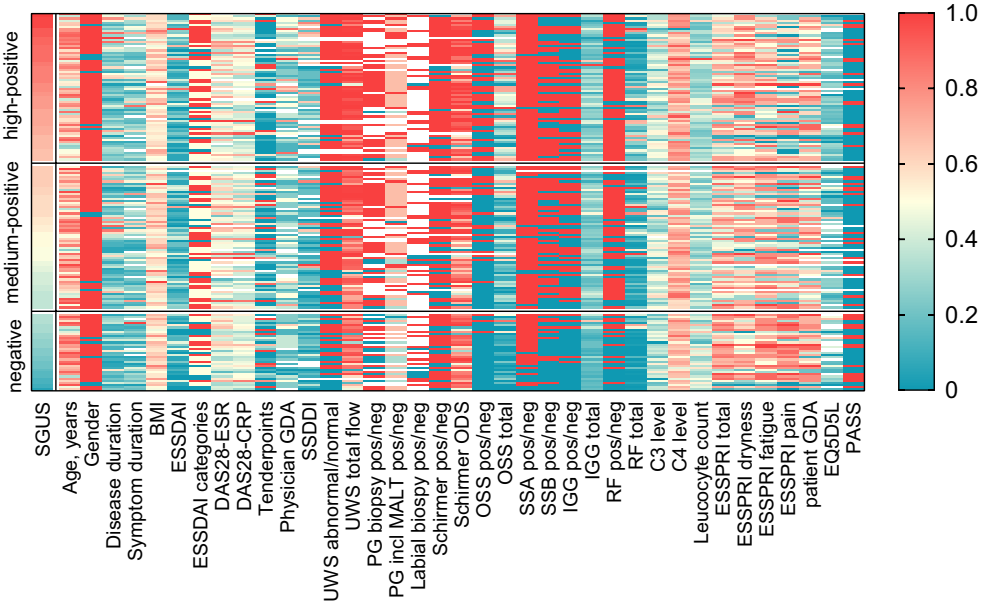

Figure 1. Patients characteristics. A heat map of the parameters for the 172 individual patients.
The values have been scaled between zero (turquoise) and one (red), with one being the worst score. Missing values are shown as white bars. BMI: body mass index; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; FS: focus score; GDA: global disease activity; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; EQ-5D-5L: 5-level EuroQoL five dimensions health status questionnaire; OSS: ocular staining score; PASS: patient acceptable symptom state; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

Table 1 shows the characteristics of the total group of pSS patients, as well as of the patients with a positive or negative SGUS. There were no significant differences in general patient characteristics between the two groups, except for disease duration, which was longer in de SGUS positive patients (figure 2A).

SGUS positive patients had significantly higher ESSDAI scores, higher DAS28-ESR and higher physician GDA compared with SGUS negative patients, indicating higher disease activity (table 1; figure 2B-D; supplementary figure 1). Moreover, a parotid gland focus score ≥1, UWS ≤0.1ml/min and OSS ≥5 were more often seen in SGUS positive patients (table 1). SSDDI, UWS, Schirmer and OSS also differed significantly between both groups, with more damage and worse salivary and lacrimal gland function in SGUS positive patients (table 1; figure 2E-H).

Regarding the serological parameters, anti-SSA and anti-SSB antibodies were more often present in SGUS positive patients. Furthermore, SGUS positive patients showed higher levels of IgG and RF, lower complement C3 and C4 levels and lower leucocyte counts compared with SGUS negative patients (table 1; figure 2I).

Regarding PROs, SGUS positive patients scored significantly lower on ESSPRI fatigue and pain, and more often found their disease state acceptable, which indicates that SGUS positive patients experienced less symptoms (table 1).

Results were confirmed with univariate logistic regression analyses (table 2). The explained variance of individual parameters varied from 0.1% for body mass index (BMI) to 22.4% for parotid gland biopsy (focus score ≥1).

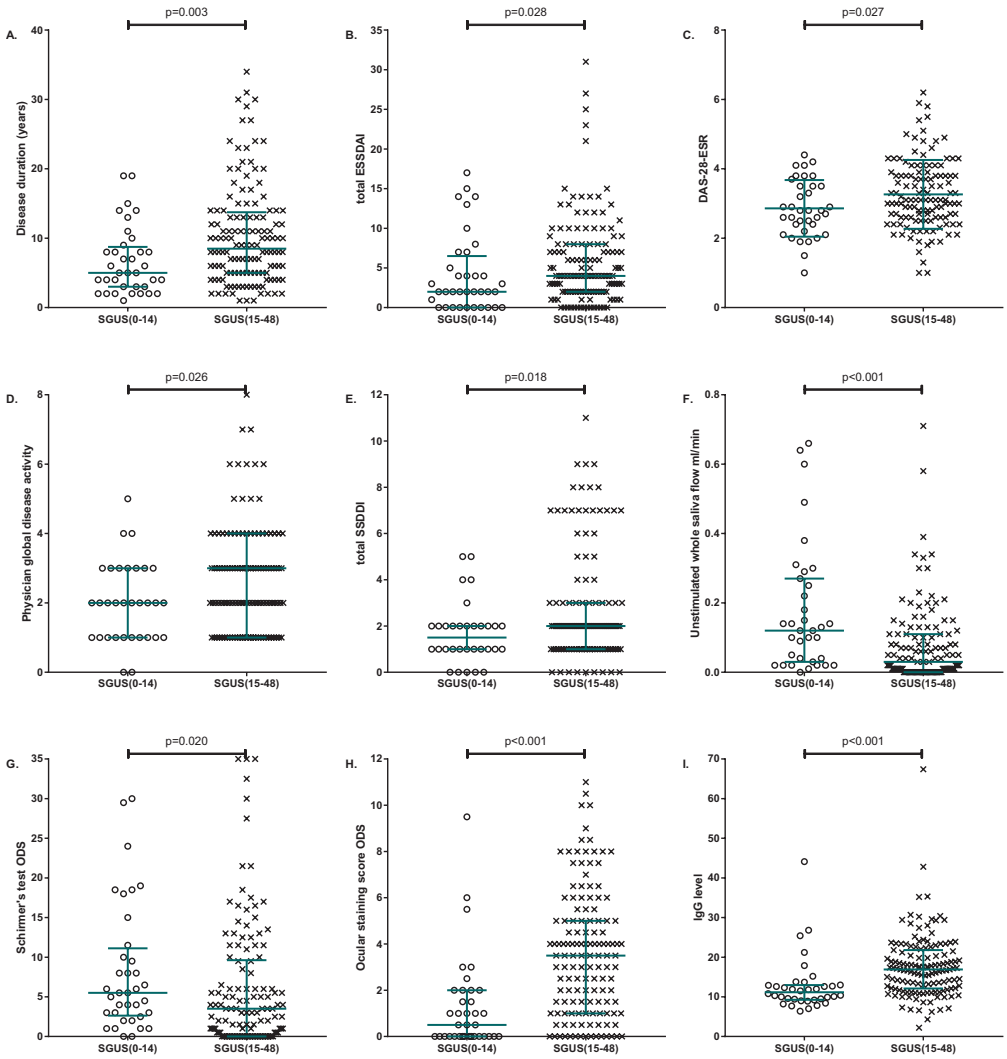

Figure 2. Ultrasound total score (negative/positive) compared with A. Disease duration; B. total ESSDAI; C. DAS28-ESR; D. Physician global disease activity; E. total SSDDI; F. Unstimulated whole saliva flow; G. Schirmer's test; H. Ocular staining score and I. total IgG level.

DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; GDA: global disease activity; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index.

Table 2. Logistic regression analyses of demographic, clinical, serological and patient reported outcome parameters to predict SGUS outcome

Characteristic	Univariate analysis OR (95% CI)	P value	R^2
General characteristics			
Age, years	1.022 (0.993-1.051)	0.13	0.021
Females	1.833 (0.593-5.662)	0.29	0.009
Disease duration, years	0.902 (0.837-0.973)	**0.007**	0.082
Symptom duration, years	0.965 (0.923-1.009)	0.12	0.028
BMI (kg/m^2)	1.012 (0.927-1.104)	0.79	0.001
Clinical parameters			
ESSDAI total score[a]	0.696 (0.503-0.962)	**0.028**	0.046
DAS28-ESR	0.622 (0.405-0.955)	**0.030**	0.048
DAS28-CRP	0.784 (0.454-1.355)	0.38	0.008
Tenderpoints	1.024 (0.969-1.082)	0.40	0.007
Physician GDA	0.679 (0.489-0.942)	**0.020**	0.064
SSDDI total score	0.737 (0.572-0.949)	**0.018**	0.079
UWS ≤0.1 mL/min	3.525 (1.622-7.663)	**0.001**	0.094
UWS flow, mL/min	103.799 (7.237-1488.828)	**0.001**	0.120
Parotid gland biopsy, FS ≥1	9.125 (3.089-26.953)	**<0.001**	0.224
Labial gland biopsy, FS ≥1	2.727 (0.696-10.684)	0.15	0.049
Schirmer's test ≤5mm/5min	1.408 (0.621-3.194)	0.41	0.006
Schirmer ODS, mm/5min[b]	1.520 (1.062-2.178)	**0.022**	0.051
OSS ≥5	7.658 (2.236-26.227)	**0.001**	0.141
OSS ODS total score	0.626 (0.499-0.785)	**<0.001**	0.212
Serological parameters			
Anti-SSA antibodies	5.292 (1.872-14.956)	**0.002**	0.084
Anti-SSB antibodies	4.788 (2.088-10.984)	**<0.001**	0.136
IgG level >16.0 g/mL	0.125 (0.046-0.342)	**<0.001**	0.192
IgG level g/mL	0.886 (0.823-0.954	**0.001**	0.121
RF level >5.0 IU/mL	0.155 (0.070-0.345)	**<0.001**	0.192
RF level IU/mL	0.980 (0.965-0.996)	**0.012**	0.094
Complement C3 level (g/L)	7.576 (1.489-38.564)	**0.015**	0.055
Complement C4 level (g/L)	37.907 (0.502-2861.647)	0.10	0.024
Leucocyte count 10^9/L	1.322 (1.088-1.607)	**0.005**	0.075
Patient-reported outcome measurements			
ESSPRI total score	1.229 (0.999-1.512)	0.051	0.038
ESSPRI dryness[b]	0.595 (0.282-1.258)	0.17	0.016
ESSPRI fatigue	1.195 (1.002-1.426)	**0.047**	0.040
ESSPRI pain[b]	2.635 (1.245-5.574)	**0.011**	0.075
Patient GDA[a]	1.237 (0.654-2.341)	0.51	0.004
EQ-5D-5L	0.095 (0.004-2.072)	0.14	0.026
PASS, acceptable	2.212 (1.018-4.809)	**0.045**	0.036

[a]SQRT transformation; [b]LN transformation. BMI: body mass index; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; EQ-5D-5L: 5-level EuroQoL five dimensions health status questionnaire; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; FS: focus score; GDA: global disease activity; OSS: ocular staining score; PASS: patient acceptable symptom state; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

Comparison of patients with medium-positive or high-positive SGUS scores

Compared with patients with a medium-positive SGUS score, patients with a high-positive SGUS score significantly more often had an UWS ≤0.1ml/min, Schirmer's test ≤5mm/5min and OSS ≥5 (table 3). Furthermore, SSDDI, UWS, Schirmer and OSS differed significantly between medium- and high-positive SGUS patients, showing more damage and a worse salivary and lacrimal gland function in the high-positive patients (table 3).

Patients with high-positive SGUS scores experienced significantly more dryness, but less fatigue and pain compared with patients with a medium-positive SGUS score (table 3).

Correlations of SGUS total score

Significant associations were found between SGUS total score and disease duration (ρ=0.279), symptom duration (ρ=0.234), ESSDAI (ρ=0.196), DAS28-ESR (ρ=0.159), physician GDA (ρ=0.217), SSDDI (ρ=0.398), UWS (ρ=-0.551), Schirmer (ρ=-0.349) and OSS (ρ=0.532) (supplementary table 1; figure 3A-D). Furthermore, significant associations were found between SGUS total score and IgG level (ρ=0.264), RF level (ρ=0.343), complement C4 level (ρ=-0.200) and leucocyte count (ρ=-0.244) (supplementary table 1; figure 3E,F). Moreover, SGUS total scores showed significant association with PROs; ESSPRI total score (ρ=-0.157), dryness (ρ=0.223), fatigue (ρ=-0.209) and pain (ρ=-0.314) (supplementary table 1; figure 3G-I).To summarize, an increase in SGUS abnormalities is associated with longer disease duration, more damage and worse gland function, and with more dryness symptoms.

SGUS – hypoechogenic areas only

When using only hypoechogenic areas to define SGUS positivity[9], multiple parameters showed similar results as when total Hocevar score was applied, except that no significant differences were found for: ESSDAI, DAS28-ESR, physician GDA, complement C3 and C4 levels, leucocyte counts and PASS (supplementary tables 2&3).

Table 3. Comparison of SGUS positive patients with medium or high SGUS scores

Characteristic	SGUS 15-26 (n=67)	SGUS 27-41 (n=69)	P value
General characteristics			
Age, years	53.1 (13.6)	51.0 (13.9)	0.39
Females	63 (94.0%)	62 (89.9%)	0.53
Disease duration, years	8.0 (4.0-14.0)	9.0 (6.0-13.5)	0.35
Symptom duration, years	14.5 (8.0-21.8)	16.0 (11.0-22.0)	0.22
BMI (kg/m^2)	24.8 (4.7)	24.8 (4.0)	0.99
Clinical parameters			
ESSDAI total score	4.0 (2.0-8.0)	4.0 (2.0-8.0)	0.76
ESSDAI categories			0.92
ESSDAI=0	7 (10.6%)	8 (11.6%)	
ESSDAI=1-4	30 (45.5%)	29 (42.0%)	
ESSDAI ≥5	29 (43.9%)	32 (46.4%)	
DAS28-ESR	3.3 (1.0)	3.3 (1.0)	0.88
DAS28-CRP	2.3 (1.7-2.7)	2.3 (2.0-2.7)	0.59
Tenderpoints	2.0 (0.0-9.0)	0.0 (0.0-5.8)	0.19
Physician GDA	2.0 (1.0-3.0)	3.0 (1.0-4.0)	0.28
SSDDI total score	2.0 (1.0-3.0)	2.0 (2.0-5.8)	**0.001**
UWS ≤0.1 mL/min	40 (60.6%)	55 (90.1%)	**<0.001**
UWS flow, mL/min	0.08 (0.01-0.15)	0.01 (0.00-0.04)	**<0.001**
Parotid gland biopsy, FS ≥1	36 (85.7%)	37 (94.9%)	0.27
Labial gland biopsy, FS ≥1	18 (81.8%)	18 (90.0%)	0.67
Schirmer's test ≤5mm/5min	41 (67.2%)	55 (84.6%)	**0.022**
Schirmer ODS, mm/5min	5.0 (1.0-12.0)	2.0 (0.0-5.3)	**0.017**
OSS ≥5	17 (25.8%)	38 (55.9%)	**<0.001**
OSS ODS total score	2.0 (1.0-4.0)	4.0 (2.5-6.4)	**<0.001**
Serological parameters			
Anti-SSA antibodies	60 (90.9%)	67 (97.1%)	0.16
Anti-SSB antibodies	38 (57.6%)	45 (65.2%)	0.36
IgG level >16.0 g/mL	37 (56.1%)	39 (56.5%)	0.96
IgG level, g/mL	16.8 (12.0-19.9)	17.4 (12.1-22.6)	0.57
RF level >5.0 IU/mL	47 (71.2%)	56 (81.2%)	0.17
RF level, IU/mL	15.5 (3.0-36.3)	32.0 (8.5-57.5)	**0.037**
Complement C3 level (g/L)	1.10 (0.23)	1.10 (0.22)	0.88
Complement C4 level (g/L)	0.19 (0.15-0.24)	0.18 (0.13-0.22)	0.16
Leucocyte count 10^9/L	5.3 (1.6)	5.1 (2.0)	0.64
Patient-reported outcome measurements			
ESSPRI total score	6.0 (4.3-7.2)	5.7 (4.0-6.7)	0.30
ESSPRI dryness	6.0 (4.0-8.0)	7.0 (5.0-8.0)	**0.050**
ESSPRI fatigue	7.0 (5.0-8.0)	6.0 (4.0-7.0)	**0.042**
ESSPRI pain	6.0 (3.0-7.0)	4.0 (2.0-6.0)	**0.019**
Patient GDA	6.0 (4.0-7.5)	6.0 (4.0-8.0)	0.80
EQ-5D-5L	0.78 (0.14)	0.78 (0.11)	0.94
PASS, acceptable	45 (73.8%)	51 (77.3%)	0.65

Data are expressed as number of patients (%), mean (SD) or median (IQR). BMI: body mass index; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; EQ-5D-5L: 5-level EuroQoL five dimensions health status questionnaire; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; FS: focus score; GDA: global disease activity; OSS: ocular staining score; PASS: patient acceptable symptom state; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

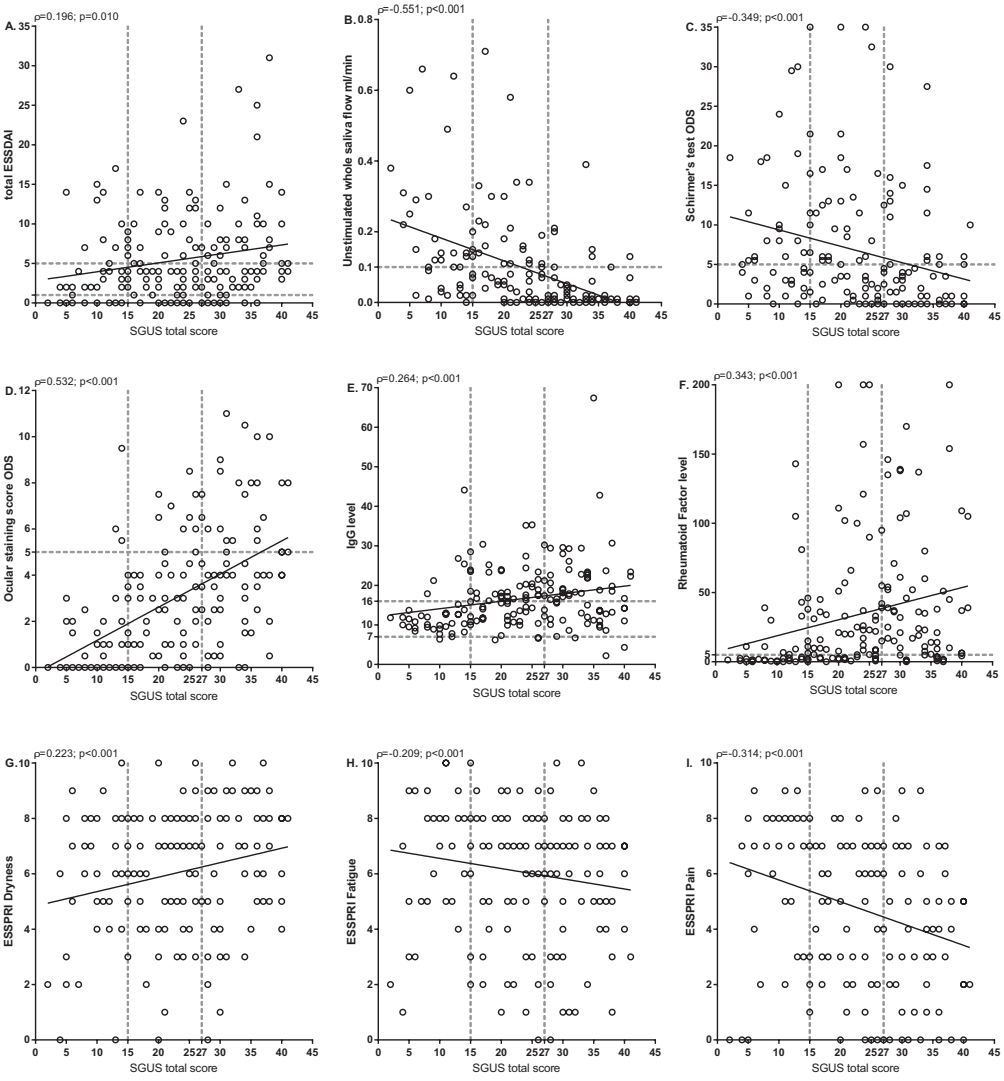

Figure 3. Scatterplots of ultrasound total score compared to A. total ESSDAI; B. Unstimulated whole saliva flow; C. Schirmer's test; D. Ocular staining score; E. IgG level; F. Rheumatoid Factor level; G. ESSPRI dryness; H. ESSPRI fatigue and I. ESSPRI pain.

For Schirmer ODS and OSS ODS, the mean score of both eyes was calculated. ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; SGUS: salivary gland ultrasonography.

DISCUSSION

In our prospective observational RESULT cohort, we showed that SGUS positive patients have a distinct clinical phenotype compared with SGUS negative patients. This difference was found in all aspects of the disease; clinical, functional, serological and PROs. SGUS could give an overall indication about the severity of pSS.

SGUS positive patients have higher systemic disease activity, measured by ESSDAI, DAS28-ESR and physician GDA, compared with SGUS negative patients. Of interest, SGUS positive patients score significantly worse on all individual items of the ACR-EULAR criteria, i.e. parotid gland biopsy, anti-SSA antibodies, Schirmer, OSS and UWS, compared with SGUS negative patients. Overall, total SGUS score showed the strongest association with OSS and UWS. In addition to these differences, SGUS positive patients score worse on SSDDI and serological parameters. These results show that SGUS enables us to identify patients with higher clinical and serological disease activity and more damage due to pSS.

Interestingly, SGUS positive patients experienced less fatigue and pain, both measured by ESSPRI, and more often found their disease state acceptable, which implies that these patients have a lower symptom burden. Perhaps patients who already have pSS (or symptoms) for several years are more used to it and developed their own coping strategy or they adjusted their expectations.

Another possibility is that there are indeed different phenotypic clusters of pSS patients. Very recently, Tarn et al.[26] defined four subgroups of pSS patients based upon the patient-reported outcomes dryness, fatigue, pain, anxiety and depression. Our data suggest that patients with high SGUS scores belong to a subgroup of patients with low symptom burden. Unfortunately, the Hospital Anxiety and Depression Scale (HADS) is not part of the questionnaires within our RESULT cohort. Therefore we were unable to verify whether SGUS scores also differ within these four subgroups of patients.

In the current study, we not only compared SGUS negative and positive patients based on a previously defined diagnostic cut-off point[8], but also zoomed in on the broad range of SGUS positive patients. As expected, patients with a high-positive SGUS score showed more pSS-related damage (SSDDI) and lower salivary and lacrimal gland function and more glandular damage, compared with patients with a medium-positive SGUS score. Interestingly, there were no differences in the percentage of patients with a positive biopsy or presence of anti-SSA antibodies between both groups. This could be because most patients within our cohort score positive on these items, which makes it more difficult to see differences within subgroups of patients and data on both items was collected as absent/present rather than on a continuous scale. Furthermore, the differences in ESSPRI fatigue and pain remain, with

less patient symptoms in the high-positive group. In contrast however, high-positive SGUS patients do indeed experience more dryness compared with the medium-positive patients, which is logical considering the relationship between SGUS and glandular function.

The association between SGUS and disease duration suggests that there is an increase in ultrasonographic abnormalities over time. In contrast, when looking solely at the SGUS positive patients, there is no difference in disease duration between patients with medium-positive or high-positive scores. This raises the question how long it takes for these SGUS abnormalities to develop and how long these abnormalities continue to worsen. Gazeau et al.[27] showed that a nearly two-year interval between consecutive SGUS examinations was not enough to see significant progression over time in a group of 49 suspected pSS patients. A possible explanation for the lack of difference in disease duration in medium-positive and high-positive SGUS patients might be inter-observer differences, as it was previously shown that SGUS scores between different observers show more variability when total score exceeds 20[12]. Alternatively, it could be postulated that after a certain disease duration SGUS lesions stabilize, as is the case with the production of saliva[28].

In our previous studies, we have shown that for diagnostic purposes it suffices to only measure hypoechogenic areas in one parotid and one submandibular gland[9] and that optimal cut-off for a positive SGUS is ≥1.5[25]. Since the use of SGUS to stratify pSS patients is essentially different from the use of SGUS for diagnostic purposes, we assessed whether results would be similar when using total SGUS score compared with only measuring hypoechogenic areas. Regarding UWS, Schirmer's test, OSS and disease damage measured by SSDDI, results were the same when only the component hypoechogenic areas was taken into account. This suggests that evaluation of hypoechogenic areas can be used to identify patients with glandular dysfunction and overall pSS-related damage. However, no differences in ESSDAI, physician GDA and DAS28-ESR were found when SGUS positivity was solely based on hypoechogenic areas, although there were significant differences in serological activity. Therefore, it remains unclear whether patients with high disease activity can be identified by evaluating only hypoechogenic areas. For this purpose, a more comprehensive scoring system, like the Hocevar scoring system[6], may be preferred above a scoring system including only 1 component.

Previously, several groups studied associations between SGUS and clinical, serological, and patient-reported parameters[29–35]. However, there are considerable differences between some of these studies and our current study. The most important difference is that most studies focus on the possible diagnostic purposes of SGUS rather than its possible use for stratification of already classified pSS patients[30,33–35]. In our study, differences between the SGUS negative and positive patients cannot be attributed to the fact that there are non-SS sicca controls included, as we only included pSS patients in this study. In comparison with the previous studies, we included a considerable higher number of pSS patients. Nevertheless, previous studies found

significant differences between SGUS negative and positive patients regarding ESSDAI[31], tear- and saliva production[29–32], presence of anti-SSA antibodies and/or anti-SSB antibodies[29–32], RF positivity[30,31], VAS dry mouth[32] and ESSPRI dryness[29], and, with the exception of the patient-reported dryness symptoms, we were able to confirm these results. In contrast, other studies did not find differences in ESSDAI[29,30] and SSDDI[30] between SGUS negative and positive patients. In a study including pSS as well as non-SS sicca controls, SGUS positive patients had higher labial gland focus score and more often had an OSS ≥3, UWS ≤0.1mL/min, were anti-SSA/SSB and RF positive and had hypergammaglobulinemia, compared with SGUS negative patients[33]. Other studies also found associations between SGUS and ESSDAI[34] and several serological parameters[34,35], but again in a mixed population of pSS and non-SS sicca controls.

Other differences between previously performed studies and our current study relate to the applied SGUS scoring system and criteria set used for classification. Some studies, including this current study, applied the Hocevar scoring system[6], but different cut-off points were applied[29,30]. Furthermore, we applied the ACR-EULAR classification criteria, as did Kim et al.[33], whereas in all other studies, including the more recent ones, the AECG criteria were applied[29–32,34,35].

To confirm our results in different populations, a consensus scoring system with a validated cut-off is needed. Nevertheless, our results emphasize the important role SGUS could play, not only for diagnostic purposes, but also for the selection of subgroups of patients for instance for clinical trials.

Our prospective observational cohort revealed that the majority of patients is SGUS positive. These patients have a longer disease duration, a higher disease activity and more pSS-related damage compared with SGUS negative patients, whereas SGUS negative patients experience more fatigue and pain. SGUS could be considered as a selection method for clinical trials, as it gives an overall indication of the disease.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ETHICAL APPROVAL INFORMATION

This study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Medical Ethics Committee of the UMCG; METC 2014/491. Al subjects provided informed consent.

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Supplementary Table 1. Associations of SGUS with demographic, clinical, serological and patient reported outcome measurements

Characteristic	Spearman's ρ	P value
General characteristics		
Age, years	-0.085	0.27
Disease duration, years	0.279	**<0.001**
Symptom duration, years[c]	0.234	**0.003**
BMI (kg/m^2)[a]	0.005	0.95
Clinical parameters		
ESSDAI total score[a]	0.196	**0.010**
DAS28-ESR[b]	0.159	**0.045**
DAS28-CRP[b]	0.091	0.25
Tenderpoints[b]	-0.080	0.31
Physician GDA[c]	0.217	**0.007**
SSDDI total score[c]	0.398	**<0.001**
UWS flow, mL/min[b]	-0.551	**<0.001**
Schirmer ODS, mm/5min[b]	-0.349	**<0.001**
OSS ODS total score[a]	0.532	**<0.001**
Serological parameters		
IgG level, g/mL[a]	0.264	**<0.001**
RF level, IU/mL[a]	0.343	**<0.001**
Complement C3 level (g/L)[a]	-0.089	0.25
Complement C4 level (g/L)[a]	-0.200	**0.009**
Leucocyte count 10^9/L[a]	-0.244	**0.001**
Patient-reported outcome measurements		
ESSPRI total score[a]	-0.157	**0.043**
ESSPRI dryness[a]	0.223	**0.004**
ESSPRI fatigue[a]	-0.209	**0.007**
ESSPRI pain[a]	-0.314	**<0.001**
Patient GDA[b]	-0.033	0.68
EQ-5D-5L[d]	0.037	0.67

[a]<5% missing data; [b]5-10% missing data; [c]10-15% missing data; [d]22% missing data. For Schirmer ODS and OSS ODS, the mean score of both eyes was calculated. BMI: body mass index; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; EQ-5D-5L: 5-level EuroQoL five dimensions health status questionnaire; GDA: global disease activity; OSS: ocular staining score; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

Supplementary table 2. Comparison of SGUS negative and positive patients based on presence of hypoechogenic areas

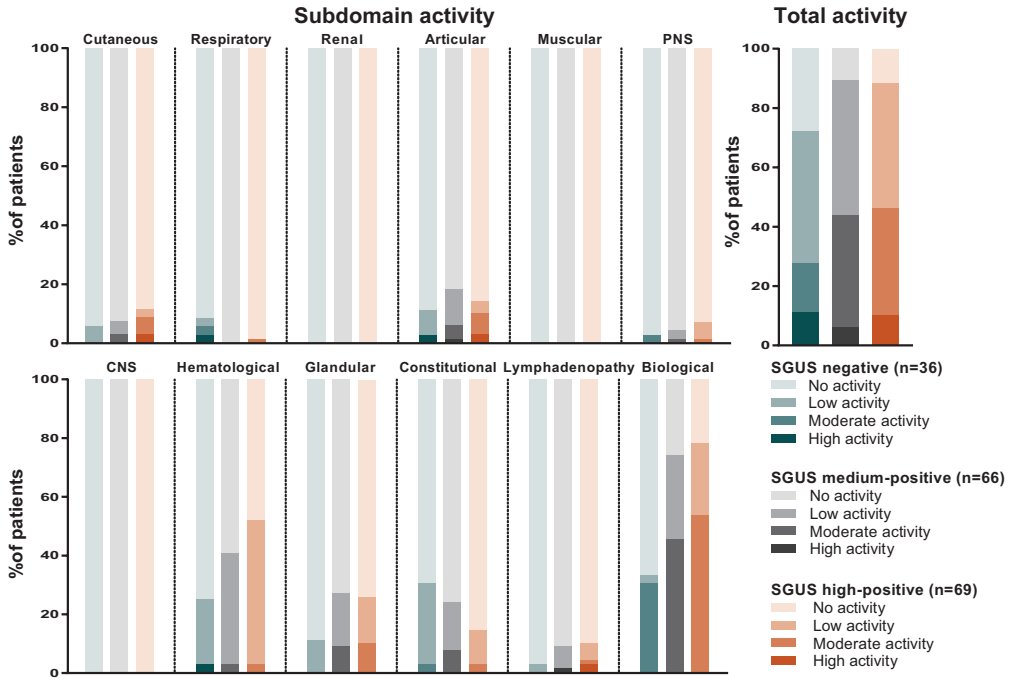
Characteristic	SGUS HYPO <1.5 (n=38)	SGUS HYPO ≥1.5 (n=134)	P value
General characteristics			
Age, years	55.2 (14.7)	52.2 (13.6)	0.25
Females	32 (84.2%)	124 (92.5%)	0.12
Disease duration, years	5.5 (3.0-9.5)	8.5 (5.0-13.3)	**0.011**
Symptom duration, years	11.0 (6.3-19.0)	15.5 (10.0-22.0)	**0.050**
BMI (kg/m^2)	25.4 (4.3)	24.7 (4.1)	0.38
Clinical parameters			
ESSDAI total score	2.0 (0.8-7.3)	4.0 (2.0-8.0)	0.12
ESSDAI categories			0.18
ESSDAI=0	9 (23.7%)	16 (12.0%)	
ESSDAI=1-4	16 (42.1%)	59 (44.4%)	
ESSDAI ≥5	13 (34.2%)	58 (43.6%)	
DAS28-ESR	2.9 (1.0)	3.2 (1.0)	0.10
DAS28-CRP	2.3 (2.0-2.5)	2.3 (1.8-2.7)	0.97
Tenderpoints	2.0 (0.0-10.0)	1.0 (0.0-8.0)	0.55
Physician GDA	2.0 (1.0-3.0)	3.0 (1.0-3.3)	0.08
SSDDI total score	1.0 (1.0-2.0)	2.0 (1.0-3.0)	**0.015**
UWS ≤0.1 mL/min	16 (43.2%)	95 (76.0%)	**<0.001**
UWS flow, mL/min	0.13 (0.04-0.26)	0.03 (0.00-0.11)	**<0.001**
Parotid gland biopsy, FS ≥1	15 (60.0%)	70 (87.5%)	**0.002**
Labial gland biopsy, FS ≥1	10 (66.7%)	37 (86.0%)	0.10
Schirmer's test ≤5mm/5min	24 (64.9%)	97 (77.6%)	0.12
Schirmer ODS, mm/5min	5.5 (2.8-14.0)	3.0 (0.0-9.0)	**0.006**
OSS ≥5	3 (7.9%)	55 (41.7%)	**<0.001**
OSS ODS total score	0.5 (0.0-2.0)	3.5 (1.1-5.4)	**<0.001**
Serological parameters			
Anti-SSA antibodies	28 (73.7%)	126 (94.7%)	**<0.001**
Anti-SSB antibodies	10 (26.3%)	82 (61.7%)	**<0.001**
IgG level >16.0 g/mL	8 (21.1%)	73 (54.9%)	**<0.001**
IgG level, g/mL	11.5 (9.5-14.2)	16.7 (12.1-21.7)	**<0.001**
RF level >5.0 IU/mL	13 (34.2%)	102 (76.7%)	**<0.001**
RF level, IU/mL	2.2 (0.8-7.5)	22.0 (5.4-48.5)	**<0.001**
Complement C3 level (g/L)	1.19 (0.24)	1.10 (0.23)	0.09
Complement C4 level (g/L)	0.21 (0.18-0.24)	0.18 (0.14-0.23)	0.30
Leucocyte count 10^9/L	6.1 (1.8)	5.2 (1.9)	0.06
Patient-reported outcome measurements			
ESSPRI total score	6.7 (5.0-7.9)	5.7 (4.3-7.0)	**0.007**
ESSPRI dryness	7.0 (5.0-8.0)	6.0 (5.0-8.0)	0.91
ESSPRI fatigue	8.0 (5.0-8.0)	7,0 (4.0-8.0)	**0.025**
ESSPRI pain	7.0 (4.5-8.0)	5.0 (2.0-7.0)	**<0.001**
Patient GDA	7.0 (4.5-8.0)	6.0 (4.0-8.0)	0.21
EQ-5D-5L	0.75 (0.13)	0.78 (0.14)	0.41
PASS, acceptable	24 (64.9%)	93 (73.8%)	0.29

Data are expressed as number of patients (%), mean (SD) or median (IQR). BMI: body mass index; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; EQ-5D-5L: 5-level EuroQoL five dimensions health status questionnaire; GDA: global disease activity; PASS: patient acceptable symptom state; OSS: ocular staining score; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

Supplementary table 3. Comparison of SGUS patients with medium or high SGUS scores based on presence of hypoechogenic areas

Characteristic	SGUS HYPO 1.5-2.0 (n=72)	SGUS HYPO 2.5-3.0 (n=62)	P value
General characteristics			
Age, years	52.7 (13.7)	51.7 (13.7)	0.69
Females	68 (94.4%)	56 (90.3%)	0.51
Disease duration, years	8.5 (4.0-13.8)	8.5 (5.0-13.3)	0.55
Symptom duration, years	16.0 (8.0-22.0)	15.0 (11.0-22.0)	0.45
BMI (kg/m^2)	24.6 (4.3)	24.8 (3.9)	0.80
Clinical parameters			
ESSDAI total score	4.0 (2.0-6.0)	5.0 (3.0-10.0)	**0.006**
ESSDAI categories			**0.044**
ESSDAI=0	11 (15.5%)	5 (8.1%)	
ESSDAI=1-4	36 (50.7%)	23 (37.1%)	
ESSDAI ≥5	24 (33.8%)	34 (46.6%)	
DAS28-ESR	3.2 (1.0)	3.3 (0.9)	0.74
DAS28-CRP	2.2 (1.7-2.7)	2.4 (2.0-2.6)	0.44
Tenderpoints	2.0 (0.0-10.0)	0.0 (0.0-5.3)	0.29
Physician GDA	2.0 (1.0-3.0)	3.0 (1.0-4.0)	0.07
SSDDI total score	2.0 (1.0-2.3)	2.0 (2.0-6.0)	**0.001**
UWS ≤0.1 mL/min	47 (67.1%)	48 (87.3%)	**0.009**
UWS flow, mL/min	0.07 (0.01-0.14)	0.01 (0.00-0.05)	**<0.001**
Parotid gland biopsy, FS ≥1	39 (83.0%)	31 (93.4%)	0.18
Labial gland biopsy, FS ≥1	21 (84.0%)	16 (88.9%)	1.00
Schirmer's test ≤5mm/5min	47 (70.1%)	50 (86.2%)	**0.032**
Schirmer ODS, mm/5min	3.5 (1.0-11.5)	1.8 (0.0-5.1)	**0.019**
OSS ≥5	21 (29.6%)	34 (55.7%)	**0.002**
OSS ODS total score	2.5 (1.0-4.0)	4.0 (2.5-6.0)	**<0.001**
Serological parameters			
Anti-SSA antibodies	66 (93.0%)	60 (96.8%)	0.45
Anti-SSB antibodies	42 (59.2%)	40 (64.5%)	0.53
IgG level >16.0 g/mL	34 (47.2%)	39 (62.9%)	0.08
IgG level, g/mL	15.9 (11.7-18.9)	17.7 (13.1-22.9)	0.06
RF level >5.0 IU/mL	53 (74.6%)	49 (79.0%)	0.55
RF level. IU/mL	20.0 (4.9-37.0)	24.5 (6.3-65.0)	0.12
Complement C3 level (g/L)	1.10 (0.23)	1.10 (0.22)	0.93
Complement C4 level (g/L)	0.19 (0.15-0.22)	0.18 (0.14-0.23)	0.31
Leucocyte count 10^9/L	5.3 (1.8)	5.1 (2.0)	0.49
Patient-reported outcome measurements			
ESSPRI total score	6.0 (4.3-7.0)	5.3 (4.0-6.9)	0.58
ESSPRI dryness	6.0 (4.0-7.0)	7.0 (5.0-8.0)	**0.002**
ESSPRI fatigue	7.0 (5.0-8.0)	6.0 (3.5-7.0)	0.06
ESSPRI pain	5.0 (3.0-7.0)	4.0 (2.0-6.0)	**0.038**
Patient GDA	6.0 (4.0-7.3)	6.0 (4.0-8.0)	0.69
EQ-5D-5L	0.77 (0.16)	0.79 (0.11)	0.44
PASS, acceptable	47 (71.2%)	46 (76.7%)	0.49

Data are expressed as number of patients (%), mean (SD) or median (IQR). BMI: body mass index; CRP: C-reactive protein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; EQ-5D-5L: 5-level EuroQoL five dimensions health status questionnaire; GDA: global disease activity; PASS: patient acceptable symptom state; OSS: ocular staining score; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index; UWS: unstimulated whole saliva.



Supplementary figure 1. Comparison of total and subdomain ESSDAI activity between SGUS negative (total score 0-14), medium-positive (total score 15-26) and high-positive (total score 27-48) patients.
CNS: central nervous system; PNS: peripheral nervous system; SGUS: salivary gland ultrasonography.

PART III

Systemic treatment

CHAPTER 8

Abatacept treatment for patients with early active primary Sjögren's syndrome: a single-centre, randomised, double-blind, placebo-controlled, phase 3 trial (ASAP-III study)

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ABSTRACT

Background. Several small open-label studies have suggested efficacy of abatacept—a co-stimulation inhibitor—in patients with primary Sjögren's syndrome. These promising results warranted further evaluation. We therefore aimed to further assess the safety and efficacy of abatacept compared with placebo in patients with primary Sjögren's syndrome.

Methods. We did a single-centre, randomised, double-blind, placebo-controlled, phase 3 trial at the University Medical Center Groningen (Groningen, Netherlands). We included patients with primary Sjögren's syndrome fulfilling the American-European Consensus Group criteria, aged 18 years or older, with positive salivary gland biopsies, time from diagnosis of 7 years or less, and a European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI) score of 5 or more. Independent pharmacists randomly allocated patients (1:1) to either the abatacept group or placebo group using a computer-generated sequence stratified by previous use of disease-modifying anti-rheumatic drugs. Patients received at-home subcutaneous injections of abatacept (125 mg) or placebo once a week for 24 weeks. The primary outcome was the between-group difference in ESSDAI score at week 24. Efficacy was analysed in patients who received at least one drug dose and for whom post-baseline data were collected. Safety was analysed in all patients who received at least one drug dose.

Findings. Between Aug 14, 2014, and Aug 23, 2018, 580 patients were reviewed for eligibility, of which 80 patients were randomly assigned to receive study treatment. Efficacy was analysed in 40 patients receiving abatacept and 39 patients receiving placebo (one patient in this group was lost to follow-up). The primary outcome did not significantly differ between the treatment groups. The adjusted mean difference in ESSDAI score at week 24 between the abatacept group and placebo group was -1.3 (95% CI -4.1 to 1.6). No deaths or treatment-related serious adverse events occurred. In 38 (95%) of 40 patients in the abatacept group, 103 adverse events occurred, including one serious adverse event and 46 infections. In 38 (95%) of 40 patients in the placebo group, 87 adverse events occurred, including four serious adverse events and 49 infections.

Interpretation. On the basis of this trial, we cannot recommend abatacept treatment as standard of care to reduce systemic disease activity in patients with primary Sjögren's syndrome. Further studies should evaluate whether patients with specific clinical manifestations and biological characteristics might benefit from abatacept treatment.

RESEARCH IN CONTEXT

Evidence before this study

No systemic treatment options have been approved for patients with primary Sjögren's syndrome. Several studies confirmed the efficacy and safety of abatacept in rheumatoid arthritis. We searched PubMed with the terms "Sjögren's syndrome", "abatacept", and "therapy" to identify trials of abatacept therapy for primary Sjögren's syndrome up to Dec 8, 2019. No randomised, double-blind trials were found. Three small open-label studies were identified. The first study, in which intravenous abatacept was given to 15 patients with primary Sjögren's syndrome, short disease duration, and active disease, reported improvements in the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI) and Patient-Reported Index (ESSPRI), laboratory parameters, fatigue, and health-related quality of life. The second study reported amelioration of symptoms in some patients, although no validated questionnaires were used. The third study reported improvement of ESSDAI and salivary flow.

Added value of this study

The Abatacept Sjögren Active Patients phase III (ASAP-III) study is, to our knowledge, the first randomised, double-blind trial to compare the efficacy and safety of subcutaneous administration of abatacept with placebo over 24 weeks in patients with primary Sjögren's syndrome that have a short disease duration and active systemic disease. Abatacept therapy did not result in a significant difference in ESSDAI score compared with placebo treatment at week 24. No significant differences in ESSPRI score were found between abatacept and placebo-treated patients, although abatacept-treated patients showed slightly larger improvements in ESSPRI score, resulting in a higher proportion of ESSPRI responders. Abatacept attenuated B-cell hyperactivity, as shown by a decrease in IgG and rheumatoid factor concentrations. Sexual function in female patients was improved in the abatacept group, but no difference was seen for dryness symptoms, fatigue, and quality of life. Salivary and tear gland function were not significantly improved. In this trial, abatacept treatment showed a good safety profile (no deaths or suspected unexpected serious adverse reaction was reported) in patients with primary Sjögren's syndrome.

Implications of all the available evidence

On the basis of our results, and considering the high cost of abatacept, we cannot recommend the use of abatacept as standard of care to alleviate systemic disease activity in patients with primary Sjögren's syndrome. Further studies could assess whether patients with specific clinical manifestations and biological characteristics might benefit from abatacept treatment and whether longer treatment duration will improve efficacy of abatacept.

INTRODUCTION

Primary Sjögren's syndrome is a systemic autoimmune disease, characterised by lymphocytic infiltration of the salivary and tear glands, with an estimated prevalence of 0.06%[1,2]. Patients with primary Sjögren's syndrome experience a wide range of symptoms, including glandular enlargement, sicca symptoms, disabling fatigue, arthritis, skin involvement, renal and lung involvement, and peripheral neuropathy. Despite the major impact of this syndrome on health-related quality of life and socioeconomic status[3], treatment for primary Sjögren's syndrome mostly focuses on symptom relief, and an unmet need exists for systemic treatment options. Efficacy of traditional and biological disease-modifying anti-rheumatic drugs (DMARDs) in primary Sjögren's syndrome has not been confirmed in large randomised controlled trials[4].

Abatacept is a fully human soluble fusion protein of cytotoxic T-lymphocyte antigen 4 coupled to the Fc tail of IgG. This biological DMARD prevents T-cell activation by inhibiting co-stimulation. Because T-cell-dependent B-cell hyperactivity has a central role in the pathogenesis of primary Sjögren's syndrome, inhibition of co-stimulation is a promising treatment approach[5]. In an open-label study of intravenous abatacept in 15 patients with primary Sjögren's syndrome (time since diagnosis ≤5 years and active disease), patients showed improvements in the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI) and the European League Against Rheumatism Patient Reported Index (ESSPRI), laboratory parameters, fatigue, and health-related quality of life after 24 weeks[6]. Another open-label study found that some patients reported amelioration of their symptoms during 28 weeks of abatacept treatment, although no validated questionnaires were used[7]. In 2019, a third group reported improvement of ESSDAI and salivary flow after 24 months of intravenous abatacept treatment[8]. Abatacept has also shown efficacy in an open-label study of patients with rheumatoid arthritis and associated Sjögren's syndrome[9].

These promising results warranted further evaluation of abatacept for the treatment of primary Sjögren's syndrome. We therefore aimed to assess the safety and efficacy of subcutaneous abatacept compared with placebo in patients with primary Sjögren's syndrome.

METHODS

Study design and participants

We did a single-centre, randomised, double-blind, placebo-controlled, phase 3 trial (the Abatacept Sjögren Active Patients phase III [ASAP-III] study). This study was done in the multidisciplinary tertiary referral expertise centre for primary Sjögren's syndrome at the University Medical Center Groningen (UMCG; Groningen, Netherlands). We obtained ethical approval from the UMCG institutional review board (METc2014.118). Patients were selected

from the UMCG population or referred by other Dutch clinics for trial participation.

Our main inclusion criteria for patients for this study were fulfilment of the American-European Consensus Group criteria for primary Sjögren's syndrome[10], age 18 years or older, positive gland biopsy, time since diagnosis of 7 years or less, and an ESSDAI score of 5 or more[11]. Anti-Sjögren's-syndrome-related antigen A (anti-SSA) positivity was not required. Retrospectively, all patients also fulfilled the American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) Classification criteria[12]. Patients who had used prednisone (>10 mg/day), non-biological DMARDs including hydroxychloroquine, or pilocarpine for 1 month or less before enrolment were excluded. Previous use of biological DMARDs was not allowed. 6 months after trial commencement, the protocol was amended to allow patients who previously used abatacept (≥6 months before inclusion) or rituximab (≥12 months before inclusion) to participate, as some of these patients showed high disease activity and were eager to join our trial. The protocol (online supplement) shows a full list of the inclusion and exclusion criteria. We obtained written informed consent from all participants.

Randomisation and masking

Patients were screened for eligibility (JFvN, EM, GSvZ, RFW, HB). Following enrolment, participants received a study number and were randomly allocated (1:1) to either the abatacept group or placebo group by independent pharmacists according to a computer-generated sequence, which was only known to the pharmacists. Allocation was stratified by previous use of DMARDs (including hydroxychloroquine), using block randomisation with a block size of four. Block size was pre-specified in the protocol and therefore could be known to the investigators and outcome assessors. Abatacept and placebo injections were identical in appearance. Participants, investigators, outcome assessors, and care providers were masked to the treatment groups, until the last patient completed week 24 and the database was locked.

Procedures

Patients received instructions to administer subcutaneous injections at home, once a week, for 24 weeks. Injections contained 125 mg of abatacept or placebo. Concomitant use of pilocarpine or DMARDs was not permitted. A stable low dose of prednisone (≤10 mg) was permitted. Non-steroidal anti-inflammatory drugs and topical treatments were allowed, but patients were asked to discontinue non-steroidal anti-inflammatory drugs 3 days before each visit, and eye drops 1 h before ocular examinations. Rescue therapy with prednisone or cyclophosphamide was permitted after week 12. Patients visited the UMCG at baseline and weeks 4, 8, 12, and 24. Clinical assessments including laboratory parameters and safety outcomes were done by JFvN, EM, GSvZ, RFW, or HB at each visit. At each visit, patients also completed online questionnaires. Glandular function tests were done by oral medicine specialists and ophthalmologists during the screening visit, week 12, and week 24. Patients

from both treatment groups received open-label abatacept for another 24 weeks after the double-blind phase. Safety and efficacy results of the extension phase will be analysed separately.

Outcomes

The primary outcome was the difference in ESSDAI score between the abatacept and placebo groups at week 24[11]. Secondary clinical outcomes were ESSDAI score at other timepoints, physician global assessment of disease activity using a numeric rating scale (range 0-10), and Disease Activity Score 28 joint count (DAS-28). Secondary laboratory outcomes were rheumatoid factor, IgG, IgA, IgM, erythrocyte sedimentation rate, and complement C3 and C4. Secondary patient-reported outcomes were the ESSPRI score[13], patient global assessment of disease activity, and Patient Acceptable Symptom State at each timepoint; ocular and oral dryness (numeric rating scale [range 0-10]), Multidimensional Fatigue Inventory, Short-Form 36 (SF-36), EuroQoL five dimensions health status questionnaire (EQ-5D-5L), and Work Participation and Activity Impairment Questionnaire (WPAI) at weeks 12 and 24. Physical and mental component scores of the SF-36 were calculated using the QualityMetric Health Outcomes Scoring Software, version 5.1. EQ-5D-5L index values were calculated using syntax provided by EuroQoL, based on a set of weights for the Netherlands. Additionally, EuroQoL five dimensions (EQ-5D) visual analogue scale for general health was used as an outcome. For female participants, patient-reported outcomes included vaginal dryness (numeric rating scale [range 0-10]) at weeks 12 and 24, and Female Sexual Function Index at week 24. Secondary glandular function outcomes were unstimulated whole salivary flow and citric acid-stimulated whole salivary flow, ocular staining score[14], tear break-up time, and Schirmer's test without anaesthesia. A full description of the study outcomes is included in the protocol (online supplement).

Exploratory outcomes included salivary gland ultrasound, histology, gut microbiome, and additional laboratory outcomes, which will be analysed and reported separately to provide an in-depth translational evaluation of the efficacy of abatacept.

ESSDAI and ESSPRI response rates were not included in the original protocol, as the minimally clinically important change of ESSDAI and ESSPRI had not yet been published. These were added as secondary outcomes in the statistical analysis plan before unmasking. ESSDAI response was defined as a decrease of three points or more from baseline, and ESSPRI response as a decrease of one point or more or a decrease of 15%[15]. Post-hoc analysis included the evaluation of Sjögren Syndrome Responder Index, which defines response as a 30% improvement or more from baseline in two or more of the five domains (fatigue, oral dryness, ocular dryness, unstimulated whole salivary flow, and erythrocyte sedimentation rate)[16].

Data for adverse events were collected by clinicians from baseline to week 24, by open-ended questioning and clinical examination, and clinicians specifically inquired about infections. Investigators assessed all adverse events for severity and potential causality[17]. Adverse events were coded according to the Medical Dictionary for Regulatory Activities (version 19.0). Other safety endpoints included laboratory tests and treatment discontinuation.

Statistical analysis

The sample size was based on a difference of two points in the ESSDAI score between the abatacept group and placebo group, as the minimally clinically important change of three points or more had not yet been determined at the time the protocol was written[15]. A sample size of 44 patients per treatment group was needed to detect a two point difference in ESSDAI (estimated SD of three points on the basis of results from previous trials[6,18,19]) between the abatacept group and placebo group at 24 weeks, with a two-sided 5% significance level and power of 80%, allowing for a 15% dropout rate. However, after enrolment of 61 patients, the dropout rate appeared to be much lower (<5%), after which the predefined sample size was adjusted to 40 patients per group, in consultation with our data and safety monitoring board. An independent data and safety monitoring board reviewed unblinded study data once a year to monitor safety and overall conduct of the clinical trial. There were no predefined stopping rules. No formal interim analysis for efficacy was done.

Before unmasking, a detailed analysis plan was prepared (online supplement). According to the modified intention-to-treat principle, all participants who received at least one dose of study medication were included in the efficacy analysis, irrespective of protocol violations, with exception of one patient for whom no post-baseline efficacy data were available (lost to follow-up before week 4). We planned to do a per-protocol analysis of the primary endpoint only if there was a difference of more than 10% between the modified intention-to-treat and per-protocol population. The safety analysis included all participants who received at least one dose of study medication.

Data collected during visits were considered non-valid and coded as missing when three or more injections were skipped within 4 weeks before a visit, when cyclophosphamide was used, or when a dose of 5 mg or more of prednisone or equivalent was used within 2 weeks before the visit, unless corticosteroid dose was stable since baseline.

We considered p values less than 0.05 statistically significant. For the primary endpoint and all secondary efficacy outcomes, except the Patient Acceptable Symptom State and response according to ESSPRI, ESSDAI, and Sjögren's Syndrome Responder Index, the difference between abatacept and placebo groups was evaluated using linear generalised estimating equations. Missing data were not imputed. The generalised estimating equations model included previous DMARD use, baseline values of the efficacy outcome, treatment

(abatacept or placebo), visits, and interactions of treatment by visits. In case of residuals with non-Gaussian distribution, the variable was transformed (second power for ESSPRI total, ESSPRI fatigue, ESSPRI pain, and oral and vaginal dryness; square root for IgG; and natural logarithm for erythrocyte sedimentation rate). Different correlation structures (exchangeable, M-dependent, unstructured) were tested and the model with the lowest information criterion was used, which was the exchangeable correlation structure for all variables. The comparison of main interest was the difference between groups at 24 weeks (treatment-by-visit interaction). Differences at 4, 8, and 12 weeks were assessed to investigate early treatment efficacy. Differences in Patient Acceptable Symptom State between treatment groups were similarly evaluated using logistic generalised estimating equations.

Response according to ESSDAI, ESSPRI, and Sjögren's Syndrome Responder Index were evaluated using binary logistic regression with treatment and previous use of disease-modifying anti-rheumatic drugs as factors. For response analyses, missing values were imputed as non-responses. Baseline characteristics, ESSDAI subdomains, and safety endpoints were summarised descriptively.

We did all the statistical analyses using IBM SPSS Statistics (version 23). This study is registered with ClinicalTrials.gov, number NCT02067910.

Role of the funding source

The funder of the study (Bristol-Myers Squibb) had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Between Aug 14, 2014, and Aug 23, 2018, 580 patients were reviewed for eligibility of which 81 patients were enrolled (figure 1). One enrolled patient was excluded before being randomly allocated to a treatment group because their disease activity became too severe to be treated with study medication (abatacept or placebo) between the screening and baseline visits. After randomisation, 40 patients received abatacept and 40 received placebo. The last visit of the double-blind phase was completed on Feb 21, 2019. Table 1 shows the baseline characteristics.

Table 1. Baseline characteristics of participants

Characteristics	Abatacept (n=40)	Placebo (n=40)	Total (n=80)
Age (years)	48 (15)	49 (16)	49 (16)
Men	3 (8%)	3 (8%)	6 (8%)
Women	37 (93%)	37 (93%)	74 (93%)
Time since diagnosis (years)	2 (0-4)	2 (1-4)	2 (1-4)
Time since symptom started (years)	11 (4-14)	8 (4-14)	8 (4-14)
Anti-Ro/SSA	34 (85%)	37 (93%)	71 (89%)
Anti-La/SSB	20 (50%)	23 (58%)	43 (54%)
Baseline SWS ≥ 0.05 ml/min	31 (78%)	28 (70%)	59 (74%)
Previous use of DMARDs	18 (45%)	16 (40%)	34 (43%)
Oral corticosteroids	10 (25%)	10 (25%)	20 (25%)
Hydroxychloroquine	15 (38%)	14 (35%)	29 (36%)
Methotrexate	3 (8%)	2 (5%)	5 (6%)
Abatacept[a]	2 (5%)	3 (8%)	5 (6%)
Rituximab[a]	1 (3%)	0	1 (1%)
Current medication use			
Corticosteroids	0	1 (3%)	1 (1%)
NSAIDs	16 (40%)	11 (28%)	27 (34%)
Artificial tears	32 (80%)	33 (83%)	65 (81%)
Corticosteroid eye-drops	6 (15%)	5 (13%)	11 (14%)
Artificial saliva	7 (18%)	7 (18%)	14 (18%)
ESSDAI subdomain activity[b]			
Constitutional	20 (50%)	17 (43%)	37 (46%)
Lymphadenopathy	10 (25%)	13 (33%)	23 (29%)
Glandular	36 (90%)	37 (93%)	73 (91%)
Articular	23 (58%)	24 (60%)	47 (59%)
Cutaneous	11 (28%)	7 (18%)	18 (23%)
Pulmonary	3 (8%)	2 (5%)	5 (6%)
Renal	0	0	0
Muscular	1 (3%)	0	1 (1%)
Peripheral nervous system	2 (5%)	5 (13%)	7 (9%)
Central nervous system	0	0	0
Haematological	14 (35%)	20 (50%)	34 (43%)
Biological	32 (80%)	31 (78%)	63 (79%)

Data are mean (SD), median (IQR), or n (%). Some percentages do not add up to 100 because of rounding. [a]Patients with previous abatacept use were participants in the open-label ASAP study[6]. Time between previous treatment with rituximab or abatacept and inclusion was at least 2 years. [b]Total number of patients with low, moderate or high activity in ESSDAI subdomains. DMARDs: disease-modifying anti-rheumatic drugs; ESSDAI: EULAR Sjögren syndrome disease activity index; NSAIDs: non-steroidal anti-inflammatory drugs; SWS: stimulated whole salivary flow.

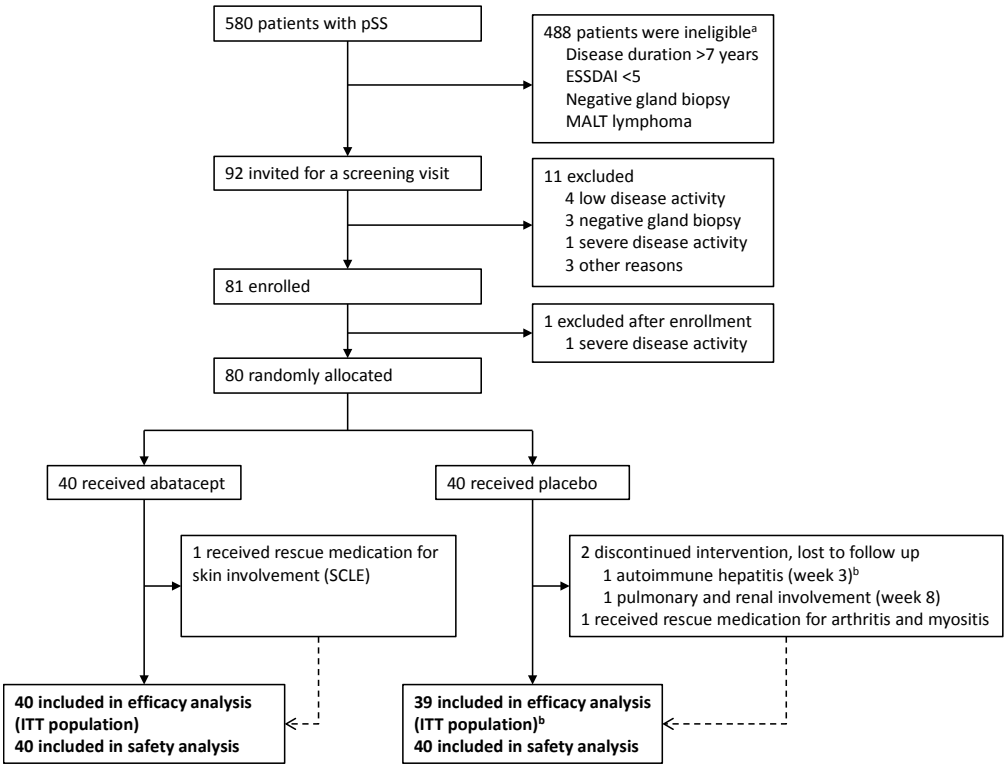

Figure 1. Trial profile.

[a]Numbers of patients fulfilling different exclusion criteria are not available because patients often fulfilled more than one criterion and only the first one that was detected was noted. [b]One patient in the placebo group was excluded from the efficacy analysis because she was lost to follow-up in week 3 and no post-baseline was collected. ESSDAI: European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; ITT: intention-to-treat; MALT: mucosa-associated lymphoid tissue; SCLE: subacute cutaneous lupus erythematosus.

None of the abatacept-treated patients withdrew from treatment or were lost to follow-up (figure 1). Two placebo-treated patients withdrew from treatment because of adverse events, of whom one was excluded from the efficacy analysis, as no post-baseline data were available. The modified intention-to-treat population consisted of 40 patients in the abatacept group and 39 in the placebo group. The per-protocol population consisted of 40 patients in the abatacept group and 37 in the placebo group. As the difference between modified intention-to-treat and per-protocol populations was less than 10%, no per-protocol analysis was done.

Week 12 data for one patient in the placebo group were coded as missing for the efficacy analysis, as this patient skipped three injections between week 8 and week 12. One patient used a maintenance dose of prednisone (5 mg/day) throughout the trial because of auto-immune hepatitis, which was in remission at inclusion. Although rescue therapy was allowed after week 12 according to protocol, two patients received corticosteroids before week 12, intended as bridging therapy while awaiting the effect of study treatment. The first patient

received abatacept and used corticosteroids between week 10 and week 17 because of active cutaneous involvement. The second patient received placebo and was treated with corticosteroids between weeks 2-10 and 12-24 for severe arthritis and myositis. Data from week 12 of the first patient and data from weeks 4, 8, and 24 of the second patient were therefore coded as missing for the efficacy analysis. No additional patients received rescue therapy after week 12. After coding non-valid data as missing, all variables had less than 5% missing values per visit, with exception of IgG at week 4, ocular staining score, tear break-up time, Schirmer's test, unstimulated whole salivary flow, and stimulated whole salivary flow at week 12, and stimulated whole salivary flow at week 24, which had 6-10% missing values.

The primary endpoint (the difference in ESSDAI score at week 24) did not significantly differ between treatment groups (table 2, figure 2, supplementary table 1). Differences in ESSDAI score between treatment groups, adjusted for baseline values and previous DMARD use, were -2.4 (95% CI -4.7 to -0.1) at week 12 and -1.3 (-4.1 to 1.6) at week 24. ESSDAI score at week 12 was significantly lower in patients receiving abatacept than in those receiving placebo. No significant differences were found in proportions of patients reaching the minimally clinically important change for ESSDAI (figure 3). At week 12, 19 (48%) of 40 patients in the abatacept group and 13 (33%) of 39 in the placebo group were ESSDAI responders (odds ratio [OR] 1.8, 95% CI 0.7-4.5; p=0.207). At week 24, 23 (58%) of 40 patients in the abatacept group and 20 (51%) of 39 in the placebo group were ESSDAI responders (OR 1.3, 95% CI 0.5-3.1; p=0.600). The largest decrease in activity was seen in the articular, glandular, and constitutional ESSDAI subdomains (figure 3, supplementary table 2). Improvements in these domains occurred in both treatment groups, although improvement in the articular domain was larger in the abatacept group. ESSDAI scores of individual patients over time are shown in supplementary figure 1.

No significant differences between groups were found for ESSPRI score (table 2, figure 2, supplementary table 1), although abatacept did increase the number of patients reaching the minimally clinically important change for ESSPRI (figure 3). At week 12, 21 (53%) of 40 patients in the abatacept group and 11 (28%) of 39 in the placebo group were ESSPRI responders (OR 2.8, 95% CI 1.1-7.1; p=0.031). At week 24, 23 (58%) patients in the abatacept group and eight (21%) in the placebo group were ESSPRI responders (OR 5.7, 95% CI 2.0-15.7; p=0.001).

The Female Sexual Function Index at week 24 was significantly better in patients receiving abatacept than in those receiving placebo (adjusted difference 3.8, 95% CI 0.1-7.4; p=0.042; table 2, supplementary table 1, supplementary figure 2). No differences were found for oral, ocular, and vaginal dryness, patient global disease activity, fatigue (Multidimensional Fatigue Index domains), or health-related quality of life (SF-36 physical and mental component scores, EQ-5D-5L, EQ-5D visual analogue scale) at any timepoint. No differences were found in proportions of patients with acceptable symptoms at week 12 (OR 0.4, 95% CI 0.1-1.7; p=0.221)

Table 2. Baseline values and differences between groups in week 12 and 24

	Baseline		Week 12			Week 24		
Variable	**Abatacept**	**Placebo**	**AD**	**95% CI**	**P value**	**AD**	**95% CI**	**P value**
ESSDAI score	14.0 (9.0-16.8)	13.0 (8.0-18.0)	-2.4	-4.7 to -0.1	**0.039**	-1.3	-4.1 to 1.6	0.385
ESSPRI score[a]	7.0 (5.4-7.7)	7.3 (5.3-8.0)	-4.7	-12.5 to 3.0	0.232	-5.0	-12.7 to 2.8	0.208
Dryness	7.0 (5.3-8.0)	7.0 (7.0-8.0)	-0.3	-1.0 to 0.3	0.311	-0.3	-0.9 to 0.4	0.455
Fatigue[a]	7.5 (7.0-8.0)	8.0 (6.0-9.0)	-2.0	-12.5 to 8.6	0.716	-2.6	-14.1 to 9.0	0.664
Pain[a]	7.0 (5.0-8.0)	7.0 (3.0-8.0)	-4.5	-14.7 to 5.7	0.389	-5.2	-14.5 to 4.2	0.279
Ocular dryness (NRS)	6.0 (3.3-7.0)	7.0 (6.0-8.0)	0.4	-0.5 to 1.2	0.388	0.0	-0.8 to 0.9	0.972
Oral dryness (NRS)[a]	7.0 (5.0-8.0)	7.0 (6.0-8.0)	-3.8	-11.6 to 4.1	0.345	-0.6	-10.1 to 8.9	0.902
Vaginal dryness (NRS)[a]	5.0 (3.0-8.0)	6.5 (5.0-8.8)	-1.4	-10.2 to 7.3	0.751	-0.1	-10.0 to 9.7	0.981
Patient GDA	8.0 (7.0-8.0)	7.0 (7.0-9.0)	-0.8	-1.7 to 0.0	0.063	-0.4	-1.3 to 0.6	0.442
MFI								
General fatigue	16.5 (13.3-19.0)	16.0 (13.0-20.0)	-0.4	-2.0 to 1.2	0.583	-0.8	-2.8 to 1.2	0.448
Physical fatigue	15.5 (14.0-17.0)	15.0 (13.0-18.0)	-0.1	-1.4 to 1.2	0.907	-1.3	-3.0 to 0.4	0.144
Reduced activity	14.0 (11.0-15.0)	13.0 (11.0-16.0)	-0.5	-1.9 to 1.0	0.509	-0.4	-2.1 to 1.3	0.619
Reduced motivation	12.0 (9.0-13.0)	11.0 (9.0-14.0)	-0.6	-2.2 to 1.0	0.487	-1.3	-2.9 to 0.2	0.099
Mental fatigue	12.0 (9.3-15.0)	13.0 (8.0-16.0)	-0.9	-2.2 to 0.5	0.216	-0.4	-1.6 to 0.9	0.554
Short Form 36								
PCS	37.1 (7.9)	38.2 (9.8)	2.5	-0.2 to 5.2	0.065	2.0	-0.7 to 4.7	0.141
MCS	44.0 (38.0-52.1)	44.4 (36.5-51.6)	-1.9	-5.5 to 1.8	0.312	-1.0	-4.6 to 2.6	0.594
EQ-5D-5L	0.71 (0.60-0.80)	0.71 (0.50-0.79)	-0.04	-0.11 to 0.03	0.273	-0.03	-0.10 to 0.04	0.343
EQ-5D VAS	60.5 (42.3-70.0)	60.0 (45.0-71.0)	6.2	-1.5 to 13.9	0.117	3.1	-5.0 to 11.3	0.454
FSFI	11.5 (4.4-23.2)	14.7 (5.1-23.4)	NA	NA	NA	3.8	0.1 to 7.4	**0.042**
DAS-28 (ESR)	4.82 (1.19)	5.00 (1.48)	-0.37	-0.80 to 0.06	0.089	-0.46	-1.02 to 0.09	0.101
DAS-28 (CRP)	4.35 (2.63-5.08)	4.20 (2.90-5.10)	-0.44	-0.87 to -0.02	**0.041**	-0.46	-1.00 to 0.08	0.097
Physician GDA	5.7 (1.5)	5.7 (1.6)	-0.7	-1.3 to -0.1	**0.022**	-0.6	-1.4 to 0.2	0.144
IgG (g/L)[a]	17.4 (13.4-26.7)	18.7 (14.8-24.7)	-0.1	-0.2 to 0.0	0.215	-0.1	-0.2 to -0.01	**0.028**
RF (IU/mL)	32.5 (2.1-71.0)	24.0 (6.8-83.0)	-11.7	-16.6 to -6.8	**<0.0001**	-13.8	-20.7 to -6.0	**<0.0001**
Complement C3 (g/L)	1.2 (1-1.3)	1.1 (1.0-1.3)	0.0	-0.1 to 0.1	0.564	0.0	-0.1 to 0.1	0.451
Complement C4 (g/L)	0.19 (0.09)	0.20 (0.07)	0.01	-0.01 to 0.03	0.393	0.01	-0.01 to 0.03	0.381
ESR (mm/h)[a]	28.0 (13.3-47.0)	33.0 (17.0-54.0)	-0.01	-0.20 to 0.18	0.881	-0.20	-0.41 to 0.02	0.068
Ocular staining score[b]	4.0 (0.5-6.5)	4.5 (2.0-7.0)	-0.9	-1.9 to 0.1	0.071	0.0	-1.0 to 1.0	0.993
Schirmer's test[b]	3.5 (0.6-14.0)	2.5 (0.0-8.5)	0.1	-2.9 to 3.2	0.927	1.1	-1.2 to 3.3	0.367
TBUT (s)	5.3 (2.5-7.5)	4.0 (2.0-7.0)	-0.4	-1.7 to 0.9	0.548	-0.6	-2.1 to 0.9	0.435
UWS (mL/min)	0.05 (0.01-0.12)	0.05 (0.01-0.13)	-0.01	-0.04 to 0.03	0.744	0.02	-0.02 to 0.05	0.375
SWS (mL/min)	0.16 (0.06-0.33)	0.10 (0.02-0.43)	0.03	-0.05 to 0.10	0.481	0.01	-0.08 to 0.09	0.861

Baseline data are mean (SD) or median (IQR). Raw outcome data for week 12 and week 24 are shown in supplementary table 3. The adjusted difference is the unstandardised regression coefficient from the linear generalised estimating equations models, which represents the difference between the treatment groups (abatacept–placebo), adjusted for baseline values and previous disease-modifying anti-rheumatic drug use. Vaginal dryness and the Female Sexual Function Index include only female patients. [a]Raw values were transformed before generalised estimating equations analysis. Estimates of the adjusted differences in weeks 12 and 24 therefore represent differences on the transformed scale (square root for IgG, natural logarithm for ERS and second power for ESSPRI total, ESSPRI fatigue, ESSPRI pain, oral dryness, and vaginal dryness). [b]Average of right and left eye.
AD: Adjusted difference; CRP: C-reactive protein; DAS-28: disease activity score 28 joint count; ESR: erythrocyte sedimentation rate; ESSDAI: European League Against Rheumatism Sjögren Syndrome Disease Activity Index; ESSPRI: European League Against Rheumatism Sjögren Syndrome Patient Reported Index; EQ-5D: EuroQoL five dimensions; EQ-5D-5L: EuroQoL five dimensions health status questionnaire; FSFI: Female Sexual Function Index; GDA: global disease activity; MCS: mental component summary; MFI: Multidimensional Fatigue Inventory; NA: not available; NRS: numerical rating scale; PCS: physical component summary; RF: rheumatoid factor; SWS: stimulated whole salivary flow; TBUT: tear break-up time; UWS: unstimulated whole salivary flow; VAS: visual analogue scale.

or week 24 (1.1, 0.2-5.3; p=0.893; supplementary table 1). As only 20 (50%) of 40 patients in the abatacept group and 21 (54%) of 39 patients in the placebo group had paid employment at baseline, the number of patients with complete WPAIs was small and differences in WPAI outcomes were not statistically tested. At week 24, 21 (53%) of 40 patients in the abatacept group and 19 (53%) of 36 patients in the placebo group had paid employment.

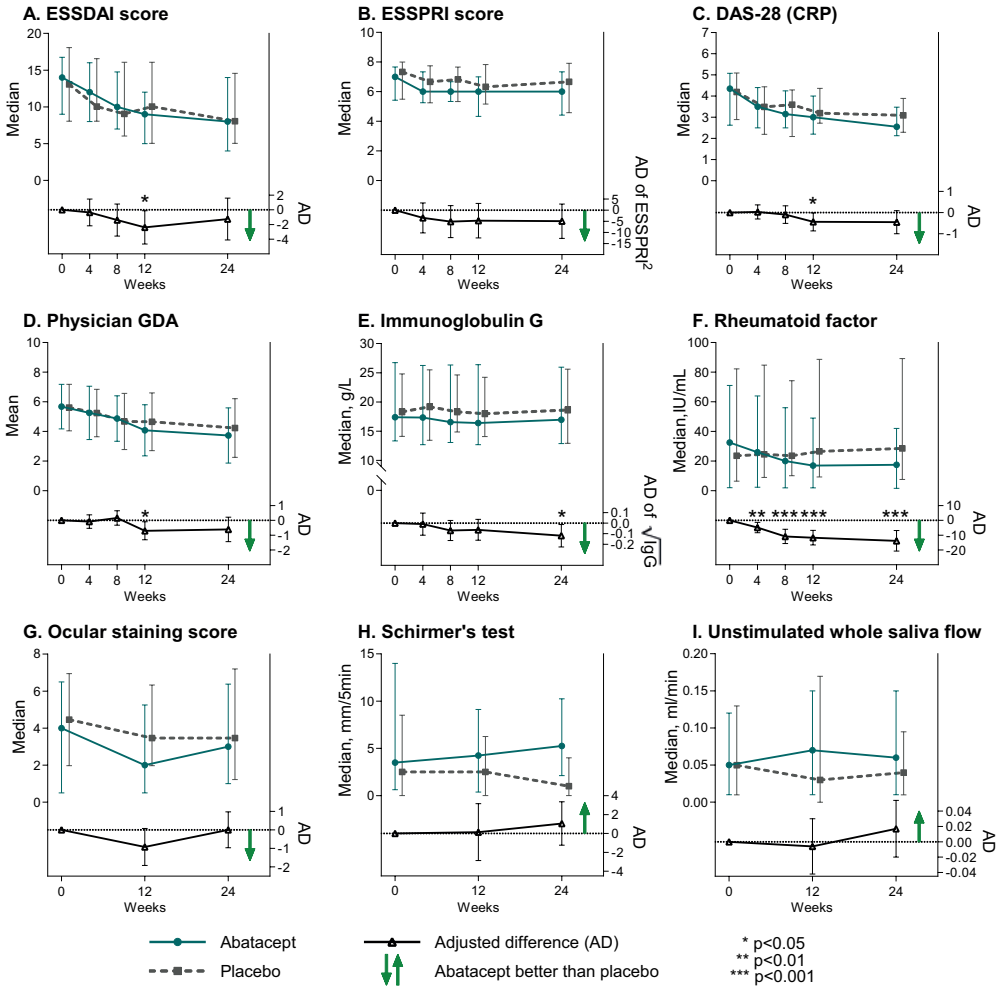

Figure 2. ESSDAI score and secondary efficacy outcomes.
The difference between the treatment groups are adjusted for baseline values and previous disease-modifying anti-rheumatic drug use. Error bars are IQR for medians, SD for means, and 95% CI for the ADs. AD: adjusted difference; CRP: C-reactive protein; DAS-28: Disease Activity Score 28 joint count; ESSDAI: European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; ESSPRI: European League Against Rheumatism Sjögren's Syndrome Patient-Reported Index.

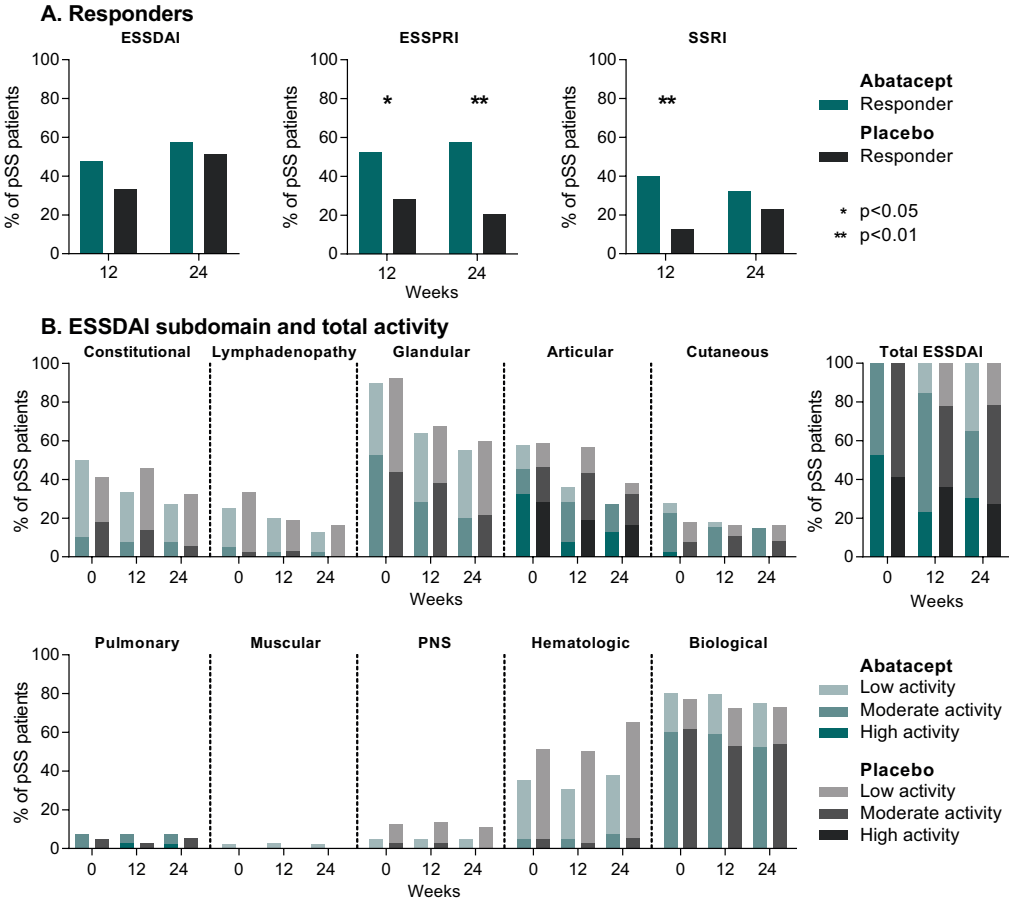

Figure 3. Categorical efficacy outcomes.

(A) Response according to the ESSDAI, ESSPRI, and Sjögren's Syndrome Responder Index. (B) ESSDAI subdomain and total activity. ESSDAI response is a decrease of three points or more from baseline[15]. ESSPRI response is a decrease of one point or more, or a decrease of 15% from baseline[15]. Response according to the Sjögren Syndrome Responder Index is an improvement of 30% or more from baseline in two or more of the five domains (fatigue, oral dryness, ocular dryness, unstimulated whole salivary flow, and erythrocyte sedimentation rate)[16]. ESSDAI: European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; ESSPRI: European League Against Rheumatism Sjögren's Syndrome Patient-Reported Index; PNS: peripheral nervous system; SSRI: Sjögren's syndrome responder index.

Abatacept significantly decreased physician global disease activity compared with placebo at week 12 (adjusted difference -0.7, 95% CI -1.3 to -0.1; p=0.022), but not at week 24 (table 2, figure 2, supplementary table 1). Although both DAS-28 (C-reactive protein) and DAS-28 (erythrocyte sedimentation rate) were decreased by abatacept treatment, a significant difference was only seen in DAS-28 (C-reactive protein) at week 12 (adjusted difference -0.44, 95% CI -0.87 to -0.02; p=0.041). IgG was significantly lower in patients receiving abatacept than in those receiving placebo at week 24. IgA and IgM did not significantly differ between groups (data not shown). Rheumatoid factor was significantly lower in patients in the abatacept group than in those in the placebo group at week 4 and all following visits. With the exception of rheumatoid factor, other laboratory efficacy outcomes did not show significant differences at weeks 4 and 8. No differences were seen

in erythrocyte sedimentation rate or complement. No significant differences between treatment groups were found in tear or salivary gland function (table 2, figure 2, supplementary table 1).

No deaths or unexpected serious adverse reaction occurred (table 3). One serious adverse event occurred in the abatacept group, which was deemed not treatment related, whereas four serious adverse events occurred in the placebo group (supplementary table 3). None of the patients receiving abatacept withdrew from treatment because of adverse events. One patient in the placebo group discontinued treatment in week 3 because of the development of autoimmune hepatitis. Another patient in the placebo group discontinued treatment in week 8 because of severe exacerbation of disease activity, with glomerulonephritis, pulmonary involvement, cutaneous vasculitis, and development of high anti-double-stranded DNA concentrations, after which the patient's diagnosis was changed to Sjögren's syndrome combined with systemic lupus erythematosus (SLE). A total of 103 adverse events (including one serious adverse event and 46 infections) occurred in 38 (95%) of 40 patients in the abatacept group compared with 87 adverse events (including four serious adverse events and 49 infections) in 38 (95%) of 40 in the placebo group. Most adverse events were mild. The most common adverse events were infections, occurring in 29 (73%) patients in the abatacept group and 28 (70%) in the placebo group (supplementary table 4). General and administration site conditions (including fatigue, malaise, and pyrexia) occurred in 12 (30%) patients in the abatacept group and four (10%) in the placebo group. Gastrointestinal disorders (including abdominal pain, dyspepsia, nausea, and diarrhoea) occurred in 11 (28%) patients in the abatacept group and five (13%) in the placebo group. Laboratory safety outcomes are shown in supplementary figure 3.

Table 3. Summary of adverse events by intervention group

	Number of events		Number of patients with events	
	Abatacept	Placebo	Abatacept (n=40)	Placebo (n=40)
Death	0	0	0	0
SUSAR	0	0	0	0
SAE (total)[a]	1	4	1 (3%)	4 (10%)
SAE with possible relation to intervention[b]	0	1	0	1 (3%)
AE, severe[c]	1	3	1 (3%)	3 (8%)
AE, moderate[d]	3	7	3 (8%)	7 (18%)
AE, total	103	87	38 (95%)	38 (95%)
AE with possible relation to intervention[b]	80	66	36 (90%)	32 (80%)
Infection	46	49	29 (73%)	28 (70%)
Treatment withdrawal due to AE	0	2	0	2 (5%)
Temporary treatment discontinuation due to AE	6	12	5 (13%)	8 (20%)

Data are n (%), unless otherwise specified. [a]A definition of serious adverse events is shown in the protocol (online supplement). [b]Possible, probable, or definite relation to treatment, as assessed by the investigators during the trial. [c]Severe adverse events were defined as those that make activities of daily living impossible and usually require treatment or other interventions. [d]Moderate adverse events were defined as those that limit activities of daily living and for which treatment or another intervention is sometimes necessary. AE: adverse event; SAE: serious adverse event; SUSAR: suspected unexpected serious adverse reaction

Post-hoc analysis showed significantly more responders according to the Sjögren's Syndrome Responder Index in the abatacept group than in the placebo group at week 12, but not week 24 (figure 3). In week 12, 16 (40%) of 40 patients in the abatacept group and five (13%) of 39 in the placebo group were responders (OR 4.5, 95% CI 1.5-14.1; p=0.009). At week 24, 13 (33%) patients in the abatacept group and nine (23%) in the placebo group were responders (OR 1.7, 95% CI 0.6-4.6; p=0.311).

DISCUSSION

In the ASAP-III study, we found no significant difference between the abatacept group and placebo group in the primary endpoint of ESSDAI score after 24 weeks of treatment. Abatacept significantly decreased ESSDAI score compared with placebo at week 12, although no difference between treatment groups was found in the proportion of patients reaching the minimally clinically important change of three points or more in ESSDAI. Physician global disease activity was significantly lower in abatacept-treated patients than in placebo-treated patients only at week 12. These results indicate that while disease activity was lowered in both treatment groups, improvements in ESSDAI and physician global disease activity were seen at earlier timepoints in patients receiving abatacept.

In previous open-label trials, ESSDAI score was significantly decreased during abatacept treatment[6,8]. In the current trial, improvement of ESSDAI score was found in both treatment groups. The improvements seen over 24 weeks in the placebo and abatacept groups might partly be explained by regression to the mean. A study in which patients were treated with standard of care therapy showed that high baseline ESSDAIs were associated with improvement of ESSDAI after 12 months[20]. As only patients with moderate or high ESSDAI scores were included in the ASAP-III study, a natural decrease in ESSDAI scores is expected to occur for some patients, regardless of the treatment group.

Sjögren's syndrome is a highly heterogeneous disease. Therefore, not all patients might respond equally to a particular DMARD, as was shown for rituximab[21]. Our trial was not powered to show effects on specific systemic manifestations. Further studies should assess whether abatacept is effective in patients with specific characteristics—e.g., high articular activity.

Besides ESSDAI, the patient symptom index ESSPRI, which includes questions regarding sicca symptoms, fatigue, and pain, is an important outcome, because both indices are complementary. Abatacept significantly increased the number of ESSPRI responders, based on the minimally clinically important change, compared with placebo at weeks 12 and 24, despite the absence of significant differences in ESSPRI score (which is a continuous variable).

This finding shows that although abatacept-treated patients reached minimally clinically important change more often, the difference between groups was small. Female abatacept-treated patients showed improved sexual function at week 24, which might be important, considering the negative effect of primary Sjögren's syndrome on sexual function[22]. No differences were found in other patient-reported outcomes including global disease activity, dryness, fatigue, and health-related quality of life.

No significant differences were found in glandular function. During our previous open-label trial[6], glandular function also did not improve, but stimulated whole salivary flow remained stable during treatment and deteriorated after treatment discontinuation. Abatacept reduced the number of germinal centres in parotid gland biopsies, but no changes were found in focus score or size of infiltration[23]. As a recent open-label study found improvement of salivary flow after 24 months of abatacept treatment[8], longer treatment might be needed to improve salivary gland function. Evaluation of long-term efficacy of abatacept on glandular function in the ASAP-III open-label extension phase, and the effect on ultrasound and histological findings, will be of interest.

In line with previous results[6], abatacept attenuated B-cell hyperactivity, as reflected by the decrease in rheumatoid factor and IgG. This decrease in B-cell hyperactivity might be caused by effects of abatacept on T-follicular helper (Tfh) cells[24]. Abatacept decreased the number of circulating Tfh-cells, and expression of inducible T-cell co-stimulator, a marker of T-cell activation[24]. Decreased expression of inducible T-cell co-stimulator was associated with ESSDAI improvement[24]. Abatacept also decreased Bruton's tyrosine kinase concentrations in naive and memory B cells[25]. Unfortunately, this biological effect did not translate into improvement of systemic disease activity, raising the question whether future trials should focus on different treatment targets or on different clinical endpoints, or both. Ongoing in-depth analysis of the biological effects of abatacept might contribute to a better understanding of the molecular pathways involved in primary Sjögren's syndrome.

The choice of endpoints can greatly influence the conclusion of a trial, as previously described in SLE[26]. Although development of the ESSDAI, 10 years ago, has been a major step forward in the assessment of primary Sjögren's syndrome, it has certain limitations as a primary endpoint. The ESSDAI does not reflect prominent symptoms experienced by patients (sicca, fatigue, and pain). Furthermore, it is not always possible to separate signs of active disease from irreversible damage, and consequently some of the ESSDAI domains are likely not to improve, such as the pulmonary and peripheral nervous system domains. Finally, the ESSDAI can be insensitive to partial improvements within subdomains, which has also been described for similar outcome measures in SLE[27]. For example, a patient with an IgG of more than 20 g/L might show a reduction of IgG, but if IgG does not drop below 20 g/L, the ESSDAI biological subdomain activity does not improve. For these reasons, it is of great importance to develop validated

composite endpoints for primary Sjögren's syndrome, which have high sensitivity to change, and adequately reflect disease activity as well as symptoms of primary Sjögren's syndrome. The composite endpoint Sjögren Syndrome Responder Index was created and validated using data from trials with rituximab and infliximab, and includes fatigue, oral and ocular dryness, unstimulated whole salivary flow, and erythrocyte sedimentation rate[16]. At week 12, the number of responders for the Sjögren Syndrome Responder Index was significantly higher in the abatacept group than in the placebo group. The development of a validated response index is the focus of the recently initiated NECESSITY project[28].

Abatacept was well tolerated by patients with primary Sjögren's syndrome during the ASAP-III study. No treatment-related serious adverse events or treatment withdrawals occurred in the abatacept group. Prevalence of infections was not increased during abatacept treatment, and most adverse events were mild.

The results of a sponsor-initiated, multicentre trial of abatacept treatment for primary Sjögren's syndrome have recently been presented[29]. Similar to our results, despite favourable effects on biological activity, abatacept was no better than placebo for improving ESSDAI, ESSPRI, or stimulated whole salivary flow after 24 weeks of treatment. There are some important differences between the two studies. The sponsor-initiated trial allowed hydroxychloroquine as concomitant treatment and included only patients positive for SSA with no limit to disease duration; by contrast, in our investigator-initiated trial, the use of hydroxychloroquine was not allowed and only patients with positive biopsies and disease duration of 7 years or less were included. The number of patients using concomitant corticosteroid treatment in the ASAP-III study was very low compared with previous trials of primary Sjögren's syndrome.

Our trial has some limitations. Because our sample size was based on the primary endpoint, we cannot rule out that clinically relevant differences in secondary endpoints with large variance, such as salivary flow rate[30], were not detected with the current sample size. Second, because of the large number of secondary outcomes, some statistically significant findings might result from chance. Third, as none of the included patients had renal disease or CNS involvement, the effect of abatacept on these domains could not be assessed. Finally, only a small proportion of our patient population was eligible for participation. Efficacy of abatacept treatment in other patients with primary Sjögren's syndrome—e.g., with longer disease duration or low ESSDAI scores—remains unknown.

To conclude, abatacept was well tolerated by patients with primary Sjögren's syndrome. Despite the beneficial biological effects of abatacept, the ASAP-III study does not support the use of abatacept as standard of care to reduce systemic disease activity. As primary Sjögren's syndrome is a highly heterogeneous disease, further studies could evaluate whether patients with specific clinical manifestations and biological characteristics might benefit from abatacept treatment.

DECLARATION OF INTERESTS

JFvN is a speaker and consultant for Bristol-Myers Squibb. GSvZ is a speaker for Roche. FGMK received an unrestricted grant from Bristol-Myers Squibb, is a consultant for Bristol-Myers Squibb, and a speaker for Bristol-Myers Squibb, Roche, and Janssen-Cilag. HB received unrestricted grants from Bristol-Myers Squibb and Roche; is a consultant for Bristol-Myers Squibb, Roche, Novartis, Medimmune, and Union Chimique Belge; a speaker for Bristol-Myers Squibb and Novartis; and is a member of the advisory board of Bristol-Myers Squibb, Novartis, and Sanofi. All other authors declare no competing interests.

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Supplementary table 1. Efficacy outcomes in abatacept and placebo treated patients

	Baseline		Week 12		Week 24	
	Abatacept	Placebo	Abatacept	Placebo	Abatacept	Placebo
ESSDAI score	14.0 (9.0-16.8)	13.0 (8.0-18.0)	9.0 (5.0-12.0)	10.5 (7.0-16.0)	8.0 (4.0-14.0)	8.0 (5.0-14.5)
ESSPRI score	7.0 (5.4-7.7)	7.3 (5.3-8.0)	6.0 (4.3-7.0)	6.3 (5.2-7.8)	6.0 (4.4-7.3)	6.7 (4.6-7.9)
Dryness	7.0 (5.3-8.0)	7.0 (7.0-8.0)	6.0 (4.0-7.0)	7.0 (6.0-8.0)	6.0 (4.0-7.0)	7.0 (5.0-8.0)
Fatigue	7.5 (7.0-8.0)	8.0 (6.0-9.0)	7.0 (4.0-8.0)	7.0 (5.0-8.0)	6.0 (5.0-8.0)	7.0 (4.3-8.0)
Pain	7.0 (5.0-8.0)	7.0 (3.0-8.0)	6.0 (3.0-7.0)	6.0 (3.5-8.0)	6.0 (4.3-8.0)	6.0 (3.3-7.8)
Ocular dryness NRS	6.0 (3.3-7.0)	7.0 (6.0-8.0)	6.0 (4.0-7.0)	7.0 (5.5-8.0)	5.0 (3.3-7.0)	7.0 (5.0-8.8)
Oral dryness NRS	7.0 (5.0-8.0)	7.0 (6.0-8.0)	6.0 (4.0-8.0)	7.0 (5.5-8.0)	6.0 (4.0-7.0)	6.0 (5.0-8.0)
Vaginal dryness NRS	5.0 (3.0-8.0)	6.5 (5.0-8.8)	5.0 (3.0-7.0)	5.5 (3.0-8.0)	5.0 (3.0-7.5)	6.0 (3.0-8.0)
Patient GDA	8.0 (7.0-8.0)	7.0 (7.0-9.0)	6.0 (5.0-7.0)	7.0 (6.0-8.0)	6.0 (5.0-7.8)	7.0 (5.0-8.0)
MFI						
General fatigue	16.5 (13.3-19.0)	16.0 (13.0-20.0)	15.0 (12.0-18.0)	14.0 (12.0-19.0)	15.5 (13.0-17.8)	16.0 (11.3-18.8)
Physical fatigue	15.5 (14.0-17.0)	15.0 (13.0-18.0)	14.0 (12.0-16.0)	14.0 (11.0-17.0)	14.0 (11.3-16.0)	14.5 (11.0-18.0)
Reduced activity	14.0 (11.0-15.0)	13.0 (11.0-16.0)	13.0 (10.0-14.0)	12.0 (8.0-16.0)	12.5 (9.3-15.0)	13.0 (10.3-16.0)
Reduced motivation	12.0 (9.0-13.0)	11.0 (9.0-14.0)	11.0 (7.0-14.0)	10.0 (8.0-14.0)	10.0 (8.0-12.0)	12.0 (7.3-14.0)
Mental fatigue	12.0 (9.3-15.0)	13.0 (8.0-16.0)	12.0 (8.0-15.0)	14.0 (7.5-17.0)	12.0 (9.5-14.0)	13.0 (8.0-16.0)
SF36						
PCS	37.1 (7.9)	38.2 (9.8)	40.1 (8.6)	39.1 (9.6)	39.3 (8.6)	38.7 (11.1)
MCS	44.0 (38.0-52.1)	44.4 (36.5-51.6)	49.2 (39.3-52.2)	47.3 (40.8-55.6)	47.0 (39.7-53.7)	50.0 (42.4-54.0)
EQ-5D-5L	0.71 (0.60-0.80)	0.71 (0.50-0.79)	0.71 (0.65-0.81)	0.77 (0.67-0.84)	0.74 (0.57-0.82)	0.75 (0.59-0.81)
EQ-5D VAS	60.5 (42.3-70.0)	60.0 (45.0-71.0)	63.0 (51.0-72.0)	59.0 (39.5-73.0)	61.0 (52.0-75.0)	56.5 (40.3-67.8)
FSFI	11.5 (4.4-23.2)	14.7 (5.1-23.4)	NA	NA	18.8 (5.1-25.7)	14.9 (3.7-22.2)
DAS-28 (ESR)	4.82 (1.19)	5.00 (1.48)	4.04 (1.13)	4.58 (1.55)	3.74 (0.88)	4.30 (1.29)
DAS-28 (CRP)	4.35 (2.63-5.08)	4.20 (2.90-5.10)	3.00 (2.20-4.00)	3.20 (2.73-4.38)	2.50 (2.10-3.40)	3.10 (2.30-3.90)
Physician GDA	5.7 (1.5)	5.7 (1.6)	4.1 (1.7)	4.7 (2.0)	3.7 (1.9)	4.3 (2.0)
IgG, g/L	17.4 (13.4-26.7)	18.7 (14.8-24.7)	16.4 (12.7-26.4)	18.4 (14.8-24.2)	17.0 (12.9-26.0)	19.0 (13.7-25.5)
RF, IU/ml	32.5 (2.1-71.0)	24.0 (6.8-83.0)	17.0 (1.9-49.0)	27.0 (9.7-89.5)	17.5 (1.7-42.0)	29.0 (8.0-90.0)
Complement C3, g/L	1.2 (1-1.3)	1.1 (1.0-1.3)	1.2 (1.0-1.4)	1.2 (1.0-1.3)	1.2 (1.0-1.4)	1.1 (1.0-1.2)
Complement C4, g/L	0.19 (0.09)	0.20 (0.07)	0.20 (0.08)	0.21 (0.07)	0.21 (0.07)	0.20 (0.08)
ESR, mm/hour	28.0 (13.3-47.0)	33.0 (17.0-54.0)	35.0 (11.0-60.0)	30.5 (21.3-59.0)	30.0 (15.0-57.0)	44.0 (21.0-66.0)
OSS[a]	4.0 (0.5-6.5)	4.5 (2.0-7.0)	2.0 (0.5-5.3)	3.5 (2.0-6.4)	3.0 (1.0-6.4)	3.5 (1.3-7.3)
Schirmer's test[a]	3.5 (0.6-14.0)	2.5 (0.0-8.5)	4.3 (0.4-9.1)	2.5 (0.0-6.3)	5.3 (2.1-10.3)	1.0 (0.0-4.0)
TBUT, seconds[a]	5.3 (2.5-7.5)	4.0 (2.0-7.0)	4.0 (2.9-7.6)	4.0 (2.6-7.9)	4.0 (2.5-6.9)	4.0 (2.0-7.5)
UWS, ml/min	0.05 (0.01-0.12)	0.05 (0.01-0.13)	0.07 (0.01-0.15)	0.03 (0.00-0.17)	0.06 (0.01-0.15)	0.04 (0.01-0.10)
SWS, ml/min	0.16 (0.06-0.33)	0.10 (0.02-0.43)	0.21 (0.05-0.41)	0.15 (0.03-0.35)	0.20 (0.08-0.43)	0.10 (0.03-0.29)
PASS[b], n (%)	16 (40%)	17 (44%)	18 (46%)	23.0 (62%)	18 (45%)	17 (47%)
ESSDAI responder, n (%)	NA	NA	19 (48%)	13 (33%)	23 (58%)	20 (51%)
ESSPRI responder, n (%)	NA	NA	21 (53%)	11 (28%)	23 (58%)	8 (21%)
SSRI responder, n (%)	NA	NA	16 (40%)	5 (13%)	13 (33%)	9 (23%)

Values are mean (SD), median (25th-75th percentile) unless otherwise indicated. Vaginal dryness and FSFI include only female patients (n=74). [a]Average of right and left eye. [b]Number (%) of patients with acceptable symptoms. CRP: C-reactive protein; DAS-28: disease activity score 28 joint count; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren syndrome disease activity index; ESSPRI: EULAR Sjögren syndrome patient-reported index; EQ-5D-5L: summary index value of the five level version of the EuroQoL five dimensions health status questionnaire; FSFI: Female Sexual Function Index; GDA: global disease activity; IgG: immunoglobulin G; NA: not applicable; NRS: numeric rating scale (range 0-10); MCS: Mental component summary; MFI: multidimensional fatigue inventory; OSS: ocular staining score; PASS: patient acceptable symptom state; PCS: physical component summary; RF: rheumatoid factor; SF-36: Short-Form 36; SSRI: Sjögren syndrome responder index; SWS: stimulated whole salivary flow; UWS: unstimulated whole salivary flow; VAS: visual analogue scale (range 0-100).

Supplementary table 2. ESSDAI subdomain and total activity

Domains	Baseline		Week 12		Week 24	
	Abatacept	Placebo	Abatacept	Placebo	Abatacept	Placebo
Activity	n=40	n=39	n =39	n=37[a]	n=40	n =37
Constitutional						
None	20 (50)	23 (59)	26 (67)	20 (54)	29 (73)	25 (68)
Low	16 (40)	9 (23)	10 (26)	12 (32)	8 (20)	10 (27)
Moderate	4 (10)	7 (18)	3 (8)	5 (14)	3 (8)	2 (5)
Lymphadenopathy						
None	30 (75)	26 (67)	35 (90)	30 (81)	35 (88)	31 (84)
Low	8 (20)	12 (31)	3 (8)	6 (16)	4 (10)	6 (16)
Moderate	2 (5)	1 (3)	1 (3)	1 (3)	1 (3)	0
High	0	0	0	0	0	0
Glandular						
None	4 (10)	3 (8)	14 (36)	12 (32)	18 (45)	15 (41)
Low	15 (38)	19 (49)	14 (36)	11 (30)	14 (35)	14 (38)
Moderate	21 (53)	17 (44)	11 (28)	14 (38)	8 (20)	8 (22)
Articular						
None	17 (43)	16 (41)	25 (64)	16 (43)	29 (73)	23 (62)
Low	5 (13)	5 (13)	3 (8)	5 (14)	0	2 (5)
Moderate	5 (13)	7 (18)	8 (21)	9 (24)	6 (15)	6 (16)
High	13 (33)	11 (28)	3 (8)	7 (19)	5 (13)	6 (16)
Cutaneous						
None	29 (73)	32 (82)	32 (82)	31 (84)	34 (85)	31 (84)
Low	2 (5)	4 (10)	1 (3)	2 (5)	0	3 (8)
Moderate	8 (20)	3 (8)	6 (15)	4 (11)	6 (15)	3 (8)
High	1 (3)	0	0	0	0	0
Pulmonary						
None	37 (93)	37 (95)	36 (92)	36 (97)	37 (93)	35 (95)
Low	0	0	0	0	0	0
Moderate	3 (8)	2 (5)	2 (5)	1 (3)	2 (5)	2 (5)
High	0	0	1 (3)	0	1 (3)	0
Muscular						
None	39 (98)	39 (100)	38 (97)	37 (100)	39 (98)	37 (100)
Low	1 (3)	0	1 (3)	0	1 (3)	0
Moderate	0	0	0	0	0	0
High	0	0	0	0	0	0
PNS						
None	38 (95)	34 (87)	37 (95)	32 (86)	38 (95)	33 (89)
Low	2 (5)	4 (10)	2 (5)	4 (11)	2 (5)	4 (11)
Moderate	0	1 (3)	0	1 (3)	0	0
High	0	0	0	0	0	0
Hematologic						
None	26 (65)	19 (49)	27 (69)	18 (50)	25 (63)	13 (35)
Low	12 (30)	18 (46)	10 (26)	17 (47)	12 (30)	22 (59)
Moderate	2 (5)	2 (5)	2 (5)	1 (3)	3 (8)	2 (5)
High	0	0	0	0	0	0
Biological						
None	8 (20)	9 (23)	8 (21)	10 (28)	10 (25)	10 (27)
Low	8 (20)	6 (15)	8 (21)	7 (19)	9 (23)	7 (19)
Moderate	24 (60)	24 (62)	23 (59)	19 (53)	21 (53)	20 (54)
Total ESSDAI						
Low	0	0	6 (15)	8 (22)	14 (35)	8 (22)
Moderate	19 (48)	23 (59)	24 (62)	15 (42)	14 (35)	19 (51)
High	21 (53)	16 (41)	9 (23)	13 (36)	12 (30)	10 (27)

Values are number (%) of patients. [a]N=36 for ESSDAI biological, hematologic and total score due to missing laboratory parameters. ESSDAI: EULAR Sjögren's syndrome disease activity index. PNS: peripheral nervous system.

Supplementary table 3. Listing of serious adverse events

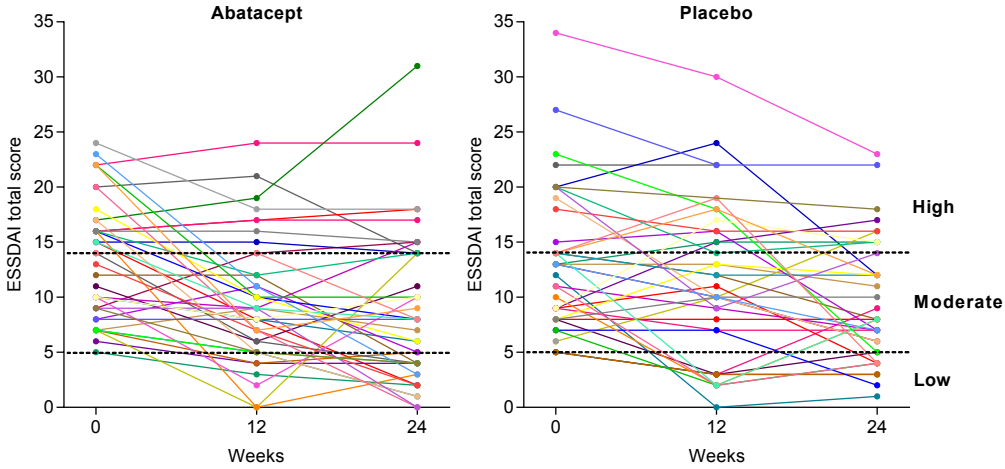
System organ class	Lower level term	Related to treatment[a]
Abatacept		
Injury, poisoning and procedural complications	Clavicle fracture	Doubtful
Placebo		
Immune system disorders	SLE flare	Doubtful
Infections and infestations	S. pneumoniae pneumonia	Possible
Nervous system disorders	Lumbar radiculopathy	Doubtful
Respiratory, thoracic and mediastinal disorders	Embolism lung	Doubtful

No suspected unexpected serious adverse reactions occurred during the study. [a]As assessed by the investigators during the trial according to Naranjo criteria. SLE: systemic lupus erythematosus.

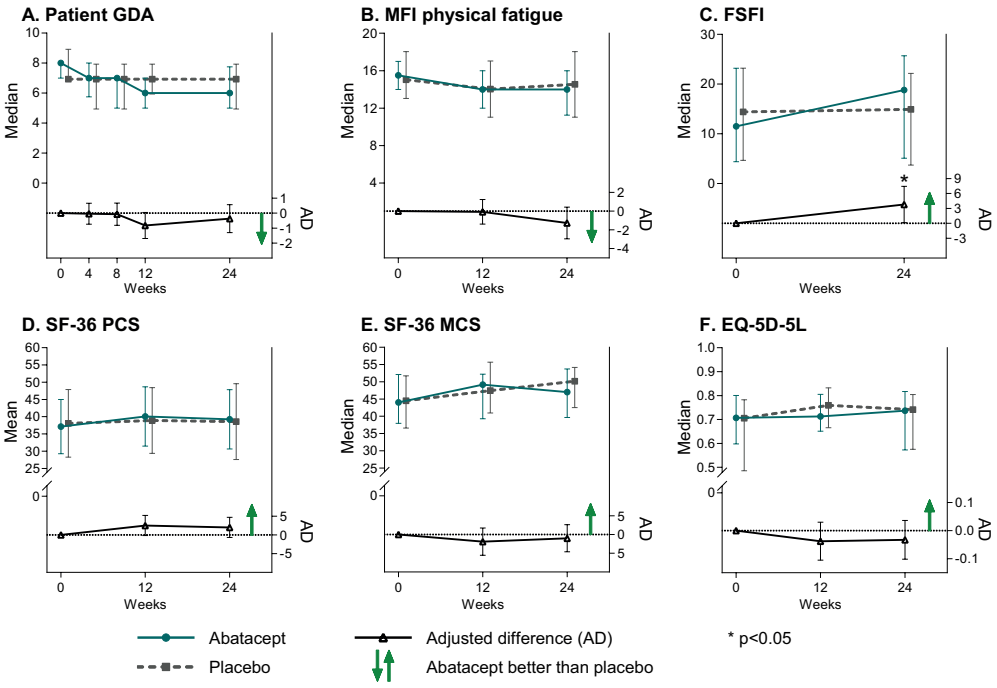
Supplementary table 4. Listing of adverse events per system organ class

System organ class	Number of events		Number of patients (%) with events	
	Abatacept	Placebo	Abatacept (n=40)	Placebo (n=40)
Infections and infestations	46	49	29 (72.5)	28 (70)
General and administration site conditions	14	4	12 (30)	4 (10)
Gastrointestinal disorders	12	6	11 (27.5)	5 (12.5)
Nervous system disorders	8	4	7 (17.5)	3 (7.5)
Musculoskeletal and connective tissue disorders	7	5	7 (17.5)	5 (12.5)
Respiratory, thoracic and mediastinal disorders	4	1	4 (10)	1 (2.5)
Cardiac disorders	3	4	3 (7.5)	4 (10)
Skin and subcutaneous tissue disorders	3	1	3 (7.5)	1 (2.5)
Injury, poisoning and procedural complications	2	1	2 (5)	1 (2.5)
Investigations	2	2	2 (5)	2 (5)
Hepatobiliary disorders	1	1	1 (2.5)	1 (2.5)
Psychiatric disorders	1	1	1 (2.5)	1 (2.5)
Eye disorders	0	2	0	2 (5)
Immune system disorders	0	1	0	1 (2.5)
Metabolism and nutrition disorders	0	1	0	1 (2.5)
Reproductive system and breast disorders	0	3	0	3 (7.5)
Surgical and medical procedures	0	1	0	1 (2.5)

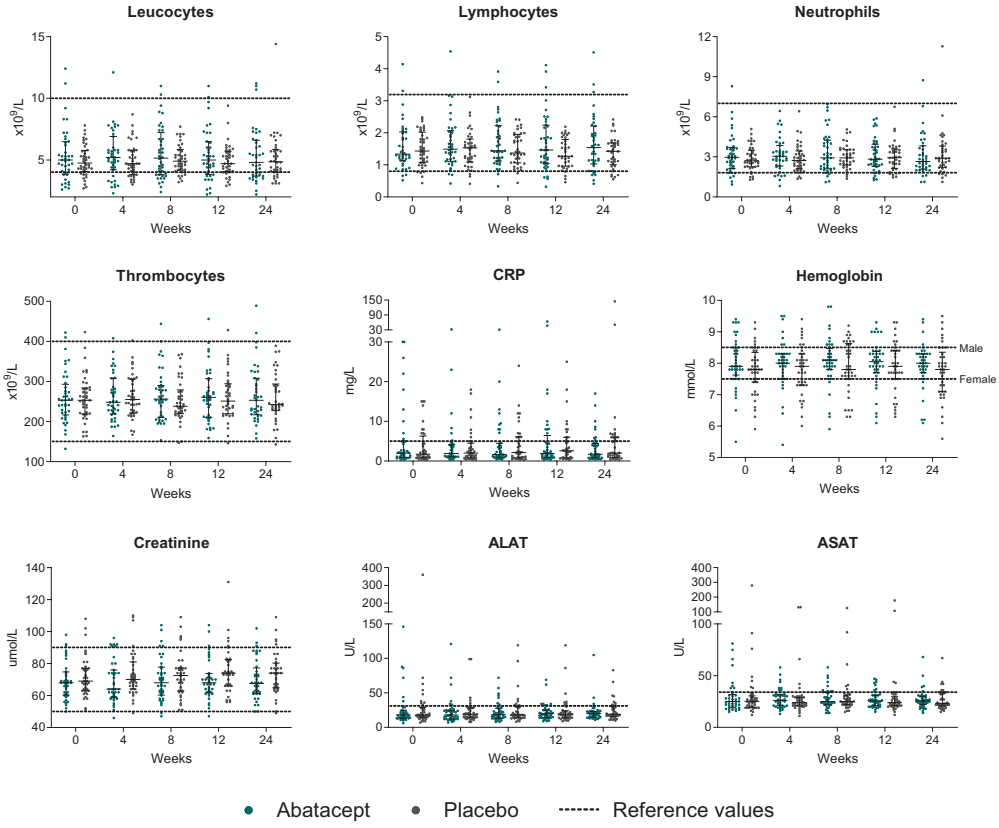
System organ classes in which no events occurred were not shown in this table.



Supplementary figure 1. ESSDAI scores of individual abatacept and placebo patients.
ESSDAI: EULAR Sjögren's syndrome disease activity index.



Supplementary figure 2. Secondary patient-reported efficacy outcomes.
The upper parts of all figures show medians or means of the abatacept and placebo group. The lower parts shows the difference between groups (abatacept-placebo), adjusted for baseline values and previous DMARD use. Error bars represent IQR for medians, SD for means and 95% CI for adjusted differences. AD: Adjusted difference; EQ-5D-5L: five level version of the EuroQoL five dimensions health status questionnaire; FSFI: Female Sexual Function Index; GDA: global disease activity; MCS: Mental component summary; MFI: multidimensional fatigue inventory; PCS: physical component summary; SF-36: Short-Form 36.



Supplementary figure 3. Laboratory safety parameters.
ALAT: Alanine aminotransferase; ASAT: aspartate aminotransferase; CRP: C-reactive protein.

CHAPTER 9

The value of rituximab treatment in primary Sjögren's syndrome

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ABSTRACT

The rationale for B-cell depletion therapy with rituximab in primary Sjögren's syndrome (pSS) relies upon the well-established role of B-cell hyperactivity in immunopathogenesis. In line with this notion, several biomarkers of B-cell activity are significantly affected by treatment, both in the target organs and periphery. In contrast to most biological outcomes, clinical outcomes are not consistent between studies. Although two large RCTs did not meet their primary endpoint, several beneficial clinical effects of treatment have been shown. As discussed in this review, differences in study design and patient characteristics could explain the variation in results. Interestingly, a newly developed composite endpoint of subjective and objective outcomes did show a significant effect of rituximab in one of the large RCTs. Response predictors need to be identified to define more targeted inclusion criteria and achieve precision medicine. The positive effects seen on biological and clinical parameters warrant future studies to investigate this promising treatment modality.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease with a heterogeneous clinical presentation. Predominant symptoms of pSS are dryness of mouth and eyes, but many patients also suffer from extraglandular symptoms, including chronic fatigue, arthralgia and involvement of lungs, skin, kidneys and the nervous system. Dysfunction of exocrine glands is accompanied by periductal mononuclear infiltration of these glands, mainly by $CD4^+$ T cells and B cells. Involvement of B cells in pSS pathogenesis is further illustrated by the presence of autoantibodies directed against SS-A/Ro and/or SS-B /La, elevated levels of rheumatoid factor (RF), hypergammaglobulinemia, elevated levels of Bruton's tyrosine kinase in B cells and a significantly increased risk of Non-Hodgkin B cell lymphoma, predominantly mucosa-associated lymphoid tissue (MALT) lymphoma[1,2].

The prominent role of B cell hyperactivity in pSS pathogenesis provides a rationale for the use of rituximab, a humanized anti-CD20 monoclonal antibody, to treat this disease. Binding of rituximab to CD20-expressing B cells results in a significant depletion of these cells via antibody-dependent cellular cytotoxicity, complement mediated cytotoxicity and apoptosis[3]. Plasma cells are not directly depleted by rituximab, because expression of CD20 is downregulated when B cells differentiate towards plasma cells, but formation of new plasma cells may be impaired by B cell depletion therapy. Although initial studies in pSS showed improvement of both subjective and objective parameters[4–7], two large placebo-controlled trials[8,9] did not confirm all promising results of the earlier studies. Possible explanations for this discrepancy are heterogeneity in patient characteristics, primary end points and background medication use, which will all be discussed in this review. Consensus on the efficacy of rituximab in pSS is currently lacking, but treatment results in several clinical, biological and histological improvements. Furthermore, treatment studies with rituximab in pSS provided insights in the pathogenic mechanisms of the disease and post-hoc analyses of biological parameters have identified possible biomarkers that can predict response. These biomarkers may characterize subgroups of pSS patients that benefit from rituximab before start of treatment. Future studies with B cell-targeting therapy can contribute to identification of new predictors of response, as well as development of sensitive and accurate outcome measures for future clinical trials in pSS.

EFFECTS OF RITUXIMAB ON B CELL HYPERACTIVITY

Systemic markers of B cell hyperactivity

Several biomarkers of B cell activation, including gammaglobulins, autoantibodies (RF, anti-SS-A/Ro, anti-SS-B/La), β2-microglobulin, free light chains and B cell activating factor (BAFF/Blys) have been studied in the context of rituximab treatment in pSS (figure 1). A small but

significant gradual decrease in total serum IgG after 24 weeks of treatment is seen in larger studies (Table 1)[4,8,10]. At the same time, a decrease in RF levels (up to 50%) is observed (Table 1)[4,6,10–12]. Interestingly, Dass et al. found that a non-responder had less reduction in RF after treatment compared with responders[6]. Following B cell repopulation, RF levels rise again and this rise can predict relapse of clinical symptoms[5,12]. Similar to findings in rheumatoid arthritis (RA), combined presence of RF and disease–specific autoantibodies (anti-SS-A/Ro, anti-SS-B/La) may result in higher disease activity in pSS as well[13]. The mechanism behind this synergistic effect in unknown, but crosslinking and/or stabilization of immune complexes, consisting of autoantigens and autoantibodies, by RF is likely involved. In combination with the finding that higher RF levels seem to increase the risk of lymphoma[14], these data suggest that lowering RF levels by rituximab treatment is of clinical importance, as it may protect against disease progression and/or lymphoma development in pSS.

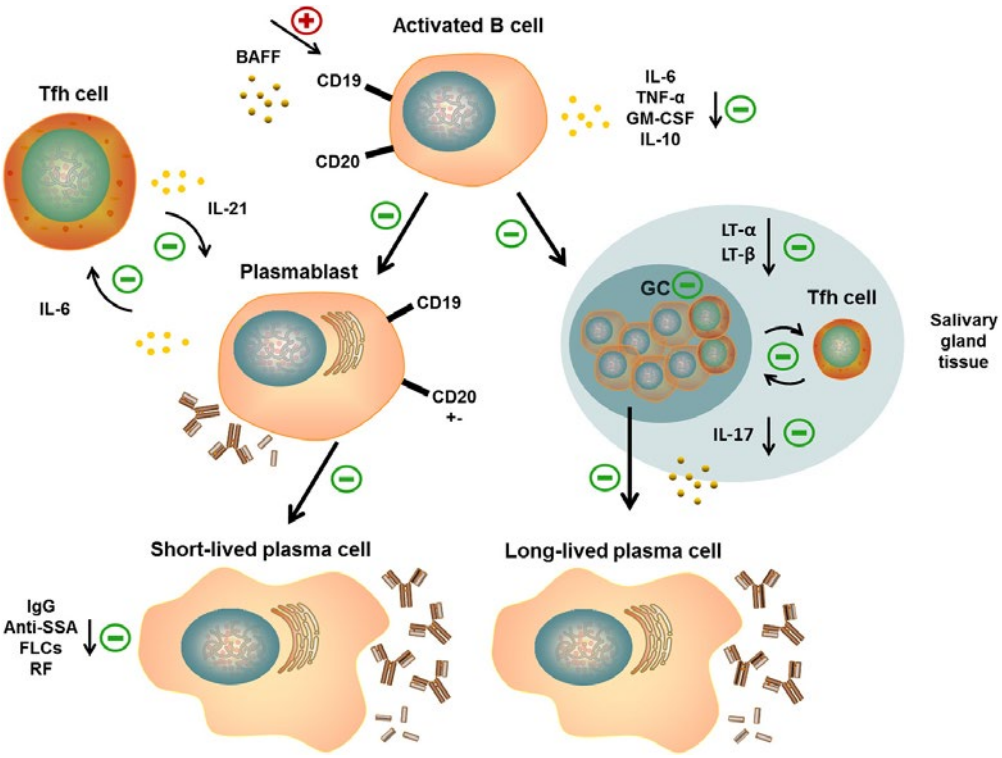

Figure 1. Effects of rituximab treatment on immunopathogenesis of pSS.
Cytokine production by B cells and (T cell-dependent) formation of plasmablasts and short-lived plasma cells are impaired by B cell depletion therapy with rituximab. B cell hyperactivity is reduced, as reflected by lowering of serum IgG, anti-SSA, free light chains and rheumatoid factor. Numbers of circulating Tfh cells and serum levels of IL-21 are also decreased. In salivary gland tissue, formation of ectopic lymphoid tissue and germinal centers is impaired. FLCs: free light chains; RF: rheumatoid factor.

Several studies assessed the effect of rituximab on anti-SS-A/Ro or anti-SSB/La serum levels in pSS patients. While three studies did not find significant changes in anti-SS-A/Ro or anti-SS-B/La autoantibodies after treatment[7,15,16], we found a significant reduction of ±25% in anti-SS-A/Ro and anti-SS-B/La titers at 16 weeks after treatment (Table 1)[17]. The discrepancy between studies may be explained by differences in study population size, baseline systemic disease activity, time point of measurements, or differences in reliability of the immunoassay, but methods for anti-SS-A/Ro and anti-SS-B/La measurement were not specified in most studies. The observed reduction in autoantibodies is likely a result of decreased generation of short-lived plasma cells, due to depletion of $CD20^+$ precursor cells, and/or direct depletion of CD20-expressing (short-lived) plasma cells. There is evidence that anti-SSA/Ro60 antibody production depends –at least partially- on clonal turnover of short-lived plasma cells and this may also be true for other autoantibodies[18]. B cell depletion therapy can therefore directly affect autoantibody production in pSS patients.

Table 1. Main biological effects of rituximab treatment analyzed in prospective clinical studies

Study population	Patients treated (n)	IgG	RF	Anti-SSA titer	Glandular B cells	Patients on concomitant immunomodulatory drugs (n (%))	
						DMARDS	Steroids
Pijpe et al.[11]	15	=	↓[a]	NA	↓	3 (20)	3 (20)
Devauchelle-Pensec et al.[15]	16	=	=	↓[b]	↓	0 (0)	0 (0)
Dass et al.[6]	8	↓[b]	↓	NA	NA	NA	NA
Meijer et al.[69]	5	NA	↓	NA	NA	0 (0)	0 (0)
Meijer et al.[4]	20	↓	↓	NA	↓	0 (0)	0 (0)
Gottenberg et al.[50]	78	NA	NA	NA	NA	29 (37)	17 (22)
Carubbi et al.[7]	19	=	=	=	↓	0 (0)	19 (100)
St. Clair et al.[16]	12	NA	↓[b]	=	NA	8 (67)	3 (25)
Devauchelle-Pensec et al.[8]	63	↓	NA	NA	↓	10 (19)	17 (32)
Meiners et al.[5]	28	↓	↓	↓	NA	0 (0)	0 (0)
Bowman et al.[9]	67	NA	NA	NA	NA	39 (58)	7 (10)

Arrows indicate a decrease. [a]Only in patients with MALT/pSS. [b]Not statistically significant. NA: not available.

In addition to gammaglobulin and autoantibody levels, other indicators of B cell hyperactivity in pSS are also affected by rituximab treatment. β2-microglobulin levels show a 'delayed' drop at 16 weeks after treatment[8,12], which was not seen at 6 or 12 weeks after rituximab treatment[8,11]. Serum immunoglobulin free light chains (FLCs) are also affected by rituximab and decrease significantly from week 5 up to week 48 after treatment (unpublished data), in line with findings in RA[19]. Both β2-microglobulin and FLC baseline levels in serum of pSS patients are positively correlated to ESSDAI scores[20], suggesting that there is a link between the degree of B cell activation and systemic disease activity. Serum levels of several B cell-associated cytokines, including IL-6, GM-CSF, TNF-α and IL-10, are also lowered by rituximab[21]. Whether this decrease is the consequence of removal of cytokine-producing B cells, or is caused by indirect effects of B cell depletion on cytokine production by other cells is not

yet known. In contrast to the B cell-associated cytokines mentioned above, serum BAFF levels increase after B cell depletion therapy, likely due to unavailability of BAFF receptors as a consequence of the absence of B cells[22]. This rise in BAFF levels may be unfavorable for the patient due to enhanced survival of autoreactive B cell clones and skewing of newly formed B cells towards an autoreactive phenotype[23,24]. Therefore, the efficacy of therapy combining B cell depletion and BAFF-blockade is currently under investigation (NCT02631538).

In summary, most biomarkers of B cell activation in the circulation are decreased by B cell depletion therapy (figure 1). Lowering of B cell activation likely contributes to amelioration of systemic disease activity in pSS patients, due to lower levels of autoantibodies and pro-inflammatory cytokines.

Histological markers of B cell hyperactivity

B cells infiltrate the glandular tissue of pSS patients, accumulate around the ductal epithelium and, together with stromal cells and follicular dendritic cells, orchestrate formation of ectopic lymphoid tissue. Importantly, rituximab clearly reduces the total number and proportion of infiltrating B cells in both minor and major salivary glands (Table 1)[7,25,26]. In addition, as shown in minor salivary gland tissue, rituximab decreases mRNA expression of lymphotoxin (LT)-α and –β, important for lymphoid organogenesis [7]. The reduction of lymphotoxin is likely a direct result of lower B cell numbers in the glands, as the heterodimer LTα1β2 is mainly produced by B cells[27]. Lowering of B cell numbers is further accompanied by a decline in germinal centers located within ectopic lymphoid tissue of the glands[28]. This decline is likely caused both by direct depletion of B cells, as well as reduced presence of Tfh cells (figure 1)[17].

B cells often infiltrate the ductal epithelium of the salivary glands, resulting in the development of lymphoepithelial lesions. Most of these intra-epithelial B cells belong to a unique subset of cells expressing FcRL4 and these cells possibly function as precursor cells for MALT lymphoma[29]. We have found that intra-epithelial FcRL4+ B cells are almost completely depleted by rituximab[29]. Furthermore, rituximab treatment reduces the severity of lymphoepithelial lesions, and concomitantly leads to restoration of the epithelium[25]. It would be of value to study whether rituximab-treated patients develop MALT-lymphoma less frequently than untreated patients.

As expected, plasma cells can persist in parotid glands of pSS patients despite B cell depletion therapy, since they lack expression of CD20[30]. However, it is not known if absolute numbers of plasma cells in salivary glands are affected by rituximab and whether this is associated with response to treatment. In synovial tissue of RA patients, a larger decrease in synovial plasma cells was observed in responders versus non-responders[31]. Therefore, it is of interest to study local plasma cell numbers in pSS patients after rituximab.

EFFECTS OF RITUXIMAB ON THE CD4$^+$ T CELL COMPARTMENT

Depletion of B cells abrogates antigen presentation and cytokine production by these cells and rituximab treatment may therefore affect other cell types, in particular CD4$^+$ T cells (figure 1)[32]. Patients with pSS have elevated proportions of circulating T follicular helper (cTfh) cells, defined as CXCR5$^+$PD-1$^+$CD45RA$^-$CD4$^+$ cells, compared with healthy controls[17,33,34]. The B cell hyperactivity that is present in pSS patients may favor differentiation of Tfh cells through secretion of IL-6 by activated B cells in conjunction with high expression of co-stimulatory molecules (e.g., CD40, ICOS-L)[35,36]. Tfh cells subsequently activate B cells and promote germinal center formation and plasma cell formation[36], providing a positive-feedback loop. We have recently shown that cTfh cells, and to a smaller extent also Th17 cells, are reduced by rituximab[17]. The decrease in cTfh cells correlates with lowering of ESSDAI scores, emphasizing their potential role in the disease process. Reduced frequencies and numbers of cTfh cells and Th17 cells during B cell depletion are accompanied by decreased serum levels of IL-21 and IL-17. Th17 cells in minor salivary glands are also reduced by rituximab, but the effect on local Tfh cells is not known yet[37,38]. Depletion of the small fraction of Th17 cells that co-expresses CD20 may contribute to the decrease in Th17 cells[39]. Thus, taking all the biological effects of rituximab on (T cell-mediated) B cell hyperactivity together, these findings may –at least in part- underlie beneficial clinical outcomes of rituximab in pSS patients.

CLINICAL EFFICACY OF RITUXIMAB IN PRIMARY SJÖGREN'S SYNDROME

Several open-label and randomized controlled trials have been performed to date, including two larger RCTs: the TEARS and TRACTISS trials[8,9]. In tables 2 and 3, population characteristics and clinical outcomes of all prospective clinical trials reported in literature are summarized. Despite the generally acknowledged beneficial effects of rituximab treatment on biological parameters, clinical outcomes vary between studies.

Effects on exocrine gland function and sicca symptoms

Objective measures of salivary gland function include unstimulated whole salivary flow (UWS) and stimulated whole salivary flow (SWS). UWS depends mainly on submandibular gland function, while SWS depends on both submandibular and parotid gland function. The ratio of parotid and submandibular saliva in SWS depends on the method of stimulation (mechanical vs. citric acid stimulation). UWS and SWS are both outcomes of interest. However, it is important to realize that patients show substantial intra-individual variability in salivary flow, resulting in a large standard deviation[40,41]. Therefore, adequate sample sizes are needed to show the effect of treatment on salivary gland function.

Table 2. Study population characteristics

	Study design	Patients (n)		Age (years)	Disease duration (years)	ESSDAI	Anti-SSA and/or –SSB positive (%)	IgG (g/L)	Unstimulated salivary flow (ml/min)	Stimulated salivary flow (ml/min)
		RTX	Control							
Pijpe et al.[11] (*Early pSS group*)	Open label	8	0	46 ± 12	2 ± 1	NA	100	19 ± 5	0.04 (0–0.19)	0.38 (0.2–1.38)
Pijpe et al.[11] (*MALT + pSS group*)	Open label	7	0	54 ± 10	7 ± 4	NA	100	13 ± 6	0 (0–0.5)	0.01 (0–0.47)
Devauchelle-Pensec et al.[15,70]	Open label	16	0	55 ± 13	13 ± 10	NA	81	20 ± 13	0.1 ± 0.1	NA
Dass et al.[6]	RCT pilot	8	9	51 (22–64)	7 (1–18)	NA	100	19 (12–29)	NA	NA
Meijer et al.[69]	Open label *Re-treatment*	5	0	[a]	[a]	[a]	[a]	[a]	NA	0.09 ± 0.07[b]
Meijer et al.[4], and Moerman et al.[51]	RCT	20	10	43 ± 11	5 ± 4	8 (4–13)	100	23 ± 8	0.17 ± 0.19	0.70 ± 0.57
Gottenberg et al.[50]	Registry	78	0	60 (29-83)	12 (3-32)	11 (2-31)	69	NA	NA	NA
Meiners et al.[5]	Open label *Re-treatment*	28	0	43 ± 14	7 ± 4	8 ± 5	100	23 ± 7	0.16 ± 0.18	0.42 ± 0.37
Carubbi et al.[7]	Open label	19	22	40 (27-53)	1 (1-2)	20 (6-41)	NA	NA	0.08 ± 0.04	NA
St. Clair et al.[16]	Open label	12	0	51 (34-69)	8 (2-18)	NA	83	NA	0.03 (0.0–0.22)	0.05 (0.0–0.65)
Devauchelle-Pensec et al.[8]	RCT	63	57	53 ± 13	5 ± 5	10 ± 7	81	16 ± 6	0.2 (0.4)	NA
Bowman et al.[9]	RCT	67	66	54 ± 12	5 ± 5	5 ± 5	99	18 ± 7	0.08 (0.08)	NA

Results are presented as mean ± SD or median (range). For controlled studies, patient characteristics are presented for the rituximab-treated group. [a]See Pijpe et al.[11]. [b]Stimulated submandibular/sublingual flow rate.

Table 3. Main clinical effects of rituximab treatment in prospective clinical studies

Study	Study design	Patients (n)		Follow-up (weeks)	RTX dose	Salivary gland function	Tear gland function	Dryness VAS	Fatigue	Pain	ESSDAI	ESSPRI	SF-36
		RTX	Control										
Pijpe et al.[11] *Early pSS group*	Open label	8	0	12	High	UWSF = Stim SM/SL ↑	RB ↓ Schirmer =	Oral ↓ Ocular =	MFI GF ↓	SF-36 BP =	NA	NA	Physical functioning ↑ Vitality ↑ Health change ↑ Other domains =
Pijpe et al.[11] *MALT + pSS group*	Open label	7	0	12	High	UWSF = Stim SM/SL =[a]	RB ↓ Schirmer =	Oral = Ocular =	MFI GF =	SF-36 BP =	NA	NA	All domains =
Devauchelle-Pensec et al.[15,70]	Open label	16	0	36	Low	UWSF =	Schirmer =	↓	VAS ↓	VAS ↓	NA	NA	All domains except physical functioning ↑ MCS ↑ PCS ↑
Dass et al.[6]	RCT pilot	8	9	26	High	UWSF =	Schirmer =	NA	VAS ↓[d] PROFAD-SSI ↓[d]	VAS =	NA	NA	Social functioning ↑[f] MCS =[e] PCS =
Meijer et al.[69]	Open label *Re-treatment*	5	0	48	High	SWSF ↑	NA	Oral ↓	MFI GF ↓	NA	NA	NA	Physical functioning ↑
Meijer et al.[4], and Moerman et al.[51]	RCT	20	10	48	High	UWSF ↑[d] SWSF ↑[e,f]	LG ↓[d] Schirmer =[e]	Oral ↓[d] Ocular ↓[e,f]	MFI GF ↓	NA	↓[f]	NA	Total score ↑[d] Vitality ↑[d]
Gottenberg et al.[50]	Registry	78	0	152[b]	High	NA	NA	NA	NA	NA	↓	NA	NA
Meiners et al.[5]	Open label *Re-treatment*	28	0	48	High	SWSF =	NA	Oral=[c] Ocular =[c]	MFI GF ↓[c]	NA	↓	↓	NA
Carubbi et al.[7]	Open label	19	22	120	High	UWSF ↑[d,f]	Schirmer ↑[f]	↓[f]	VAS ↓[f]	VAS ↓	↓[f]	NA	NA
St. Clair et al.[16]	Open label	12	0	52	High	UWSF = SWSF =	Schirmer =	Oral subscores ↓ Ocular =	VAS ↓	VAS =	NA	NA	Vitality ↑ MCS= PCS =
Devauchelle-Pensec et al.[8]	RCT	63	57	24	High	UWSF =	Schirmer =	↓[f]	VAS ↓[f]	VAS =	=	NA	MCS= PCS=
Bowman[9]	RCT	67	66	48	High	UWSF =[e]	Schirmer =	=	VAS = PROFAD-SSI =	VAS =	=	=	All domains =

High-dose: 375 mg/m2/week (4x) or 1000 mg/m^2/two weekly (2x). Low-dose: 375 mg/m2/week (2x). Green arrows indicate significant improvements. [a]Stim SM/SL improved in patients with baseline SWSF >0.1 ml/minute (n=2). [b]Median follow up time. [c]In 15/28 patients, reported in Meiners et al., 2015 (REF). [d]No significant change in control group, compared to baseline. [e]Deterioration in control group, compared to baseline. [f]Significant difference between RTX group and control group. RTX: rituximab. NA: not available. VAS: visual analogue scale. ESSDAI: European League Against Rheumatism (EULAR) Sjögren's syndrome (SS) disease activity index. ESSPRI: EULAR SS patient reported index. UWSF: Unstimulated whole salivary flow. Stim SM/SL: Stimulated submandibular/sublingual salivary flow. SWSF: Stimulated whole salivary flow. RB: Rose Bengal. LG: Lissamin Green. MFI GF: Multidimensional Fatigue Index, General Fatigue domain. PROFAD: Profile of Fatigue and Discomfort—Sicca Symptoms Inventory (PROFAD-SSI). SF-36: Short-form 36-item Health Survey. SF-36 BP: SF-36 Bodily pain domain. MCS: mental component summary score. PCS: physical component summary score.

Meijer et al. and Carubbi et al. showed significant improvement in UWS after rituximab treatment[4,7]. In other trials, including the TEARS trial, no effect on UWS was observed[6,8,11,15]. Although the mean baseline UWS in the TEARS trial was comparable to the study of Meijer et al., the standard deviation was twice as high, which may influence the power of their analysis. St. Clair et al. did not find an effect on UWS, but included patients with low to absent UWS at baseline, who therefore may have had irreversible destruction of glandular parenchyma[16]. Recently, Bowman et al. showed that UWS of patients in the rituximab group of the TRACTISS trial remained stable, while the placebo group deteriorated[9].

Only few studies measured the effect of rituximab on SWS. Pijpe et al. showed that rituximab improved stimulated submandibular/sublingual salivary flow only in patients with residual salivary gland function at baseline (SWS >0.10 ml/minute)[11]. Similarly, in the RCT by Meijer et al. only patients with a SWS ≥0.15 ml/minute were included, and SWS was significantly increased in the rituximab group, while it deteriorated in the placebo group[4]. Unfortunately, recent RCTs did not measure SWS.

Currently, there is a growing interest in salivary gland ultrasound for assessment of the salivary gland structure, as it is non-invasive and inexpensive. The first study using ultrasound showed a reduction in size of the parotid and submandibular glands after rituximab treatment[42]. In a sub-analysis of the TEARS study, parotid parenchyma echostructure improved in 50% of the rituximab-treated patients versus 7% in the placebo group, visualizing histological changes induced by rituximab (referentie?)[43].

In summary, there seems to be a beneficial effect of rituximab on salivary gland function and structure, but the effect size is small and varies between studies. Echostructure of the gland seems to improve by rituximab, in line with the histological effects. The observed decrease in glandular B cells and (partial) restoration of the ductal epithelium in patients after treatment may contribute to the increase in salivary flow, but additional factors that affect salivary flow in pSS patients need to be identified. Lastly, it should be considered that severe destruction of parenchyma may not be reversed by immunomodulatory treatment, but such treatment could halt further damage in patients with residual gland function.

Tear gland function was assessed by Schirmer's test in most studies. Only one out of seven studies showed significant improvement in Schirmer's test after treatment (table 3)[7]. The TEARS study showed a stable Schirmer's test result in the rituximab group, whereas the placebo group tended to deteriorate[8]. Of note, Schirmer's test may not be suitable to detect small changes over time, as it shows low to moderate reliability[44]. Measurement of the epithelial integrity of the ocular conjunctiva by rose Bengal or lissamin green, and cornea by fluorescein staining is more reliable to evaluate keratoconjunctivitis sicca[45]. Interestingly, studies using these ocular surface staining methods did show improvement after treatment[4,11]. This improvement may

be caused by effects of rituximab on tear gland morphology and function, composition of tear fluid, as well as effects on the inflammatory micro-environment of the ocular surface. For example, B cell-derived IL-6 levels in tears correlate with the severity of ocular surface disease, reflected by a higher extent of ocular pain, irritation and staining[46]. More knowledge about the effect of rituximab on lacrimal gland inflammation would be valuable, and ocular surface staining should be evaluated in all clinical studies, instead of using Schirmer's test only.

Besides objective measures of dryness, patient-reported outcomes (PROs) with visual analogue scales (VAS) were used in most studies to assess subjective symptoms. Positive results on total dryness scores or subscores for oral and ocular dryness were seen in most studies (table 3). Although no decrease in dryness VAS was seen in de TRACTISS study, improvement was seen in the TEARS study[8,9]. VAS dryness scores improved significantly among patients in the rituximab group, although less than 30 mm, which was set as minimum to achieve the primary end-point. Furthermore, in a post-hoc analysis, the SS Responder Index (SSRI) was developed, which includes VAS scores for fatigue, oral dryness and ocular dryness, as well as UWS and ESR. Using this composite endpoint, the proportion of patients with a 30% improvement was significantly higher in the rituximab group, compared to the placebo group[47].

Altogether, both subjective symptoms and objective measures of dryness seem to improve or at least stabilize during rituximab treatment in most studies. These findings are in accordance with histological improvements observed in the salivary glands. A lack of robust objective tests and the poor correlation between objective tests and symptoms in pSS may underlie the reported variation in study results[48].

Effects on extraglandular manifestations

Fatigue has a major impact on quality of life in pSS patients and is therefore an important target for treatment. However, fatigue is a complex and poorly understood feature of the disease and can only be measured subjectively[49]. Most studies measured fatigue by VAS, but more detailed instruments such as the multi-dimensional fatigue inventory (MFI) and the Profile of Fatigue and Discomfort (PROFAD) questionnaire were also used. Importantly, most studies show that fatigue is reduced in pSS patients. All studies, except for the TRACTISS study and a small group of patients with advanced disease and MALT lymphoma, showed a positive effect of treatment on fatigue (table 3). The largest decrease in fatigue is often seen at early time points (week 4 in Meijer et al. and week 6 in the TEARS study). This may explain why no effect was seen on fatigue in the TRACTISS study, as the first visit in this study was scheduled in week 16. Results at early time points may have been biased by initial prednisone treatment to prevent infusion reactions. However, fatigue also improved in the open-label study by Devauchelle-Pensec et al. where no initial prednisone treatment was given[15]. In summary, although the effect size is small, most studies did show improvements in fatigue. In contrast, symptoms of arthralgia and tendomyalgia do not seem to be ameliorated during rituximab treatment (table 3).

Rituximab is often used off-label to treat severe systemic manifestations of pSS. The effect of rituximab on systemic disease activity was assessed by ESSDAI in several studies, including a prospective registry study of off-label treatment with rituximab (table 3). Substantial heterogeneity exists within and between study populations regarding systemic disease activity (table 2). A significant decrease in ESSDAI score following treatment was seen in the RCT of Meijer et al, as reported by Moerman et al., as well as two open label trials and the registry study[4,7,8,50,51]. Improvement was predominantly seen in the glandular, articular, hematological and biological domains[5], possibly because these ESSDAI domains are more likely to change[52]. The efficacy of rituximab on articular involvement was also confirmed using the 28-joint disease activity score (DAS-28)[53]. Results from the registry study and extrapolation of efficacy data from other autoimmune conditions further support the use of rituximab in pSS patients with vasculitis and pulmonary involvement[50,54]. Therefore, these specified clinical settings for rituximab treatment were recently included in the clinical practice guidelines of the Sjögren's Syndrome Foundation[54]. In contrast with earlier findings, no significant effect on ESSDAI score was seen in the TEARS and TRACTISS trials[8,9]. Whereas a lack of effect in the TRACTISS study can be explained by relatively low baseline ESSDAI scores (mean 5.3±4.7 for the rituximab group), the mean baseline score in the TEARS study was 10±7. Of note, in the TEARS study, the ESSDAI was determined retrospectively, which may influence the accuracy and reliability. Furthermore, in the TEARS study, the prevalence of baseline involvement in the domains that show the highest sensitivity to change, e.g. glandular, constitutional, articular, hematological and biological domains, was 29%, 25%, 48%, 38% and 57%, respectively[8]. These percentages are relatively low in comparison to the study by Moerman et al.[51], in which these domains were active in 70%, 5%, 80%, 55% and 85% of patients, respectively (unpublished data). Meiners et al. and Carubbi et al. also reported a higher rate of involvement of most of these domains at baseline[5,7]. In conclusion, four prospective studies have shown beneficial effects of rituximab on systemic involvement[5,7,50,51]. The lack of effect in recent trials may be explained by low systemic involvement at baseline or heterogeneity in clinical systemic involvement.

Effects on quality of life

Several studies investigated the effect of rituximab treatment on quality-of-life using the 36-Item Short Form Health Survey (SF-36). Effects of rituximab treatment were seen in several studies in different domains of the SF-36, but with a large variability between studies (table 3). Interestingly, vitality often improved by treatment. However, the TEARS and TRACTISS trials did not observe a significant effect of rituximab treatment on SF-36 scores, compared with placebo. This is consistent with findings that subjective symptoms improved only slightly (TEARS) or not at all (TRACTISS) in the rituximab group, as subjective symptoms are strong predictors of health-related quality-of-life in pSS patients[55].

PREDICTORS OF RESPONSE TO RITUXIMAB

As described in the previous section, the efficacy of rituximab varies substantially between studies. Therefore, it is important to detect possible predictors which enable selection of patients that are likely to respond to rituximab treatment. Several predictors of good clinical response to rituximab have, for example, already been identified in RA and SLE. In RA, these factors are RF or anti-CCP positivity, elevated serum IgG, low IFN activity, lower serum levels of BAFF and lower numbers of circulating plasmablasts[56]. Furthermore, the degree of B cell depletion was positively associated with clinical response in both RA and SLE[57,58]. SLE patients with a low-affinity FcγRIIIa genotype have less effective B cell depletion, as antibody-dependent cellular cytotoxicity, mediated by FcγRIIIa-positive effector cells (mostly NK cells), is impaired[59]. This genotype results in lower binding affinity of FcγRIIIa to anti-CD20 antibodies that are bound to the target B cells. Whether this FcγRIIIa genotype is also present in a subgroup of pSS patients is not known.

In pSS, some predictors of response to rituximab were evaluated. Baseline expression of B cell-related transcripts and presence of the IFN signature in blood or minor salivary glands were not associated with clinical response to rituximab in pSS[16,60]. Devauchelle-Pensec et al. did identify some candidate transcripts, but these need further validation[60]. Concerning response biomarkers in serum, lower serum BAFF levels at baseline were associated with clinical response to rituximab in pSS patients, as defined by a ≥30% improvement in at least two items of the SSRI[26]. As mentioned earlier, high BAFF levels may enhance the survival (and prevent the depletion) of autoreactive B-cell clones, residing in glandular tissue and/or bone marrow. Besides lower BAFF levels, responders to rituximab – based on the SSRI- seemed to have lower baseline B cell activity, as reflected by a significantly lower B cell proportion within the glandular infiltrate in the labial salivary glands and lower levels of serum anti-SSA and FLCs[26]. Responders also had a lower focus score (median 0.3) and a lower salivary gland ultrasonography grade at baseline, compared with non-responders[26,43]. Based on these characteristics, responders may have less irreversible gland destruction and respond to rituximab based on SSRI improvement, since VAS dryness scores and UWS are two of the five measures that constitute the SSRI.

Using a different definition of clinical response, i.e., a decrease of ≥3 in the ESSDAI, we have shown that both baseline absolute numbers of B cells and the B cell proportion in parotid gland tissue are higher in responders versus non-responders[25,61]. Explanations for the apparent discrepancy between the study of Cornec et al. and our study have been extensively discussed elsewhere[61,62]. Our findings that high absolute numbers and proportions of B cells in the parotid gland are associated with ESSDAI response suggest that the number of B cells in the target tissue influences systemic disease activity. Likewise, the B cell proportion in the labial gland positively correlates with markers of systemic B-cell hyperactivation[63].

Together, these data indicate that rituximab may be effective in either patients with low salivary gland inflammation, to prevent further glandular damage, or in patients with high numbers of infiltrated B cells and high systemic disease activity, to ameliorate activity in specified ESSDAI domains[61].

WHY DOES THE EFFICACY OF RITUXIMAB VARY BETWEEN STUDIES?

As discussed in the previous paragraphs, results from several trials of rituximab treatment for pSS vary. First, the use of different inclusion criteria, leading to differences in baseline patient characteristics, may explain part of this variation. Since rituximab has shown to -at least- halt further deterioration of glandular function, compared with placebo, treating patients early in the disease process may prevent progression of irreversible damage to the glands. Therefore, the majority of the studies incorporated a limited disease duration (range 2-10 years) as an inclusion criterion, but still there are large differences in disease duration between the study populations. Besides disease duration, patients characteristics such as mean age, IgG levels, and salivary flow also differ among study populations. For example, mean age is ±10 years lower in the studies by Meijer et al. and Carubbi et al., and mean IgG is higher in the study by Meijer et al., compared to other studies[4,7]. In addition, there may be other unspecified patient characteristics that influence treatment response. For example, ±80% of pSS patients show poor correlation between reported ocular dryness symptoms and objective parameters of gland function, caused by either under- or over-reporting of symptoms[48]. The number of patients under- or over-reporting their symptoms included in a trial may influence the results. Moreover, a study by Lendrem et al. identified four phenotypic clusters using hierarchical clustering of patient-reported pain, fatigue, dryness, anxiety and depression, and found significant differences in IgG, lymphocytes, ESR, ESSDAI score, and UWS between groups[64]. Presumably, these groups may show different responses to rituximab treatment.

Another possible cause of discrepancies between studies is the use of (stable) background medication. In the TEARS and TRACTISS studies, respectively 51% and 68% of the patients used either concomitant DMARDs (mostly hydroxychloroquine) or prednisone (table 1). Hydroxychloroquine and prednisone both have significant effects on the immune system, making it more difficult to show additional effects of rituximab.

Differences in statistical analysis may also contribute to the variation in reported outcomes. Several studies use paired tests between baseline and multiple time points, whereas specific methods for longitudinal data analysis are available that increase statistical power and reduce multiple testing problems. Generalized estimating equations (GEE), for example, take into account the fact that repeated measurements within one individual are correlated and GEE is therefore a more powerful tool to detect even small changes over time.

Finally, discrepancy between studies is also caused by the use of different outcome measures. No consensus has been reached about the ideal combination of outcome measures to measure treatment efficacy in pSS. The two large RCTs (TEARS and TRACTISS) have used change in subjective symptoms (VAS scores) as primary outcome measures[8,9]. Subjective symptoms such as fatigue and sicca symptoms account for a great loss in quality of life and are indeed an important target for treatment. However, the sensitivity to change of these outcome measurements has not been validated, and the response goals were set quite high (30mm change in 2 out of 4 VAS scores in TEARS, 30% change of either oral dryness or fatigue VAS score in TRACTISS). These goals may have been too high, considering that the ESSPRI has a minimal clinically important improvement of 1 point (out of 10) or 15% change. Sensitivity to change may be improved by the use of more precise PROs, such as the Patient-Reported Outcomes Measurement Information System (PROMIS), developed by the National Institutes of Health[65]. Importantly, there is a poor correlation between subjective and objective measures of dryness in pSS[48]. Until we are able to understand these discrepancies, subjective and objective measurements of dryness should be equally weighted in the evaluation of treatment efficacy. In line with this notion, Cornec et al. proposed a new data-driven composite outcome which combines objective manifestations and subjective symptoms, the SSRI[47]. This outcome was established by combination of five outcome measures that were improved by rituximab in the TEARS trial. Although the combination of subjective and objective measures as primary outcome is of interest, the SSRI needs to be refined and validated in other clinical studies.

For objective measurement of systemic activity in pSS, introduction of the ESSDAI in 2010 has been a big step forward[66]. Before that, trials did not have a validated tool to assess the effect of rituximab treatment on systemic disease activity. In later trials, most improvement was seen in domains with the highest activity at baseline[5] and a minimal clinically important improvement in ESSDAI of at least three points was determined[67]. Recent trials in pSS have therefore focused on including patients with moderate-to-high ESSDAI scores (≥5), to be able to show an effect on extraglandular manifestations.

Although the ESSDAI has been validated and is now being used in most clinical trials, there are also disadvantages regarding the use of ESSDAI as outcome measure. It is now recognized that not all ESSDAI domains show sensitivity to change[52]. Consequently, even in populations with comparable mean ESSDAI scores, differences in which ESSDAI domains are active at baseline may greatly influence response to rituximab. To prove efficacy of rituximab on systemic disease activity, future trials should therefore include patients with moderate-to-high ESSDAI scores and activity in at least one of the domains that is likely to change (biological, articular, hematological, pulmonary, and glandular domains). Prospective use of specific indices for separate domains, such as the DAS-28 for articular involvement, may provide more detailed information on efficacy. For example, it is difficult to detect moderate changes in

patients with high baseline IgG levels, using the biological domain of the ESSDAI. Additionally, researchers should be aware of the complexity of ESSDAI, which needs to be completed by rheumatologists who are trained and experienced in doing so. In a multi-center setting, this may not always be the case. A more detailed user guide has been published, which may increase the accuracy of the ESSDAI[68]. Considering that rituximab shows effect in several domains of the ESSDAI, patients with high ESSDAI scores may be the target population that we should aim for. Future trials should explore composite endpoints, which include selected domains of the ESSDAI score, besides subjective symptoms and gland function.

CONCLUSIONS AND FUTURE DIRECTIONS

Rituximab shows beneficial effects on B cell activity, glandular morphology, dryness, fatigue and several extraglandular manifestations in pSS patients. Although two large RCTs did not meet their primary endpoint, the sensitivity to change of their subjective endpoints may be limited. Future trials should evaluate clinical and biological predictors of response and explore the use of composite endpoints such as the SSRI. We believe that there is still room for new trials with anti-CD20 biologicals, as well as with other B cell-targeting therapies, such as anti-CD22 or anti-BAFF/Blys antibodies for the treatment of pSS, in well-defined populations with moderate to high ESSDAI scores. At the same time, data on long-term (>1 year) efficacy of rituximab and preventive effects on development of extraglandular manifestations and/or lymphoma are needed and may support the use of rituximab in pSS. The effectiveness of pSS has not been proven for all pSS patients, but in our opinion, rituximab is of great value to treat patients with systemic manifestations of pSS and we should not throw the baby out with the bath water.

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CHAPTER 10

Safety of treatments for primary Sjögren's syndrome

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ABSTRACT

Introduction. Primary Sjögren's syndrome (pSS) is a disabling auto-immune disease, affecting exocrine glands and several organs.

Areas covered. In this review we analyze the safety of therapies used in pSS. Symptomatic treatment is widely applied due to the good supportive effect and good safety profile. Systemic stimulation of tears and saliva can be successful in pSS. However, cumbersome adverse events can influence the tolerability of this therapy. Evidence for the effectiveness of synthetic DMARDs therapies in pSS is limited, while there is a risk of adverse events. Several studies on biologic DMARD treatment of pSS patients have shown promising efficacy and safety results.

Expert opinion. The safety of symptomatic treatment of pSS is very good. However, systemic therapy is necessary to achieve long-term relieve and prevention of organ-damage. Synthetic DMARDs have not shown much efficacy in earlier studies, and their benefits do not weigh up to the possible harms, while biologic DMARDs show promising results regarding efficacy and cause mostly mild adverse events. Many questions remain unanswered regarding safety of DMARDs in pSS. There is a need for well-designed studies, in which safety should be evaluated in a uniform manner to be able to compare the results between studies.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands leading to, among others, sicca symptoms of the eyes and mouth. Several systemic and extraglandular manifestations can develop as well including fatigue, arthritis and involvement of organs such as the skin, lungs and kidneys. Although the pathogenesis of pSS is not fully elucidated, T-cell mediated B-cell hyperactivity is thought to play an important role, as reflected by the presence of autoantibodies, cryoglobulins and hypergammaglobulinemia[1]. pSS is a disabling disease and has a large effect on health related quality of life[2]. Besides symptomatic treatments that improve dryness, no effective treatments have yet been approved for use in pSS. However, treatment with biologic disease modifying antirheumatic drugs (DMARDs) have shown promising outcomes[3].

Evaluation of treatment outcomes in pSS has been challenging in the past, due to the wide range of outcome measurements that were applied, making it difficult to compare studies. Early studies primarily focused on exocrine gland function (saliva, tears) as a primary outcome measurement, whereas later studies focused on fatigue and systemic symptoms. Furthermore, pSS has a very heterogeneous course. Most patients show a chronic progressive decrease in exocrine gland function, until a very low level or no saliva and tear production remains[4]. However, systemic symptoms can present in different patterns, as patients can show chronic involvement (e.g. polyneuropathy) and exacerbations (e.g. polyarthritis)[5,6]. The recent development and validation of disease activity indices, the European League against Rheumatism Sjögren Syndrome Disease Activity Index (ESSDAI) and Patient Reported Index (ESSPRI), have enhanced clinical research as it is now possible to reliably measure changes in disease activity and patient reported complaints[7,8].

When assessing the efficacy of new treatments, also when applying ESSDAI and ESSPRI, it remains important to keep the balance between efficacy and adverse effects in mind. Therefore, the aim of this review is to summarize the safety of treatments currently applied in pSS and to identify in which areas knowledge is still lacking. This review will discuss symptomatic treatment, synthetic DMARDs and biologic DMARDs, with a focus on the treatments that have shown promising results.

SAFETY OF SYMPTOMATIC THERAPIES

Symptomatic treatment of patients with pSS is widely applied due to the non-invasive nature, good supportive effect and good safety profile. Educating the patient with regard to lubricant use, preventive dental care and general personal hygiene, avoiding windy or low-humidity environments and exposure to irritants such as dust and cigarette smoke is

important. Attention should also be paid to several medical conditions and medications which can aggravate sicca symptoms.

Ocular manifestations

First line treatment of ocular sicca symptoms consists of topical treatment with artificial tears, gels and ointments[9]. If the effect of tear replacement is inadequate, topical immunomodulatory agents such as cyclosporine and corticosteroids, and systemic stimulation of tear production can be added to the treatment.

Artificial tears

Substitution therapy, like eye drops, gels and ointments are mainstay of the treatment of sicca symptoms. There are many different types of artificial tears available on the market based on hydroxypropyl methylcellulose, carboxymethylcellulose, hyaluronic acid, polyethylene glycol or propylene glycol as well as gel/lipid formulations, ointments and liposomal sprays. Ophthalmic gels and ointments may be used at night. Highly viscous drops, gels and ointments have longer effect duration, but they may cause visual blurring. In general, if used appropriately, artificial tears substitutes have good safety and tolerability characteristics. The most common adverse event is a temporary burning sensation. Other adverse events include eye redness, discharge, watery eyes, eye pain, foreign body sensation, itching, stinging and blurred vision[10].

Blepharitis may worsen by the use of artificial tears, especially those with high viscosity or those containing preservatives, which also can damage the corneal epithelium and disrupt the tear film[11,12]. The advantage of preservatives is that the drops are available in multidose administration bottles. However, patients can develop an adverse reaction to the preservative. The use of artificial tears containing preservatives should therefore be restricted to three times a day to prevent high concentrations of these substances. Preservative-free artificial teardrops should be used as single dose dispenser, to prevent infection risk, which in turn increases the costs of these substitutes. The choice of artificial tears should be based on individual patient characteristics.

Autologous serum

Autologous serum eye drops might be superior to artificial tear substitutes due to presence of a variety of biological factors, such as Epidermal Growth Factor (EGF), vitamin A, transforming growth factor beta (TGF-β), fibronectin, substance P, insulin-like growth factor 1 (IGF-1) and nerve growth factor (NGF). Moreover, autologous serum eye drops have an osmolarity comparable to natural tears[13].

Although there is some evidence for effectiveness and safety of autologous serum eye drops in pSS patients[14,15], large randomized controlled trials (RCT) are warranted to provide sufficient evidence on superiority of these drops over artificial tears.

The adverse events of autologous serum eye drops are mild and include increased discomfort, slight epitheliopathy, bacterial conjunctivitis and eyelid eczema[14,16,17]. Autologous serum eye drops should be prepared under a strict protocol and in sterile conditions.

Topical cyclosporine A

Topical cyclosporine A (tCsA) 0.05% ophthalmic emulsion is an immunomodulatory agent with the ability to down regulate T-cell proliferation, activity and receptor signal transduction. tCsA has an anti-inflammatory effect due to decreased formation of proinflammatory cytokines. The latter effects of tCsA contribute to the stability of the tear film by interruption of the inflammatory cascade, inhibition of apoptosis and stimulation of production of goblet cells in the corneal epithelium. Goblet cells produce mucin which serves as an interface between hydrophobic corneal epithelium and aqueous tear fluid[18–21].

tCsA is recommended for the treatment of pSS patients with moderate-to-severe inflammation of the cornea[22]. Long-term use of tCsA is well tolerated in pSS patients[23]. A variety of adverse events is reported, including burning and stinging symptoms, foreign body sensation and blurring. These adverse effects resolve with cessation of treatment[23,24]. No systemic side-effects were observed during tCsA treatment. Patients with ocular infections should discontinue tCsA use[23]. Taken together, tCsA is an important tool in the management of ocular manifestations in SS with a good tolerability, no systemic side effects and overall good safety profile. Unfortunately, tCsA is not registered for use in pSS in several countries.

Topical glucocorticoids

Non-preserved glucocorticoid eye drops are used in pSS patients with moderate to severe disease. By reducing the levels of cytokines, such as interleukin-1 and interleukin-8, topical glucocorticoids suppress the inflammatory process. Furthermore, these eye drops reduce the activity level of matrix metalloproteinase's[25]. Although the overall safety of topical glucocorticoids in clinical trials in pSS and keratoconjunctivitis sicca was considered satisfactory, prolonged use of topical glucocorticoids in pSS patients is restricted by their ability to induce glaucoma, cataract, decreased wound healing, increased risk of secondary infections and epithelial defects[26–29]. Therefore, topical glucocorticoids are only recommended for short term use when treatment with artificial tears is insufficient and rapid reduction of inflammation should be achieved.

Topical NSAIDs

Use of NSAID eye drops in pSS was evaluated in a couple of studies[30–32]. Inhibition of prostaglandins and the arachidonic acid cascade by topical NSAIDs can relieve ocular hyperalgesia. However, in patients with corneal problems, a common phenomenon in pSS patients, the use of topical NSAIDs is associated with corneal-scleral melts, perforation, and severe keratopathy[33,34]. Therefore, there is no place for topical NSAID eye drops in the

treatment of pSS patients due to potential corneal complications and inferiority to topical corticosteroids.

Oral manifestations

The treatment of a sensation of a dry mouth (xerostomia) and salivary gland hypofunction (hyposalivation) in pSS patients should be based on the following principles[35]. Stimulate the flow of saliva by gustatory and mechanical stimulation, or systemic stimulation. If the saliva cannot be adequately stimulated, decreased sicca symptoms can be achieved by coating the surfaces of the oral mucosa with saliva replacement therapy. Preserve and protect the teeth and the oral soft tissues with topical fluorides. The mainstay of this therapy is to make it as simple and as safe as possible for the patients, e.g., limit salivary stimulation therapy to gustatory and mechanical stimulation as this is accompanied by fewer side effects than systemic stimulation therapy.

Gustatory and mechanical stimulation

The combination of chewing and taste, as provided by gums, candies and mints, can be very effective in relieving symptoms for patients who have remaining salivary function. Masticatory stimulatory techniques (non-sticky chewing gums) are the easiest to implement and have few adverse events, assuming that they are sugar-free. The same accounts to sugarfree candies, mints etc., preferably with mild acids added with a low risk to harm teeth and oral mucosa, such as malic acid.

Saliva replacement therapy

Artificial saliva (saliva substitutes) is available for the treatment of moderate to severe dry mouth in patients with pSS. A variety of saliva substitutes is available, some are water-based and often short working, others are gels which preferably used when stimulation or frequent moistening is not applicable, e.g. at night. In this respect it also has to be mentioned that many pSS patients use water to moisten their mouth, which can be used freely, but is a worse moistener of the oral mucosa.

When prescribing a saliva substitute, it is important to instruct the patient properly how to use that substitute to get the maximum effect from the therapy, as it is not an exception that use of artificial saliva is not well accepted long-term by many patients, particularly when they have not been instructed how to use them. Moreover, it is very useful to try another type of substitute in a patient when a particular substitute does not sufficiently relieve xerostomia; which substitute is effective in a particular patient is often related to the preference of a patient and is not easy to predict. The safety of saliva substitutes is very good with a very small number of minor adverse events reported[36].

Topical fluorides

Topical fluorides in patients with salivary gland hypofunction are critical to control dental caries[37]. There are different fluoride therapies available, from low-concentration, over-the-counter fluoride rinses, to more potent highly concentrated prescription fluorides (e.g., 1.0% sodium fluoride). Oral health care practitioners may also utilize fluoride varnishes. The dosage chosen and the frequency of application should be based on the severity of the salivary hypofunction and the rate of caries development[37,38]. In addition, particularly in patients with severe oral dryness, non acidic gels and/or solutions should be used, as acidic sodium fluoride gels may induce a more rapid destruction of the teeth, and could cause sensitivity and pain in the gingival and oral mucosa. There is little information on the risk of adverse events in the available studies. Known adverse events of the use of fluorides are fluorosis, tooth staining/discoloration, oral allergic reactions, nausea or vomiting[39].

Systemic stimulation of tears and saliva

Pilocarpine

Pilocarpine is a cholinergic parasympathomimetic agonist with onset of action within 1 hour. It binds to muscarinic-M3 receptors of various exocrine glands to stimulate the secretion function[40]. Contraindications to use pilocarpine are uncontrolled asthma, untreated cardiovascular conditions, angle-closure glaucoma and severe hepatic impairment. Precautions should be made by patients with cholelithiasis or nephrolithiasis. The effect on saliva flow is dose-dependent and time-related with duration of effect of about 3-5 hours.

Several RCTs were conducted to evaluate the efficacy and safety of pilocarpine in SS patients[41–44]. Salivary flow rate and visual analogue scale (VAS) for dry mouth or dry eye were significantly improved in the pilocarpine groups compared to placebo. Pilocarpine showed improvement of VAS for eye dryness and Rose Bengal test compared to artificial tears alone or punctual occlusion intervention. No serious adverse events were reported. The most frequent adverse events were sweating, increased urinary frequency, headache, flu syndrome, nausea, dyspepsia, rhinitis, and dizziness. Adverse effects occurred more often at higher doses. In these studies, 0-13% of patients receiving pilocarpine discontinued treatment due to adverse events versus 0-10% of patients receiving placebo[41–44]. Recently, Kawakita and colleagues demonstrated that lower dose of 2.5 mg pilocarpine three times a day is effective in patients with SS and can diminish the adverse events[45]. Moreover, pilocarpine seems to be safe and effective in juvenile-onset Sjögren's syndrome[46]. Conclusively, pilocarpine can be successfully used in pSS patients, especially in those with sufficient remaining salivary gland function. However, common and cumbersome adverse events can influence the tolerability of this therapy.

Cevimeline

Cevimeline is a parasympathomimetic and muscarinic agonist that, just like pilocarpine, has particular effect on M3 receptors. It stimulates saliva secretion, thereby alleviating dry mouth. Cevimeline has the same contraindication profile as pilocarpine[47]. Several RCTs confirm the effectiveness and favorable safety profile of cevemeline[47–52]. Cevimeline is not yet approved for use in Europe. However, the tolerability of cevemeline seems to be better compared to pilocarpine and is associated with lower discontinuation rates during the treatment[53].

SAFETY OF SYSTEMIC IMMUNOSUPPRESSIVE THERAPIES

Several systemic immunosuppressive therapies have been studied in phase 2 and 3 trials with pSS patients[3,54]. Although no systemic treatments have yet been registered for use in pSS, the number of studies with systemic therapy in pSS is increasing. In the next section, the safety of several systemic therapies that have shown some effect in pSS will be discussed.

An important safety issue during immunosuppressive therapy in rheumatic diseases is the increased risk of serious infections[55,56]. The risk of infection in patients on systemic immunosuppressive therapy is, amongst others, influenced by comorbidity, use of other immunosuppressive medications and age. In pSS, the presence of extraglandular manifestations such as interstitial lung and renal disease may further increase the risk of infection. During treatment with any systemic DMARD, physicians should be aware of this and monitor patients for the development of infections. Careful clinical and laboratory assessments need to be carried out to minimize the potential risk of adverse effects.

Synthetic DMARD therapies

Patients treated with synthetic DMARDs have a rather high risk of developing adverse reactions. The most common adverse effects of synthetic DMARDs are infections, bone marrow toxicity, gastrointestinal symptoms and cardiovascular diseases (e.g., hypertension). Therefore, it should be assessed before onset of therapy whether the benefits of a therapy outweighs its possible adverse effects.

Most synthetic DMARDs have not been shown to be effective in patients with pSS in double blind, randomized clinical trials. Methotrexate, leflunomide and cyclosporine A have shown insufficient efficacy in clinical trials and/or their use was accompanied by unacceptable rates of adverse events[57–61]. As a consequence, most synthetic DMARDs are not used routinely for pSS. In case of severe or life-threatening organ involvement in pSS, however, synthetic DMARDS are frequently prescribed on an empiric basis or on basis of small case series.

Hydroxychloroquine

Based on efficacy experience in systemic lupus erythematosus (SLE) patients, hydroxychloroquine (HCQ) is frequently used in pSS, to treat skin involvement, e.g. purpura associated with hypergammaglobulinemia, myalgia, arthralgia, arthritis and constitutional symptoms like fever and fatigue[62]. HCQ is also effective in prevention of cardiovascular events by reducing levels of total cholesterol, increasing high-density lipoprotein cholesterol and improving the atherogenic index[63]. Moreover, a recent report has shown that HCQ impairs systemic IFNα production in pSS, which is hypothesized to play an important role in the pathogenesis of pSS[64]. It has been shown, however, that administration of HCQ did not resolve sicca signs and symptoms or extraglandular manifestations in pSS patients[65,66]. In both trials in pSS serious adverse events rarely occurred. Gottenberg et al. reported a similar frequency of serious adverse events in the HCQ group in the placebo group during the first 24 weeks. The most common adverse effects of HCQ are skin rash, hyperpigmentation of the skin, temporarily hair loss and blurred vision[62]. Furthermore, bilateral bull's-eye maculopathy is considered a serious adverse effect, resulting in loss of visual acuity, loss of peripheral vision and loss of night vision[67]. Screening for bull's-eye maculopathy should be done at start of HCQ, after five years of treatment and yearly thereafter. The risk of eye toxicity is low, unless the patient suffers from impaired kidney function or is given HCQ in a high dose (dose of >6.5 mg/kg of ideal body weight). Furthermore, as HCQ is not retained in fatty tissues, obese patients can be seriously overdosed when HCQ is dosed on basis of the patients' actual body weight instead of the ideal body weight. Another risk group is elderly patients with retinal and macular diseases. Rare adverse effects include cardiomyopathy, hearing disorders and myopathy[68–70]. HCQ is safe to be used during pregnancy and lactation[71,72]. In conclusion, HCQ has a mild adverse events profile. The efficacy of HCQ in pSS patients needs further evaluation.

Glucocorticoids

Glucocorticoids are used in pSS patients with severe organ complications, like renal involvement and myelitis. The effect of glucocorticoids on sicca symptoms and signs and extraglandular manifestations has not yet been proven in RCTs. Moreover, a prospective cohort study failed to show the effect of glucocorticoids on salivary function tests[73].

Adverse effects of glucocorticoids are common, depending on dose and duration of the therapy. Short term adverse effects include steroid induced diabetes mellitus, hypertension, peptic ulcers of the stomach, electrolyte disturbances, heart failure and mood disorders. Long term complications are susceptibility for infections, osteoporosis, steroid induced myopathy, cataract, glaucoma, Cushing syndrome, thin skin and central adiposity. The use of glucocorticoids should always be combined with prophylaxes for osteoporosis and peptic ulcers.

Cyclophosphamide

Cyclophosphamide is used in the treatment of severe and life-threatening conditions in pSS patients, e.g., severe renal involvement, vasculitis, mononeuritis multiplex, central nervous system involvement and mucosa-associated lymphoid tissue (MALT) lymphoma[54]. However, treatment efficacy regarding these extraglandular manifestations has not been assessed in RCTs and safety data is often not reported in the available pSS studies.

Hemorrhagic cystitis is more frequently seen in patients on oral cyclophosphamide than patients on IV treatment due to higher cumulative dose[74]. Importantly, even in low doses (1-2 mg/kg body weight) administration of cyclophosphamide is accompanied by significant adverse effects, such as infections, pancytopenia, hair loss, sterility, hemorrhagic cystitis, urinary bladder cancer, development of lymphoma and skin malignancies. Thus, frequent clinical and biochemical evaluations of patients treated with cyclophosphamide are mandatory. Mercaptoethane sulfonate has been added to cyclophosphamide treatment in patients with rheumatic diseases to prevent adverse effects, but conclusive evidence of its protective effect is lacking[75].

Azathioprine

Potentially, azathioprine can be useful in pSS patients, analogous to SLE patients. A retrospective case series, showed that azathioprine might be effective for progressive pulmonary involvement in pSS patients[76]. An RCT in pSS patients showed no significant change in clinical, serological or histological disease activity variables[59]. In this study, six of 25 patients, receiving azathioprine, withdrew because of adverse events. Common adverse effects are leucopenia, abnormal liver biochemistry and gastrointestinal symptoms[74]. Prolonged use of azathioprine has been associated with increased risk of skin cancer development[77]. Blood cell counts are recommended for every two weeks during the first 3 months of treatment and every 2-4 months thereafter.

Mycophenolate mofetil

In pSS, mycophenolate mofetil was evaluated in an open-label pilot trial with follow up of 24 weeks. Authors reported improvement of subjective glandular and extraglandular manifestations as well as some laboratory parameters[60]. In addition, mycophenolate mofetil might be effective for progressive interstitial lung disease in pSS patients[78]. No RCTs have yet been performed to confirm these findings.

Mycophenolate mofetil is associated with an increased risk of infections, gastrointestinal symptoms, bone marrow depression, metabolic changes (e.g., hyperlipidemia, hyperglycemia, hyperuricemia) and impairment of kidney and liver function[74].

Biologic DMARD therapies

Several biologic DMARDs have shown promising outcomes in pSS[3]. Unfortunately, for most treatments only short-term safety data from a limited number of patients is available. As some systemic treatments have been used extensively for other indications, such as rheumatoid arthritis (RA), psoriatic arthritis and SLE, long term safety data from worldwide registries are available for these indications. When applicable to pSS, these safety data will be discussed.

An important safety issue during biologic DMARD therapy are systemic infusion related reactions and injection-site reactions. The presence of anti-drug antibodies, raised against these biologics, often related to the non-human origin of the biologic DMARDs, may increase the risk of systemic reactions[79].

The risk of serious infections seems to be higher during biological DMARD therapy than during synthetic DMARD therapy for RA[80]. Therefore, before biological DMARD treatment is started, patients have to be screened for latent or active infections, such as tuberculosis, HIV, and hepatitis B and C. In case of latent infections, adequate prophylactic therapy should be initiated before onset of treatment to prevent reactivation. Furthermore, influenza, pneumococcal and hepatitis B vaccination should be considered before onset of therapy, and life attenuated vaccinations should be avoided during treatment, in accordance with the European League Against Rheumatism (EULAR) guidelines for vaccination in rheumatic diseases[81].

Whether patients treated with biologic DMARDs have an increased risk for development of malignancies is still under discussion. This relationship is confounded by the increased risk of hematological malignancies due to chronic inflammation in rheumatic diseases. Compared to conventional synthetic DMARDs, only patients with RA on TNF inhibitors have shown an increased risk of non-melanoma skin cancer[56]. As there are no indications that rituximab is associated with the occurrence of cancers, the ACR recommends rituximab treatment for RA patients with treated melanoma and lymphoproliferative malignancies as well as treated solid and non-melanoma malignancies less than 5 years ago[82]. Insufficient long-term data is available to be able to draw conclusions about an increased risk for malignancies during and after biologic immunosuppressive treatment in pSS. When patients with prior malignancies are treated with biologicals, rheumatologists should therefore be aware of the possibility of recurrence of these malignancies.

Rituximab

Rituximab therapy (anti-CD20), counteracting the B-cell hyperactivity in pSS, is widely used in the treatment of pSS-related lymphoma, often in combination with cyclophosphamide and prednisone[83,84]. B-cell depleting therapy is also regularly used off-label for pSS patients with severe extraglandular manifestations. In several populations with moderate to high

Table 1. Safety results of rituximab therapy in pSS

Author	Design	Follow up (weeks)	Patients		Serious AEs[a]		Infectious serious AEs[a]		Infusion reaction[a,d]		Serum sickness-like AE[a]		Discontinued due to AEs[a]	
			T	P	T	P	T	P	T	P	T	P	T	P
Meijer [85]	RCT	48	20	10	1 (5)	0 (0)	0 (0)	0 (0)	4 (20)	0 (0)	1 (5)	0 (0)	1 (5)	0 (0)
Devauchelle [94]	RCT	24	63	57	13 (21)	8 (14)	2 (3)	5 (9)	15 (24)	12 (21)	0 (0)[b]	0 (0)	5 (8)	1 (2)
Dass [103]	RCT pilot	52	8	9	2 (25)	0 (0)	1 (13)	0 (0)	2 (25)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)
Gottenberg [87]	Registry	152[c]	78	0	10 (13)		3 (4)		4 (5)		1 (1)		6 (8)	
Gottenberg [104]	Retrospective	36[c]	6	0	0 (0)		0 (0)		1 (17)		1 (17)		1 (17)	
Seror [88]	Retrospective	63[c]	16	0	1 (6)		0 (0)		1 (6)		2 (13)		NR	
Carubbi [86]	Open label	120	19	22[e]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pijpe [95]	Open label	12	15	0	3 (20)		0 (0)		2 (13)		3 (20)		3 (20)	
Devauchelle [102]	Open label	36	16	0	2 (12)		0 (0)		2 (12)		1 (6)		0 (0)	
St Clair [98]	Open label	52	12	0	2 (17)		0 (0)		NR		0 (0)		0 (0)	

Only articles with complete safety information and populations that were not reported in other studies were included in this table. [a]Number of patients who experienced this type of event (percentage). [b]2 patients with treatment-related purpura were reported, but human anti-chimeric-antibodies were not measured and the authors did not classify these events as serum sickness-like reactions. [c]Median follow up time. [d]Infusion reactions include non-serious as well as serious infusion reactions and exclude serum sickness-like reactions. [e]Control group received one or more synthetic DMARDs instead of placebo. AE: adverse event; P: placebo group; NR: Not reported; T: treatment group.

systemic disease activity, rituximab has shown a beneficial effect on systemic disease activity and ESSDAI scores[85–92]. Unfortunately, the effect on ESSDAI was not confirmed in two recent large RCTs[93,94]. Regarding glandular manifestations, rituximab has been reported to improve salivary flow in patients with enough residual gland function[85,86,95,96]. Other studies reported stabilization of exocrine gland function during rituximab treatment, whereas salivary gland function deteriorated in placebo patients[91,93,94,97]. In most studies, patient-reported symptoms such as fatigue and dryness were improved by rituximab treatment[86,89,91,95–99], but in recent RCTs this effect was smaller or did not differ significantly from the placebo group[93,94]. The differences between the results of these trials are likely explained by differences in the baseline characteristics of the study populations. Safety results of rituximab trials in pSS patients are summarized in table 1.

In a Cochrane review of the safety profile of biologics during treatment of several diseases, rituximab showed the lowest odds for serious infections compared to control treatment[100]. The infectious side effects of rituximab also seem to be mild in pSS, as in RCTs of rituximab treatment in pSS infection rates were comparable between treatment and placebo groups[85,94]. These results were confirmed by a prospective registry of rituximab treatment in systemic autoimmune diseases (AIR registry), in which 78 pSS patient were included with a median follow up of 34.9 months. According to the AIR registry, the rate of serious infections during rituximab treatment was lower in pSS than in SLE (1.3/100 patient years versus 6.6/100 patient years, respectively)[87,101].

Despite pretreatment with IV corticosteroids, antihistamines, and paracetamol, and cotreatment with oral corticosteroids in some studies, infusion reactions such as fever, rigors and urticaria occur more common during rituximab treatment than during placebo treatment, occurring in 8-25% of patients[85,94,95,102,103]. Specifically, Devauchelle et al. reported a higher incidence of respiratory disorders within 24 hours of injection with rituximab compared to placebo[94]. Gottenberg et al. reported serious infusion reactions in 6.4% of pSS patients in the AIR registry[87]. One study did not report any infusion reactions during long term treatment with rituximab in 19 patients[86]. However, as no safety analysis plan was included in the methods of this study, it is unclear how infusion reactions were defined.

Patients with active pSS seem to develop serum sickness-like reactions after rituximab treatment more often than patients with other rheumatologic diseases[88,95,103,104]. Serum sickness-like reactions were generally characterized by fever, purpura, arthralgia, myalgia, and sometimes low complement levels and proteinuria. Serum sickness-like reactions were often associated with the development of human anti-chimeric antibodies (HACA), which supports the diagnosis of true serum sickness[88,95]. However, the presence of HACAs was not always reported and in some cases a delayed infusion reaction may have been falsely interpreted as serum sickness. The presence of hypergammaglobulinemia in pSS might explain the higher

prevalence of serum sickness in pSS, as it might increase the chance of immune complex deposition. In more recent studies, the prevalence of serum sickness-like reactions was low, probably due to adequate pre-treatment with high dose corticosteroids[85–87,94].

Progressive multifocal leukoencephalopathy (PML) due to JC virus replication in the brain is a rare but life-threatening condition associated with rituximab treatment[105]. Rheumatologists should be aware of the risk of developing PML during B cell depleting treatment of pSS patients, as a case of PML has been reported in a pSS patient[106].

In RA, vaccination response is decreased by B-cell depleting therapy, which will probably also be the case during rituximab therapy in pSS[107]. In addition, an open label study which evaluated safety of rituximab in 12 patients with pSS reported an exaggerated adverse reaction to pneumococcal vaccination in 3 out of 8 patients who received this vaccination[98]. This might be an extra argument to administer pneumococcal and influenza vaccination prior to onset of rituximab treatment in pSS, although these results have not been confirmed by larger studies of vaccination in pSS.

In conclusion, rituximab has shown promising results regarding efficacy in populations with moderate to high systemic disease activity. Rituximab is generally safe in pSS, when adequate co-treatment is given and monitoring for infusion reactions takes place. Although patients should be monitored for development of infusion reactions and serum sickness-like disease, these adverse reactions are usually fully reversible. Long term safety effects of rituximab in pSS are still unclear and should be recorded in prospective registries.

Epratuzumab

Epratuzumab (anti-CD22) targets B-cells. An advantage of epratuzumab above rituximab is that it is fully humanized. An open label study of epratuzumab in 16 pSS patients showed a beneficial clinical response in 67% of patients, which was defined as a 20% improvement in 2 out of four domains: Schirmer score, unstimulated salivary flow, VAS fatigue and erythrocyte sedimentation rate and/or serum immunoglobulin G (IgG)[108]. Two patients discontinued treatment due to infusion reaction and one serious infectious adverse event occurred. Three patients developed a low level of anti-epratuzumab antibodies, but these were not associated with infusion reactions.

In phase 2 trials of epratuzumab in SLE patients, rates of (serious) adverse events and infusion reactions were similar between treatment and placebo arms[109]. In other words, epratuzumab has a good safety profile in SLE in a dose of 360 mg/m^2, but the tolerability of epratuzumab in pSS has to be confirmed in larger RCTs.

Belimumab

B-cell activating factor (BAFF) blockade by belimumab, a human monoclonal antibody, inhibits survival of autoreactive B-cells and could therefore be beneficial in diseases characterized by B-cell hyperactivity. In SLE, a significant greater proportion of responders according to the SLE Responder Index in belimumab plus standard therapy versus placebo plus standard therapy has been reported[110,111]. Therefore, belimumab is already registered for use in SLE. The safety of belimumab in SLE is favorable, with low rates of infection, malignancy and infusion reactions[110,111]. A 7 year follow up study of belimumab treatment in SLE did not report any additional safety concerns and showed a stable or decreasing rate of AEs and infections during follow up[112].

One open label trial has been performed in 30 pSS patients, showing a beneficial response in 60% of patients[113]. Although a significant improvement was shown in ESSDAI and ESSPRI scores, the minimal clinically important improvement was not reached for both indices[8]. One serious infection led to discontinuation of treatment, and in another patient breast cancer was diagnosed three months after the last infusion. No infusion reactions were reported in the pSS open label trial. The percentage of patients experiencing adverse events was lower in the open label pSS trial than in SLE trials (54% of pSS patients versus 93% of SLE patients), which might be due to the larger percentage of SLE patients receiving co-treatment with immunosuppressants[113,114]. A 52-week extension study of belimumab treatment in 19 pSS patients did not show any additional serious adverse events or infusion reactions[115].

In summary, belimumab is effective and well tolerated in SLE. The efficacy and safety of belimumab in pSS, although favorable in the open label trial, should be further investigated in RCTs.

Abatacept

pSS is considered to be a B-cell hyperactivity mediated disease, but co-stimulation by T-cells is needed for inducing and maintaining B-cell activation. Thus, blockade of T-cell mediated B-cell hyperactivity is an interesting approach that has to be considered in pSS treatment too. Abatacept is a fully human fusion molecule of the Fc region of IgG with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which blocks co-stimulation of B-cells by T-cells. Abatacept is currently registered for use in RA and Juvenile Idiopathic Arthritis (JIA) and has a low odds ratio for serious adverse events, serious infections and withdrawals due to adverse events compared to other biologics[100]. Due to the molecular structure of abatacept, its immunogenicity is very low[116,117]. Subcutaneous abatacept has a comparable safety profile as IV abatacept in RA patients[117].

Two small open label trials of IV abatacept treatment in pSS have shown that abatacept decreases ESSDAI, ESSPRI, IgG and rheumatoid factor, improves fatigue and decreases

glandular inflammation. In the study by Meiners et al[118], mild to moderate infusion reactions occurred in 40% of patients <1 hour of infusion (mostly hypotension and dizziness) and infections occurred in 66% of patients (mostly mild upper respiratory tract infections). Adler et al did not include a safety analysis plan in their methods, but did report that 27% of the patients experienced an adverse event, of which one was infectious (diverticulitis)[119].

In conclusion, infectious adverse events and mild infusion reactions are relatively common during abatacept treatment in pSS. However, no serious adverse events or discontinuations were reported and in general, abatacept has a good safety profile. Subcutaneous abatacept is currently studied in an RCT of 88 pSS patients (NCT02067910).

Anakinra

Anakinra is a recombinant IL-1 receptor antagonist preventing activity of IL-1α and IL-1β. Anakinra is used to treat RA, gout and JIA, among others. In RA, anakinra does not cause a significantly higher number of withdrawals, deaths, adverse events or infections compared to placebo groups, but it does cause a higher prevalence of injection site reactions[120]. An advantage of anakinra with regard to safety is the short half-life of 6 hours, which allows for prompt discontinuation in the case of adverse events.

Because anakinra has shown a beneficial effect on fatigue in RA, the effect of anakinra in pSS on fatigue was studied in an RCT of 26 patients[121]. Anakinra indeed reduced fatigue, but the study was underpowered for the primary outcome measurement and treatment duration was only 4 weeks. As expected, anakinra caused injection site reactions in a large proportion of pSS patients (54%)[121,122]. Due to the small sample size and short follow up, no definitive conclusions can be made regarding the efficacy and safety of anakinra in pSS. Further study is needed.

IVIG

Intravenous immunoglobulin G (IVIG) is applied as substitution therapy in immunodeficiency syndromes, and as immunomodulating therapy in idiopathic thrombocytopenic purpura and several neurological auto-inflammatory disorders. Furthermore, it has been shown that IVIG decreases disease activity in SLE[123]. Possible severe side effects of IVIG treatment include renal failure, trombo-embolic events and aseptic meningitis, which may be prevented by a slow infusion rate and pre-hydration[124]. A retrospective study in 19 pSS patients with peripheral neuropathy reported a beneficial effect of monthly courses of IVIG on SS-associated sensorimotor neuropathy and non-ataxic sensory neuropathy[125]. Tolerance of IVIG was good. During the median treatment duration of 7 months, only one withdrawal due to an adverse event (nausea) was reported and no serious adverse events occurred. Case reports have also suggested efficacy of IVIG in SS-associated thrombocytopenia, central nervous system involvement and congenital heart block but no safety analysis was included[126–128]. In summary,

IVIG is a promising treatment for certain systemic manifestations of pSS and seems to be well tolerated, but should be further evaluated in prospective trials.

Baminercept

Baminercept is an inhibitor of the lymphotoxin-β pathway. Although baminercept treatment did reduce the IFN signature in RA patients, baminercept treatment showed disappointing clinical results[129]. As the lymphotoxin pathway might play an important role in lymphoid tissue organization and chronic inflammation in pSS, baminercept was recently retested in a RCT in 52 pSS patients[130]. Unfortunately, baminercept again did not improve exocrine gland function, fatigue, pain or sicca symptoms. The ESSDAI score was slightly improved in the baminercept group, but the mean ESSDAI change from baseline of 1.6 points is below the minimally clinical important improvement of 3 points[8]. Furthermore, transaminase abnormalities occurred more often in the baminercept group, and 7 serious adverse events occurred, including two patients with grade 3 hepatic injury. Therefore, the benefits of baminercept treatment in pSS do not seem to outweigh possible safety concerns.

Anti-TNF

Two anti-TNF biologic DMARDs have been studied in pSS. In a pilot RCT by Sankar et al., 14 pSS patients were treated with etanercept and 14 patients with placebo[131]. The effect of infliximab on pSS was studied in an RCT of 103 patients[132]. Unfortunately, both trials did not show a significant difference between the study drug and placebo treatment. The number of treatment discontinuations due to adverse events was somewhat higher in the anti-TNF groups than in the placebo groups. Interestingly, in the study by Mariette et al., serum immunoglobulin levels were significantly increased during infliximab treatment[132]. Impaired control of the IFNα pathway and subsequent BAFF overexpression by TNF inhibition might explain the inefficacy of this group of biologic DMARDs in pSS[133].

EXPERT OPINION

Symptomatic treatment, preventive measures and patient's education are of great importance in the management of pSS patients in daily practice and were for decades the only treatment modality to reduce SS-related complaints. However, although symptomatic treatment is safe and has little adverse events, commonly only short-term symptomatic relief is achieved and this treatment does not protect patients from persistent disease activity or organ damage. Therefore, there is a need for registration of systemic therapies for the treatment of pSS.

There is limited evidence in the available literature for effectiveness of systemic conventional DMARDs therapy in pSS patients. Furthermore, the knowledge about adverse events and drug toxicity of conventional DMARDs in pSS is limited and often based on expert opinion.

Toxicity of conventional DMARDs in pSS patients does not appear to be different compared to patients with other auto-immune diseases for which these drugs are used. Based on the weak evidence of efficacy and high rate of adverse events of conventional DMARDs, these agents should not be used in pSS routinely. Importantly, RCT's on conventional DMARDs were performed in small groups, with heterogeneous patient populations, lack of uniform endpoints and adequate measure instruments. There is a great need for well-designed and large RCT's to assess the value of conventional DMARDs in pSS with regard to glandular and extraglandular manifestations, patient-reported and systemic disease activity and organ-specific outcomes. Safety issues should take an important place in the analysis of these RCT's.

Recent years have shown a fast development regarding biologic systemic therapies for pSS. Several studies on biologic DMARD treatment of pSS patients have shown promising efficacy and safety results, especially in populations with high baseline systemic disease activity. In summary, the most frequent adverse events during biologic therapy in pSS patients are infusion and injection reactions and infections, as in other rheumatologic diseases. The risk of infection does not seem to be high in pSS, in fact it is often comparable to the infection risk in patients on placebo, patients with pSS have an inherent increased risk for developing infections and thus appropriate preventative actions should be taken. Infusion reactions occur most often when chimeric DMARDs are used such as rituximab, necessitating pre- and co-treatment with corticosteroids and antihistamines.

As most biologic DMARDs have only been evaluated in phase 2 trials so far, only limited safety data is available specifically for pSS, making it necessary to deduce information from safety analyses of biologic therapy for other indications. However, patients with other rheumatologic diseases more often use concomitant immunosuppressive therapy than patients with pSS, which may influence the risk of infection and other adverse events. Furthermore, patients with pSS might respond differently to certain medications than patients with other rheumatic diseases. An example of this is the increased risk of serum-sickness like reactions seen during some studies of rituximab therapy for pSS[88,95,103]. Therefore, safety analyses should be performed specifically in pSS patients.

This review of the literature taught us that many questions regarding drug safety in pSS remain unanswered. Larger numbers of treated patients, with a longer follow-up are needed to investigate serious adverse events with a low prevalence. Further study is needed to determine the specific dosage of systemic drugs for pSS in which the benefits/harms ratio is maximal. Furthermore, for most drugs the long-term safety is still largely unknown. To answer these questions, larger RCTs are needed and off-label treatment of pSS patients should be registered in cohort studies.

New outcome parameters such as the ESSDAI and ESSPRI, with a stronger sensitivity to change, have made it easier to monitor the efficacy of DMARDs in pSS treatment. Importantly, these indices have made it possible to compare the efficacy of different therapies. Unfortunately, for safety analyses, a multitude of methods is still used in various trials, which makes it difficult to directly compare the results of different studies and drugs. For an example, authors might use different definitions of adverse events, but do not always report which definition they used. Furthermore, it should be clear if adverse events are investigated only by open-ended questioning or by specific screening for certain adverse events (e.g., by asking about it or by laboratory analysis). In addition, there are large differences in the data that authors choose to report. Some authors only report serious adverse events, or only adverse events of a certain type, while others provide a complete list of coded adverse events. Therefore, we urge authors of future therapeutic studies in pSS to follow the extension of the Consolidated Standards of Reporting Trials (CONSORT) statement, which describes guidelines for the reporting of harms in randomized trials[134].

In conclusion, the available data suggests that the safety of symptomatic treatment is very good and symptomatic treatment should be applied in all pSS patients. However, systemic therapy is necessary to achieve long-term relieve and prevention of organ-damage and exocrine gland dysfunction. Therefore, further evaluation of the effectiveness and adverse events of systemic DMARDs in pSS is important. Conventional DMARDs have not shown much efficacy in earlier studies, and their benefits do not seem to weigh up to the risk of adverse events. However, methodological problems may have influenced these results. As biologic DMARDs cause mostly mild adverse events in pSS, and show promising results regarding efficacy, the benefits of biologic DMARD therapy seem to outweigh possible safety concerns.

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CHAPTER 11

General discussion

Primary Sjögren's syndrome (pSS) is a multifaceted disease. The pathogenesis of pSS is complex, involving the glandular epithelium, activation of the interferon system, and T-cell mediated B-cell hyperactivity. While understanding of the pathogenesis of pSS has significantly increased, many questions remain unanswered. For example, although vaginal dryness is a major symptom in women with pSS, it is unknown what causes this symptom. Given the complexity and heterogeneity of the disease, it is uncertain which inclusion criteria and endpoints should be used in pSS trials. The development of the international consensus American College of Rheumatology-European League against Rheumatism (ACR-EULAR) classification criteria has been a step forward, but these criteria could be further improved by investigating the value of new diagnostic tools. Better understanding of different phenotypes within the spectrum of pSS may also improve the selection of patients for clinical trials. Due to the development of promising new therapeutic targets, in recent years many therapeutic trials have been performed in pSS patients. However, the results of different trials are often inconsistent and/or negative, and treatment options for pSS remain limited.

How does pSS affect vaginal lubrication and sexual function?

Sexual dysfunction is an important but under-recognised aspect of pSS. Patients with rheumatologic disorders often experience several physical and psychological symptoms that may affect their sexual ability. This is reflected by a high prevalence of sexual dysfunction, which might influence quality of life[1]. In pSS, vaginal dryness and pain during intercourse are common symptoms[2–8], which form an additional barrier to enjoying sexual activity. In **chapter 2**, we showed that sexual function as measured with the Female Sexual Function Index (FSFI) in women with pSS was indeed impaired, when compared to control individuals, and that patients with pSS were less often sexually active[9]. Several authors have now confirmed the impaired sexual function in pSS[10–12]. Of the six domains of the FSFI, lubrication was most evidently impaired, showing the impact of pSS on vaginal lubrication[9]. pSS patients experienced an increased level of distress regarding their sexual life, and sexual dysfunction was associated with anxiety and depression. Despite these findings, the majority of the patients with sexual dysfunction reported that they rarely discuss sexual problems with their rheumatologists. Rheumatologists should therefore actively discuss this topic with pSS patients and refer patients to a sexologist when necessary.

Studies evaluating vaginal health of women with pSS show conflicting results. Two studies found erythema of the vaginal epithelium in pSS[13,14], while others did not find any macroscopic changes to the vagina and cervix[2,11]. Only four previous studies actually assessed histological changes in the vulva, vagina or cervix of patients with pSS which may explain vaginal dryness. The first study looked at cervical biopsies in women with pSS, and observed chronic cervicitis in 48% of premenopausal and 33% of postmenopausal pSS patients[14]. However, no controls were included in this study. Two studies found peri-epithelial lymphocytic infiltrates in the underlying stroma of the vaginal epithelium of pSS patients[4,7]. A more recent study detected

lymphocytic infiltration of the vulvar epithelium in patients with pSS, but did not see any differences between pSS patients and non-SS sicca symptoms[15]. However, in this last study, only 33% of included pSS patients were SSA positive, while the proportion of patients with positive biopsies was not reported, which raises doubts about whether the study population was actually representative of pSS. Further, the authors used semi-quantitative scoring to determine the amount of infiltration, which may not be sensitive enough to detect small differences.

To address the pathogenesis of vaginal dryness in pSS, **chapter 3** describes the first quantitative analysis of immunological and histopathological markers in the vagina and cervix of premenopausal women with pSS[16]. Despite the small number of included patients and control individuals, we found significant increases in number of infiltrating T-cells in vaginal biopsies of pSS patients. Lymphocytic infiltrates showed a peri-epithelial localization and aggregates in dermal papillae, similar to the lymphocytic infiltrates in the vagina and vulva of pSS patients that were described in other studies[4,7,15].

Previous studies have shown that T-cells and antigen presenting cells are most prevalent in the cervix of healthy women, to maintain immunity to vaginal pathogens[17,18]. Indeed, in our study lymphocytic infiltration was present in the endocervix of women with pSS as well as in controls. However, we did find an increase in the number of B-cells in the endocervix of women with pSS, while B-cells are rare in the female reproductive tracts of healthy women[17,18], possibly reflecting the B-cell hyperactivity in pSS.

Vaginal fluid mostly consists of transudate formed by ultrafiltration of plasma from the capillaries in the vaginal walls, and mucous secretions from the cervical columnar epithelium, of which amounts vary throughout the menstrual cycle. During intercourse, the majority of the fluid in the vagina consists of transudate, due to dilation of the vaginal arteries in response to release of neural vasoactive intestinal peptide[19]. Interestingly, in the study described in **chapter 3**, we found a decrease in the number of smooth muscle cells in the vagina, which may indicate damage to vascular smooth muscle cells, or a decrease in the number of arterioles[16]. We further found an increase of the interferon-induced chemokine CXCL10 in the endocervical swab samples. The decrease in vaginal smooth muscle cells might be a sign of endothelial damage and vascular dysfunction, which were previously described in pSS[20–22]. Endothelial damage and vascular dysfunction in pSS might be mediated by the interferon pathway, as was shown in systemic lupus erythematosus (SLE)[23–25]. An association of sexual dysfunction with endothelial dysfunction and vascular remodelling secondary to chronic immune system activation has also been described in metabolic disorders such as hypertension, obesity and diabetes[26]. We therefore propose that vaginal dryness in pSS is caused by vascular dysfunction, possibly induced by interferon induced pathways.

Considering the involvement of exocrine glands in pSS, previous authors have proposed that vaginal dryness may be caused by inflammation of vaginal glands[13,27]. Whether these glands are affected by pSS has never actually been investigated. However, the vestibular glands (Bartholin's glands) only provide a small contribution to lubrication of the vestibule of the vagina[19], and the para-urethral glands (Skene's glands) most likely only secrete a small amount of fluid during orgasm[28]. Therefore, it seems unlikely that involvement of these glands would cause significant intra-vaginal dryness.

It has also been proposed that vaginal dryness in pSS results from vaginal atrophy, due to oestrogen deficiency[29]. The role of gonadal hormones in the development pSS may be reflected by the increased incidence of pSS in women[30], increased risk of pSS during treatment with aromatose inhibitors[31] and association of pSS with a reduced cumulative lifetime exposure to oestrogen[32]. Oestrogen deficiency probably contributes to vaginal dryness in postmenopausal women with pSS, as the prevalence of vaginal dryness in pSS increases after menopause[2]. However, plasma levels of estrogens are not reduced in pSS[33,34], and there are several clues that in premenopausal women with pSS, oestrogen deficiency is not a major factor in the aetiology of vaginal dryness. First, symptoms of vaginal dryness in pSS often already occur before menopause[2,6,35]. In line with this notion, in **chapter 2** a subgroup analysis showed that pre-menopausal patients also experience significantly impaired sexual function compared to controls[9]. Second, in the study described in **chapter 3**, we did not find any signs of vaginal atrophy in premenopausal women with pSS who have symptoms of vaginal dryness: epithelial thickness was not decreased and vaginal pH was not increased[16]. Third, in **chapter 4** we did not find any changes to the microbiota composition of women with pSS[36]. In postmenopausal women without pSS, changes are seen in the vaginal microbiome compared to premenopausal women[37]. If vaginal dryness in pre-menopausal pSS was caused by oestrogen deficiency, we would expect to find similar changes in the microbiome as seen in postmenopausal women.

In our experience, pSS patient with vaginal dryness often benefit from the use of lubricants during intercourse. Fatty ointments or products containing hyaluronic acid can be used for relieve of daily discomfort due to vaginal dryness. However, these products only give temporary relieve. If vaginal dryness in pSS is indeed caused by vascular dysfunction, secondary to chronic immune system activation, immunosuppressive treatments may improve this symptom. Although we did not find an effect of abatacept treatment on patient-reported vaginal dryness in **chapter 8**, we did find a beneficial effect of abatacept on sexual function (FSFI)[38]. Perhaps vaginal dryness as measured with a numeric rating scale of 0-10 does not have enough sensitivity to change to detect minor improvements, or does not correspond to objective improvement in vaginal lubrication. Alternatively, sexual function may also have been improved due to amelioration of other symptoms which affect sexual function, such as fatigue and pain.

Considering the small sample size of the study population described in chapter 3 and chapter 4, larger studies are needed to confirm our results, ideally using objective measurements of vaginal lubrication. Although no methods exist to robustly measure vaginal lubrication, blood flow in the vaginal epithelium can be measured by vaginal photoplethysmography[39]. Using this method, it would be interesting to assess whether vaginal blood flow is associated with the number of smooth muscle cells in the vagina and with interferon activation, and whether vaginal blood flow is objectively improved by immunosuppressive treatment. To further explore the possible role of vascular endothelial dysfunction, it would be interesting to evaluate whether vaginal dryness is associated with Raynaud's phenomenon and other extraglandular characteristics of pSS. Furthermore, future studies should also evaluate whether sexual dysfunction also occurs in male patients with pSS, as vascular dysfunction may cause erectile dysfunction.

How should we classify and stratify pSS patients?

For many years, pSS had more proposed classification criteria sets than any other rheumatologic condition[40]. Due to the lack of a gold standard for diagnosis of pSS, diagnosis is based on expert opinion. Consensus regarding the classification criteria for pSS is therefore necessary to include homogenous study populations in clinical trials. The development of the ACR-EULAR classification criteria for pSS, using validated methods recommended by the ACR and EULAR, has therefore been an important step forward in pSS research[41,42]. The ACR-EULAR criteria use a weighted scoring system. Three points are assigned for a focus score ≥1 or presence of anti-SSA antibodies, and one point for decreased unstimulated whole salivary flow, decreased Schirmer's test, or increased ocular staining score. Patients with a score of ≥4 are classified as pSS.

The study described in **chapter 5** confirmed the excellent sensitivity of the ACR-EULAR criteria to classify patients as pSS, which was 97% in our cohort, similar to the sensitivity of 96% in the original validation cohort[43]. Using labial gland biopsies, the specificity of the ACR-EULAR criteria in our cohort (83%) was lower than in the original cohort (95%). A retrospective Japanese cohort found an even lower specificity (77%)[44]. The discrepancy with specificity in the original validation cohort may be caused by differences in opinion between the experts defining the gold standard in these cohorts. In other words, some of the patients who were classified as pSS by the experts of the original validation cohort, would be classified as non-pSS by Dutch and Japanese experts.

We found that patients with ACR-EULAR scores of 4-6 were most likely to be misclassified by the ACR-EULAR criteria. Patients who were classified as pSS by the criteria but classified as non-pSS by the experts often had either a positive biopsy or presence of SSA antibodies, combined with a decreased Schirmer's test and/or unstimulated whole salivary flow (UWS). This is important to keep in mind, as in daily clinical practice classification criteria are often

used as diagnostic criteria, and the use of the ACR-EULAR criteria may lead to some false-positive diagnosis. To reduce the risk of misdiagnosing a patient as pSS, we advise to do a complete work-up of all patients suspected of pSS. Furthermore, histological analysis of the salivary glands should not only be based on the focus score, as the focus score may be false positive. Lymphocytic periductal infiltrates may also be present in elderly patients without pSS, or arise as a result of mechanical irritation (e.g. chewing), infection, irradiation or ischemia[45]. Presence of lymphoepithelial lesions and an increased ratio of IgM/IgG producing plasma cells are more specific for pSS.

The ACR-EULAR criteria include focus score in labial gland biopsies as an item, as only labial gland biopsies were used in the cohort on which the criteria were based[41,42]. The validity of the ACR-EULAR criteria when using parotid gland biopsies had not been assessed in the original validation cohort. Parotid gland biopsies have the advantage that repeated biopsies are possible, and MALT lymphoma's, which are often found in the parotid glands of pSS patients, can coincidentally be detected with parotid gland biopsies[46]. Furthermore, no permanent complications have so far been reported in the literature, in contrast to labial gland biopsies which may cause permanent loss of sensation in the lip. In the cohort described in **chapter 5** labial as well as parotid gland biopsies were simultaneously collected from most patients. When parotid gland biopsies were used as an item instead of labial gland biopsies, the overall accuracy of the ACR-EULAR criteria remained equal, but lower sensitivity (91%) and higher specificity (92%) were found compared to when labial gland biopsies were used (sensitivity 97%, specificity 83%)[43]. Thus, patients are less often falsely classified as pSS when using parotid gland biopsies instead of labial gland biopsies for classification according to the ACR EULAR criteria.

Due to the lack of a gold standard for diagnosis, the development of new criteria will always be influenced by the thought of existing popular criteria sets. When using expert opinion as gold standard, the experts may (subconsciously) classify the patients according to the AECG criteria. Therefore, it is not surprising that the ACR-EULAR criteria showed very high agreement with the AECG criteria in our cohort (98%)[43] and in a French cohort (96%)[47]. This may raise the question whether development of new criteria was actually necessary. However, the ACR-EULAR criteria could be considered as an updated version of the AECG criteria. The ocular surface staining, as measured by the Ocular Staining Score (OSS), was added as a separate item, in contrast to the AECG which combined Schirmer's test and the van Bijsterveld score in one item[48]. The exclusion criteria were renewed, leaving out lymphoma, as MALT and other types of lymphoma's are common in pSS, and including IgG4 disease which may mimic pSS. Furthermore, sialography and salivary gland scintigraphy were excluded as these diagnostic techniques are rarely used any more.

Although the ACR-EULAR classification criteria are now widely used, many researchers support the idea that salivary gland ultrasound (SGUS) might be a valuable addition to the

classification criteria for pSS, considering its good diagnostic properties[40,49–52]. Previous studies have shown that the validity of the AECG criteria and proposed ACR criteria was improved by addition of SGUS[53,54]. Four studies, including the one described in **chapter 6**, have so far evaluated the addition of SGUS to the ACR-EULAR criteria[47,55–57]. Takagi et al.[56] found that SGUS improved both sensitivity and specificity, using an SGUS scoring system developed by them, with a weight of 3 for SGUS, and cut-off for the ACR-EULAR score of ≥5. However, complete data regarding the ACR-EULAR criteria was only available in a small subset of their study population. The other three studies used simpler scoring systems, in which homogeneity or presence of hypoechogenic areas was scored on a scale of 0 to 3 or 4. Le Goff et al.[47] arbitrarily added SGUS to the ACR-EULAR criteria with a weight of 1, keeping the cut-off of ≥4 for classification as pSS the same. The study described in **chapter 6**[57], as well as a study by Jousse-Joulin et al.[55], confirmed that the optimal weight of SGUS when added to the ACR-EULAR criteria was indeed 1 and that the optimal cut-off remained ≥4. In all three studies, sensitivity was slightly improved with little loss of specificity.

Importantly, in **chapter 6** we also found that SGUS, with a weight of 1, can replace Schirmer's test, unstimulated whole saliva and ocular staining score, without decreasing the validity of the ACR-EULAR criteria[57]. Allowing clinicians to replace one of these tests with SGUS would increase the feasibility of the ACR-EULAR criteria. When replacing the biopsy or anti-SSA positivity with SGUS, the sensitivity of the criteria was substantially decreased. As a combination of a positive SGUS and presence of SSA antibodies has a high positive predictive value (97%) for classification as pSS[58], determination of SSA antibodies and SGUS evaluation could be the first step in a classification work-up. When one of these tests shows negative results, the next step should be a salivary gland biopsy.

A question that remains unanswered is whether patients who reach a score of 4 based on positivity of all minor items (UWS, OSS, Schirmer's test and SGUS), but with a focus score ≤1 and absence of anti-SSA antibodies, should be classified as pSS. Only one such patient was present in the study in **chapter 6**, and this patient was clinically diagnosed as a non-pSS patient[57]. The study described by Jousse-Joulin et al.[55] did not include any patients with a score of 4 based on minor items only, but their panel of experts recommended that at least one major item (focus score ≥1 or anti-SSA positive) should be present for classification as pSS. Future studies should evaluate whether patients with positive ACR-EULAR criteria but without a focus score ≥1 or anti-SSA positivity have salivary and tear gland dysfunction due to different causes than pSS, such as medication induced sicca syndrome, or whether these patients may develop full-blown pSS later on.

Classification criteria are developed with the goal of creating homogenous study populations in clinical trials. However, pSS is a heterogeneous disease and all patient differ from each other in signs and symptoms. Perhaps we should therefore shift our focus to identifying subgroups

of patients with similar pathophysiological and clinical characteristics, who may respond well to a certain treatment, instead of trying to include homogenous study populations. **Chapter 7** showed that patients with abnormal SGUS have higher systemic and biological activity, increased glandular inflammation, decreased glandular function and more pSS-related damage. SGUS negative patients reported more symptoms of fatigue and pain. Other studies have also described associations between SGUS and ESSDAI, rheumatoid factor, IgG, presence of anti-SSA antibodies, focal inflammation of the minor salivary glands, salivary and tear gland dysfunction, and dryness symptoms[59–65]. SGUS therefore seems to be a suitable tool to stratify clinical subgroups of pSS patients. Another way to stratify pSS patients is based on their symptoms. Tarn et al.[66] identified four subgroups of patients based on the ESSPRI and hospital anxiety and depression scale (HADS): low symptom burden, high symptom burden, dryness dominant with fatigue, and pain dominant with fatigue. These subgroups showed distinct pathobiological endotypes and different responses to immunomodulatory treatments.

Before SGUS can be widely implemented and included in classification criteria for pSS, a consensus SGUS scoring system has to be developed. For this purpose, the EULAR US-pSS Task Force has created a consensual reference atlas, containing definitions of several items (e.g., echogenicity, homogeneity, hypoechogenic areas, hyperechoic bands)[67]. However, several SGUS scoring systems are still in use. For classification purposes, a simple system scoring only hypoechogenic areas in the submandibular and parotid gland on one side is sufficient[68]. Which scoring system works best for use as inclusion criterion or efficacy outcome in clinical trials, or for monitoring longitudinal progression of pSS, remains to be evaluated, by comparing their validity, reliability and sensitivity to change.

Most studies of salivary gland ultrasonography in pSS have used conventional brightness-mode ultrasonography, but future studies should also evaluate the added value and reliability of other modes of ultrasound such as colour Doppler and elastography. Colour Doppler, which determines the degree of vascularization of the salivary glands, can be used to detect glandular inflammation, but reliability of salivary gland colour Doppler evaluation may be limited[69–71]. Elastography measures stiffness of the salivary glands, which is increased in pSS patients and associated with B-mode ultrasound score, systemic and glandular activity and salivary CXCL10 levels[72–74].

Which systemic treatments are effective and safe in pSS?

Despite the growing number of randomised controlled clinical trials (RCT) that have been performed in pSS, and the many treatment targets that have been explored, no systemic treatment for pSS has yet been approved by the Food and Drug Administration (FDA) or European Medicines Agency (EMA). The recent EULAR guidelines for the treatment of pSS recommend that systemic therapies such as glucocorticoids, hydroxychloroquine, cyclophosphamide, rituximab, belimumab and abatacept should be restricted to patients

with active systemic disease, but also state that the level of evidence for each of these therapies was low[75].

Considering the promising results of open label trials of abatacept treatment in pSS[76–78], the Abatacept Sjögren Active Patients (ASAPIII) RCT (**chapter 8**) aimed to assess the safety and efficacy of subcutaneous abatacept compared with placebo in pSS[38]. Unfortunately, the primary endpoint (ESSDAI score at week 24) was not met, and based on the ASAPIII trial we cannot recommend abatacept treatment as standard of care to reduce systemic disease activity in pSS. A large decrease in ESSDAI was seen in the placebo group as well as in the abatacept-treated group. These results were confirmed in a multicentre, sponsor-initiated RCT[79]. Although the primary endpoint was not met, some secondary endpoints in the ASAPIII trial did show significant effects of abatacept. ESSDAI, physician global disease activity and DAS-28 (CRP) were significantly lower at week 12 in abatacept treated patients compared to placebo treated patients, which indicates that improvements in systemic disease activity occurred earlier in abatacept treated patients[38]. The larger proportion of abatacept-treated patients reaching a minimal clinical important improvements in ESSPRI, and improvement of sexual function during abatacept treatment, shows that at least some patients may experience benefit from abatacept treatment. However, when interpreting these results, we need to keep in mind the large number of secondary outcomes that were evaluated in the ASAPIII trial, which increases the chance of false positive findings. No differences between treatment groups were found for other patient reported outcomes, and the multicentre RCT did not find any effect of abatacept on ESSPRI score[79].

Abatacept has shown clear biological effects in pSS. In accordance with previous open label results[76], IgG and rheumatoid factor were decreased by abatacept in the ASAPIII trial[38] (**chapter 8**) and multicentre trial[79]. In the multicentre abatacept trial[79], abatacept significantly decreased the chemokine CXCL13 and several cell subsets including ICOS expressing T-follicular helper (Tfh) cells, confirming the findings of Verstappen et al.[80].

Due to the large variance in glandular function outcomes in pSS patients, which necessitates a large sample size to find any differences between groups, no definite conclusions can be drawn regarding the effect of 24 weeks of abatacept treatment on glandular function in pSS[38] (**chapter 8**). Furthermore, a longer treatment duration may be needed, as 24 weeks of abatacept treatment showed limited effects on histopathological features in parotid gland biopsies in our open label trial[81]. Although 24 weeks of abatacept treatment decreased germinal centers, it did not reduce focus score, lymphoepithelial lesions, area of lymphocytic infiltrate, amount of follicular dendritic cell networks or numbers of T-cells or B-cells. A recent open label study did find improvement of salivary flow after 24 months of abatacept treatment in pSS patients[78]. Data from the open label extension phase of the ASAPIII trial indeed shows that long-term abatacept does improve ocular staining score and might also improve UWS[82].

Studies assessing the efficacy of rituximab in pSS show the same pattern as studies using abatacept; despite promising results of smaller open label studies and RCTs[83–86], two large RCTs[87,88] showed disappointing results (**chapter 9**)[89]. Consequently, consensus on the efficacy of rituximab is lacking. However, post-hoc analyses suggest that rituximab treatment is beneficial in selected patient subgroups. The EULAR guidelines recommend the use of rituximab in patients with severe, refractory systemic disease[75]. Considering the prominent role of B-cell hyperactivity in pSS, there is room for new trials with anti-CD20 targeted therapy and other B-cell targeting therapies.

The unexpected negative clinical results of several recent RCTs, after open label trials showing promising results, raise an important question: why do RCTs in pSS fail? Despite the negative results of recent RCTs, we can still learn from them, as these trials provide important information which may help us understand the pathophysiology of pSS, define better inclusion criteria, and develop new endpoints. To be able to show clinical efficacy of a drug, the design of a trial should meet certain conditions, which will be discussed in the following paragraphs.

First, the right therapeutic target should be selected, and the drug should be given in an adequate dosage, for a long enough period of time. Previous studies have provided sufficient evidence for the biological efficacy of abatacept[80,90] and rituximab (**chapter 9**)[89], to justify inhibition of co-stimulation and B-cell depletion as therapeutic targets. Targeting the CD40-CD154 co-stimulatory pathway is also a promising approach, as a recent exploratory placebo-controlled study showed a clinically meaningful improvement in ESSDAI during anti-CD40 treatment with iscalimab[91]. However, considering the complex pathophysiology of pSS, perhaps treatments should target more than one pathway at the same time, by combining drugs, or using drugs with multiple modes of action. After rituximab treatment, an increase of serum levels of B-cell activating factor (BAFF) is seen, which leads to B-cell regeneration, and possibly an increase of generation of autoreactive cells[92]. pSS patients who do not respond to rituximab treatment more often show high baseline serum levels of BAFF[93]. The combination of rituximab with belimumab (anti-BAFF), which may prolong B-cell depletion, is therefore currently being investigated in an RCT (clinicaltrials.gov NCT02631538). Ianalumab, an anti-BAFF-receptor monoclonal antibody, causes lysis of B-cells as well as BAFF-receptor blockade, mimicking the effect of rituximab combined with belimumab[94]. Compared to placebo, patients treated with ianalumab showed a dose-dependent reduction in ESSDAI, and a larger number of ESSDAI responders. However, the largest mean difference in ESSDAI between groups was only 1.9 points (below the minimal clinically important improvement of 3 points), and no improvements were seen in patient reported outcomes. Another example of combination therapy is the combination of leflunomide and hydroxychloroquine, which achieved stronger in vitro immune inhibition than either drug seperately[95]. A pilot RCT suggested efficacy of this combination regarding ESSDAI, ESSPRI and laboratory parameters (IgG, rheumatoid factor, CXCL13 and complement)[96], but these results have not yet been confirmed in a larger trial.

The second condition for a successful therapeutic trial, is the selection of the right study population. The study population should be well-defined, large enough, and include patients who are likely to benefit from therapy. Inclusion of a placebo-treated control group is also important, considering previous discrepancies between open label studies and RCTs in pSS. Selection of study population has been a major topic of discussion in the past years. Should we focus on patients with high symptom burden, or on patients with high risk of life threatening complications of pSS? Previous trials have tried to include pSS patients who may be more likely to respond to systemic therapy, such as patients with high systemic disease activity (ESSDAI ≥ 5 or 6). However, using strict inclusion criteria results in exclusion of a substantial proportion of the pSS population. In a prospective registry in the United Kingdom, the inclusion criteria of most recent clinical trials were fulfilled by less than half of the patients[97]. In the ASAPIII study, only 14% of patients from the UMCG pSS population was considered eligible for participation[38]. Strict inclusion criteria also make it difficult to include enough patients to reach an adequate sample size. Post-hoc analyses of translational and clinical biomarkers, which may predict response to certain treatments, are therefore essential to improve the selection of patients for future trials and prevent unnecessary exclusion of the majority of patients.

In our open label abatacept trial, a decrease in inducible T-cell co-stimulator (ICOS) expression by circulating Tfh-cells was associated with ESSDAI improvement[80]. With regard to the recently completed ASAPIII trial, we plan to evaluate whether baseline ICOS expression by circulating Tfh-cells can predict ESSDAI response. In rituximab trials, patients with high focus scores less often reached a response according to the Sjögren's syndrome response index (SSRI), which includes visual analogue scales (VAS) for oral dryness, ocular dryness, and fatigue, UWS, and erythrocyte sedimentation rate (ESR)[98,99]. Considering that three of the five items in the SSRI measure sicca symptoms or glandular function, patients with high focus scores may show an SSRI response less often because the damage to their salivary and lacrimal glands is too severe and improvement of glandular function cannot be achieved by a single course of rituximab. On the other hand, patients with higher number of B-cells in the parotid glands are more likely to show a decrease in ESSDAI score after rituximab treatment[100]. Whether patients with severe glandular inflammation should or should not be included in trials targeting B-cell hyperactivity therefore also depends on whether the aim of the study is to improve systemic disease activity or to improve sicca symptoms.

SGUS may also be a valuable and feasible tool to predict response to therapy. As SGUS-positive patients show higher disease activity and more pronounced B-cell hyperactivity, one might speculate SGUS positive patients would respond better to therapies which decrease B-cell hyperactivity. However, considering the association of SGUS to disease duration and glandular dysfunction, a part of the patients with high SGUS scores may have irreversible structural damage to their glands. Using SGUS as an inclusion criterion may therefore increase

inclusion of patients with irreversible structural damage to their glands, decreasing the change of finding improvement of gland function during treatment. In the TEARS trial, patients who showed improvements of ≥30% in oral or ocular dryness had lower baseline SGUS scores than non-responders[98]. Whether SGUS scores can predict improvement in systemic disease activity should be further evaluated in clinical trials.

The third condition for a successful therapeutic trial, is the use of endpoints, which are valid, reliable and sensitive to change, and which aim to measure outcomes which are clinically relevant to the patients. Despite the best efforts of many research groups, a suitable primary endpoint for pSS has not yet been found. Several endpoints have been used in recent RCTs, often measuring either patient reported outcomes or systemic disease activity.

Improving patient reported outcomes is important, considering the impact of pSS on health-related quality of life. Patients with pSS who were participating in a rituximab trial rated physical fatigue as the most important symptom to improve during systemic therapy[101]. In a large patient survey conducted on behalf of the Sjögren's Syndrome Foundation, 82% of patients found it extremely important that new systemic therapies address dryness symptoms[102]. The TEARS and TRACTISS rituximab trials and JOQUER hydroxychloroquine trial defined response to treatment as reductions of 30mm or 30% from baseline in varying numbers of VAS scores (including fatigue, dryness, pain or patients global disease activity)[88,103,104]. However, the minimal clinical important improvement (MCII) in ESSPRI is a decrease of only one point (corresponding to 10mm on a VAS score) or 15% from baseline[105]. Defining response as a reduction of 30mm or 30% on a VAS scale may therefore be too strict. However, when aiming to detect smaller reductions, large sample sizes are required to show a clinically relevant difference in patient reported symptoms between groups.

The development of the ESSDAI has made it possible to measure the severity of systemic manifestations of pSS[106]. Several larger RCTs failed, using the ESSDAI as primary endpoint[38,79,107]. Failure to show differences in ESSDAI score between treatment groups may in part be due to the large decrease in ESSDAI in placebo-treated patients, which was seen in the ASAPIII trial (**chapter 8**), the multicentre abatacept trial, tocilizumab trial, and ianalumab trial[38,79,94,107]. This effect may in part be caused by regression to the mean, as each of these studies included ESSDAI ≥5 or ≥6 as an inclusion criterion. Regardless of the treatment, the ESSDAI shows natural variation over time within patients, and patients with high ESSDAI scores at baseline are more likely to show a subsequent decrease in ESSDAI[108]. A better approach may be to also include patients with lower systemic disease activity, and evaluate the proportion of patients who continue to have or reach low ESSDAI scores. Other limitations of the ESSDAI include difficulty to separate active disease from irreversible damage (e.g. permanent nerve damage due to polyneuropathy or fibrosis due to interstitial lung disease), and limited sensitivity to partial improvements within certain subdomains. Some domains are dependent on

subjective reporting of symptoms by patients, such as the constitutional domain, which may be influenced by expectations of the treatment effect. Other domains, such as the glandular and lymphadenopathy domain, are based on the results of the physical examination, which may have limited reliability. For several domains, additional diagnostic tests are needed to be able to complete the score, such as a pulmonary function test and high resolution CT for the pulmonary domain. Patients should therefore undergo an comprehensive rheumatologic evaluation and assessors should be adequately trained, to be able to provide a reliable ESSDAI score.

Due to the heterogeneity of pSS patients, treatment goals may vary from patient to patient. In patients with early disease, we may primarily aim to improve glandular function and prevent further glandular damage, while in patients with irreversible glandular damage the main goal may be to reduce extra-glandular manifestations or fatigue. Different treatments may also affect different outcomes. Therefore, a composite endpoint which combines patient reported outcomes, glandular involvement, and systemic disease activity, may be more suitable to show efficacy in varying patient populations and treatments. Cornec et al.[99] proposed the SSRI, a composite endpoint which was based on data from the TEARS trial and validated in two other RCTs, assessing rituximab and infliximab. The SSRI defines response as a 30% improvement or more in at least two of the five domains (VAS oral dryness, VAS ocular dryness, VAS fatigue, UWS, and ESR). Compared to placebo treated patients, rituximab treated patients showed higher proportions of responders according to the SSRI while no difference was seen between infliximab and placebo treated patients. In the ASAPIII trial, a significant difference in the proportion of responders according to the SSRI was seen in week 12, even though no difference between groups was seen in the individual items of the SSRI[38]. However, the SSRI does not include systemic disease activity or tear gland function, which are also considered important outcomes in pSS, and ESR may not be a sensitive or specific biomarker for biological activity.

So far, SGUS has not been used as primary endpoint in pSS trials, but it is increasingly being used as a secondary endpoint. The TEARS and TRACTISS trials both found small but significant improvements of SGUS scores during rituximab treatment, compared to placebo[109,110]. The ASAPIII also included SGUS as a secondary endpoint, of which the results will become available soon.

Innovative collaboration in European Union funded projects are needed to improve the design and results of therapeutic trials in pSS. The aim of the innovative medicines initiative (IMI) project 'NECESSITY', in which the UMCG is participating, is therefore to identify and validate a new sensitive composite clinical endpoint for use in future clinical trials in pSS. NECESSITY also aims to identify and validate discriminative biomarkers for stratification of pSS patients[111]. Another international project in which the UMCG is participating is HarmonicSS, an

Horizon2020 project which aims to bring together large longitudinal cohorts of pSS patients, to harmonize them into a cloud-based integrative pSS cohort. In the future, this cohort can be used to facilitate and improve patient selection for clinical trials[112].

When deciding to treat patients with a certain drug, physicians should consider the balance between efficacy and possible side effects (chapter 10)[113]. No major safety issues have been identified for most drugs which are used off-label in pSS, such as hydroxychloroquine and rituximab. However, the quality of most trials in pSS regarding the reporting of harms is very poor and sample sizes are small. To investigate the presence of serious adverse effects with a lower prevalence, larger RCTs are needed, which follow the CONSORT guidelines for reporting of harms. Furthermore, off-label treatment of pSS patients should be registered in prospective cohorts.

Concluding remarks

The heterogeneity of pSS makes it an fascinating disease to study, with many translational and clinical facets remaining to be explored. At the same time, this heterogeneity is challenging, not only in regard to diagnosis and classification, but also when measuring the effect of systemic treatment. The growing knowledge about the pathogenesis of pSS allows us to identify and evaluate biomarkers which may predict response to treatment and develop a personalized medicine approach for the treatment of pSS. Due to the low prevalence and heterogeneity of pSS, international and multidisciplinary cooperation is required to develop prospective patient registries and larger RCTs, and thereby speed up clinical developments. The development of consensus classification criteria, and the initiation of international projects to improve patient inclusion and define study endpoints that are able to discriminate between active treatment and placebo, show that international cooperation has already started. Although many challenges remain, more and more pieces of the puzzle are being found and many new studies are underway, with the ultimate goal of improving treatment of pSS patients.

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Summary

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease, characterised by lymphocytic infiltration of the salivary and lacrimal glands, resulting in dryness of the mouth and eyes. pSS is a multifaceted disease, presenting with a wide range of symptoms, including sicca symptoms in other areas such as the skin and vagina, disabling fatigue, tendomyalgia, arthritis, and involvement of several organs. Although advancements in knowledge of the pathogenesis of pSS have offered many new targets for treatment, there is still a great unmet need for effective systemic treatment of pSS. The general aim of this thesis was to improve the understanding and management of pSS, focusing on three topics. First, the prevalence and pathogenesis of vaginal sicca symptoms and sexual dysfunction in pSS were explored. Second, new tools to classify patients with pSS were evaluated: the new ACR-EULAR classification criteria and salivary gland ultrasonography (SGUS). Finally, the efficacy and safety of abatacept treatment and other systemic treatment options for pSS were assessed.

Vaginal dryness and sexual dysfunction

In the first part of this thesis, the impact of pSS on sexual dysfunction was described, and the pathogenesis of vaginal dryness in pSS was explored. In **chapter 2,** a case-control study is performed, in which 46 patients and 43 healthy controls completed the Female Sexual function Index (FSFI) and several other questionnaires about symptoms which may affect sexual function. Women with pSS reported significantly impaired sexual function compared to healthy controls (median FSFI 20.6 vs. 30.3, $P<0.001$). Patients with pSS also reported increased distress in relation to their sexual function, and were less frequently sexually active compared to controls (76% vs. 93%, $P<0.05$). Within the group of pSS patients, we found associations of sexual dysfunction with patient reported symptoms of pSS as measured with the EULAR Sjögren's syndrome patient reported index (ESSPRI), symptoms of fatigue, anxiety and depression, relationship dissatisfaction, and lower mental quality of life. Using multivariate linear regression, we found that symptoms of depression were predictive of sexual dysfunction. No association was found with systemic disease activity.

Despite the major impact of vaginal dryness on sexual function, data on the pathogenesis of vaginal dryness in pSS are scarce. Therefore, the case-control study described in **chapter 3** assessed clinical, histopathological and soluble and cellular immunological changes in the vagina and cervix of 9 premenopausal women with pSS, in comparison with 8 age-matched premenopausal controls. As expected, women with pSS showed impaired vaginal health. Vaginal biopsies of women with pSS showed increased numbers of $CD45^+$ leucocytes and $CD3^+$ T-lymphocytes compared to vaginal biopsies of controls. Endocervical biopsies showed higher numbers of $CD20^+$ B-lymphocytes in women with pSS. We also stained biopsies for markers of blood vessels and lymphatic vessels and found that vascular smooth muscle cells were decreased in the vagina of pSS patients. Increased levels of the interferon-induced chemokine CXCL10 were found in the endocervical swabs of women with pSS. Based on

these results, we postulated that vaginal dryness in women with pSS might be caused by vascular dysfunction, induced by interferon-mediated pathways.

As oral dryness in pSS causes dysbiosis of the oral microbiome, the objective of the study presented in chapter 4 was to assess whether the vaginal microbiome is also affected by pSS, in the same population of 9 pSS patients with vaginal dryness and 8 controls described in chapter 3. No significant differences were found in the composition of the microbiome in cervicovaginal lavages and endocervical swabs of pSS patients and controls. Patient-reported vaginal dryness in premenopausal women with pSS did not correlate with the relative abundance of the three most prevalent genera and therefore does not appear to negatively influence homeostasis of the vaginal ecosystem.

Classification and stratification

In the second part of this thesis, the new American College of Rheumatology – European League Against Rheumatism (ACR-EULAR) criteria for classification of pSS patients and the additional value of the salivary gland ultrasound were evaluated. In **chapter 5,** the validity of the ACR-EULAR criteria was assessed in a prospective multidisciplinary cohort of 114 patients clinically suspected of pSS. We confirmed that the ACR-EULAR criteria could accurately discriminate pSS from non-pSS patients, regardless of whether the parotid gland or labial gland biopsies were used for classification. Using labial gland biopsy results, the ACR-EULAR classification criteria showed a sensitivity of 97% and specificity of 83%, while using parotid gland biopsy results, sensitivity was 91% and specificity 92%. Some non-SS patients were misclassified as pSS using the ACR-EULAR criteria, most of which had an ACR-EULAR score of 4-6. The validity of the ACR-EULAR criteria was equal to the American-European Consensus Group (AECG) criteria. Compared with the 2012 ACR criteria, sensitivity of the ACR-EULAR criteria was higher, while specificity was lower. When evaluating the validity of individual items of the ACR-EULAR criteria, unstimulated whole saliva and Schirmer's test showed poor discriminative value to classify patients as pSS.

Salivary gland ultrasound (SGUS) has shown good diagnostic value in pSS. We therefore studied whether SGUS can be added to the ACR-EULAR criteria, using a simple scoring system consisting of the average score for hypoechogenic areas in one parotid and one submandibular gland (**chapter 6**). In a cohort of 243 consecutive patients who underwent SGUS in the University Medical Center Groningen (UMCG), we found that the optimal weight of SGUS is 1, when added to the ACR-EULAR criteria, and that the optimal cut-off for classification as pSS remained ≥4 after addition of SGUS. The validity of the ACR-EULAR criteria remained high after incorporation of SGUS. The performance of the ACR-EULAR criteria remained equal when SGUS was added to the ACR-EULAR criteria, or when SGUS replaced the items of the ocular staining score, Schirmer's test or unstimulated whole saliva. Sensitivity

decreased substantially when salivary gland biopsy or anti-SSA positivity were replaced by SGUS. In conclusion, addition of SGUS improves the feasibility of the ACR-EULAR criteria in clinical practice, by allowing rheumatologists to choose from a larger array of tests.

The REgistry of Sjögren syndrome in Umcg – LongiTudinal (RESULT) cohort is a cohort of patients with pSS or incomplete pSS, which has been set up to identify biomarkers and clinical parameters that determine and predict the longitudinal course of pSS. In the analysis described in **chapter 7**, the results of baseline SGUS evaluations were compared to clinical, laboratory and functional characteristics of 172 patients with pSS according to the ACR-EULAR criteria participating in RESULT. SGUS positive patients showed a distinct clinical phenotype compared to SGUS negative patients. Patients with abnormalities on SGUS had higher EULAR Sjögren Syndrome Disease Activity Index (ESSDAI) scores, higher joint activity, higher levels of IgG and rheumatoid factor, lower levels of complements and lower leucocyte counts, and more often had anti-SSA antibodies and a positive biopsy. Furthermore, SGUS positive patients had longer disease duration, decreased glandular function, and more pSS-related damage. In contrast, SGUS negative patients reported more symptoms of fatigue and pain. SGUS can therefore be used as a tool to stratify patients already diagnosed with pSS.

Systemic treatment

In the final part of this thesis, the efficacy and safety of abatacept treatment in pSS was studied and the efficacy and safety of other systemic treatment options were reviewed. In **Chapter 8**, the results of the randomised, double blind Abatacept Sjögren Active Patients phase III (ASAPIII) trial were presented. The ASAPIII trial studied the efficacy and safety of abatacept treatment in 80 patients with pSS, with positive biopsies, short disease duration and active disease. Participants were randomised to receive weekly subcutaneous injections of abatacept or placebo for 24 weeks. The primary outcome, ESSDAI score at 24 weeks, did not significantly differ between abatacept and placebo treated patients (adjusted difference –1.3 (95% CI -4.1 to 1.6). Although abatacept did not significantly improve ESSPRI score as a continuous variable, we did see a higher proportion of patients who reached a minimal clinically important improvement of 1 point or 15% in ESSPRI score (58% in the abatacept group versus 21% in the placebo group, odds ratio 5.7, 95% CI 2.0-15.7, p=0.001). Considering the major impact of pSS on sexual function, the FSFI was included as an outcome measurement. Interestingly, sexual function was significantly better after 24 weeks of abatacept treatment, compared to placebo treatment. Other patient reported parameters were not significantly improved by abatacept. Salivary and tear gland function were not significantly improved by abatacept. Abatacept showed evident biological efficacy, as shown by a decrease in rheumatoid factor and IgG in the abatacept-treated group. Abatacept was well tolerated. No treatment related SAEs or deaths occurred and the number of adverse events was similar between treatment groups. Based on this trial, we cannot recommend abatacept treatment as standard of care to reduce systemic disease activity in patients with pSS. Further studies should evaluate whether

patients with specific clinical manifestations and biological characteristics might benefit from abatacept treatment.

Considering the well-established role of B-cell hyperactivity in pSS, the efficacy of B-cell depletion therapy with rituximab has been studied in a number of open label trials and randomized controlled trials (RCT) in pSS. In **Chapter 9,** the effects of rituximab on biological and clinical outcomes in pSS, and possible predictors of response to rituximab are reviewed. In multiple trials, rituximab has shown beneficial effects on B-cell hyperactivity, glandular morphology, symptoms of dryness and fatigue, and extraglandular manifestations including arthritis, haematological abnormalities, pulmonary involvement and vasculitis. However, clinical outcomes vary greatly between studies, due to differences in study populations, patient characteristics and definition of endpoints. Two RCTs did not meet their primary endpoint, which were composite endpoints of patient reported symptoms. Studies evaluating predictors of response to rituximab show that in patients with low salivary gland inflammation, rituximab treatment could prevent further glandular damage. On the other hand, rituximab ameliorates systemic disease activity in patients with high systemic disease activity and high numbers of infiltrating B-cells in the salivary glands. We concluded that rituximab treatment can be of value in a selected group of pSS patients.

Chapter 10 reviews the safety profile of several treatment options for pSS. Overall, symptomatic treatment has few side effects, and therefore is safe, but it is insufficient to achieve long-term relief and to prevent organ damage and exocrine gland dysfunction. As synthetic disease modifying anti-rheumatic drugs (DMARDs) show limited efficacy in pSS, with the exception of some patients with specific manifestations of pSS, the benefits of synthetic DMARDs often do not weigh up to the possible harms. Most biological DMARDs have shown acceptable safety profiles. However, the manner in which adverse events are reported in therapeutic trials in pSS varies greatly, which makes it difficult to compare safety results of different trials.

Samenvatting

Het primaire syndroom van Sjögren (pSS) is een systemische auto-immuun ziekte, welke wordt gekenmerkt door de infiltratie van lymfocyten (witte bloedcellen) in de speeksel- en traanklieren. pSS is een ziekte met vele facetten en kan zich presenteren met een breed spectrum aan symptomen. Naast droogheid van de mond en ogen kunnen patiënten klachten ervaren van een droge huid en vagina. Een groot deel van patiënten ervaart ernstige klachten van vermoeidheid, pijn in de spieren en peesaanhechtingen, en gewrichtsontstekingen. Ook kan er betrokkenheid zijn van diverse organen, zoals de huid, de longen en de nieren. Er is steeds meer bekend over de pathogenese van pSS en daaruit zijn vele potentiele aangrijpingspunten voor therapie naar voren gekomen. Desondanks bestaat er nog steeds een groot gebrek aan effectieve behandelingen van pSS. Het doel van dit promotieonderzoek was daarom om de kennis over pSS uit te breiden en de behandeling van pSS te verbeteren. Allereerst werden de prevalentie en pathogenese van vaginale droogte en seksueel disfunctioneren bij patiënten met pSS onderzocht, gezien er nog weinig bekend was over dit aspect van pSS. Daarnaast werd de waarde van nieuwe methoden om patiënten met pSS te classificeren geëvalueerd: de nieuwe ACR-EULAR classificatie criteria en speekselklierechografie. Tot slot werden de effectiviteit en veiligheid van behandeling van pSS met abatacept en andere systemische behandelingen onderzocht.

Vaginale droogte en seksueel disfunctioneren

In het eerste gedeelte van dit proefschrift werd de invloed van pSS op het seksueel functioneren beschreven en is de pathogenese van het ontstaan van vaginale droogte bij patiënten met pSS onderzocht. In **hoofdstuk 2** staat een patiënt-controle onderzoek beschreven waarin 46 patiënten en 43 gezonde controles de Female Sexual Function Index (FSFI) hebben ingevuld, een gevalideerde vragenlijst over seksuele functie. Daarnaast hebben de deelnemers vragenlijsten ingevuld over lichamelijke en mentale klachten die van invloed zouden kunnen zijn op het seksueel functioneren. Vrouwen met pSS bleken een significant slechtere seksuele functie te ervaren dan gezonde controle vrouwen (mediane FSFI 20,6 vs. 30,3, $p<0,001$). Vrouwen met pSS rapporteerden ook meer zorgen omtrent hun seksueel functioneren en waren minder vaak seksueel actief vergeleken met controle vrouwen (76% vs. 93%, $p<0,05$). Binnen de groep van pSS patiënten werd een verband gevonden tussen seksueel disfunctioneren en symptomen van pSS, gemeten met de EULAR Sjögren's syndrome patient reported index (ESSPRI). Ook symptomen van vermoeidheid, angst, depressie, ontevredenheid over de relatie met hun partner en een lagere mentale kwaliteit van leven waren geassocieerd met seksueel disfunctioneren. Uit een statistisch model bleek dat de aanwezigheid van symptomen van depressie voorspellend waren voor de aanwezigheid van seksueel disfunctioneren. Daarentegen werd geen verband gevonden tussen seksueel disfunctioneren en systemische ziekteactiviteit.

Ondanks de grote invloed van vaginale droogte op de seksuele functie bij vrouwen met pSS, is weinig bekend over de oorzaak van dit symptoom. In het patiënt-controleonderzoek dat

wordt beschreven in **hoofdstuk 3** werd daarom onderzocht of er klinische, histopathologische of immunologische veranderingen aanwezig zijn in de vagina en baarmoederhals van 9 premenopauzale vrouwen met pSS, vergeleken met 8 premenopauzale controle vrouwen zonder pSS. Bij vrouwen met pSS werd tijdens een gynaecologisch onderzoek een verminderde vaginale gezondheid gezien vergeleken met controles. Vaginale biopten van vrouwen met pSS toonden hogere aantallen van $CD45^+$ leukocyten (witte bloedcellen) en $CD3^+$ lymfocyten (T-cellen). In biopten uit de baarmoederhals werden hogere aantallen $CD20^+$ lymfocyten (B-cellen) gezien bij vrouwen met pSS. Na kleuring van de biopten met merkers om de cellen rondom bloedvaten en lymfevaten te kunnen onderscheiden, werd een afname van het aantal vasculaire gladde spiercellen in de vagina van pSS patiënten gevonden. Gladde spiercellen zijn belangrijk voor de bevochtiging van de vagina, doordat ze zorgen voor verwijding van de bloedvaten in de vagina tijdens opwinding. Afname van het aantal gladde spiercellen zou derhalve de bevochtiging van de vagina negatief kunnen beïnvloeden. In materiaal dat uit de binnenkant van de baarmoederhals was afgenomen, werd bij pSS patiënten een hoger gehalte van het chemokine CXCL10 gevonden. De productie van CXCL10 wordt gestimuleerd door het cytokine interferon. Een relatie tussen overactiviteit van het interferon systeem en vasculair disfunctioneren is eerder beschreven bij systemische lupus erythematosus en zou ook bij Sjögren aanwezig kunnen zijn. Deze resultaten suggereren derhalve dat vaginale droogte bij pSS mogelijk het gevolg is van disfunctioneren van de bloedvaten in de vagina ten gevolge van overactiviteit van het interferon systeem.

Het menselijke microbioom bestaat uit alle bacteriën die in en op het menselijk lichaam leven, en bestaat normaliter uit veel bacteriën van veel verschillende soorten. Uit eerder onderzoek is gebleken dat bij pSS patiënten het microbioom in de mond wordt verstoord door droogheid van de mond. Daarom werd in de in hoofdstuk 4 beschreven studie onderzocht of bij pSS patiënten ook het vaginale microbioom wordt beïnvloedt door vaginale droogte. Dit onderzoek is uitgevoerd in dezelfde populatie van 9 premenopauzale vrouwen met pSS en 8 premenopauzale controle vrouwen die in hoofdstuk 3 is beschreven. Er werden tussen patiënten en controles geen significante verschillen gevonden in de bacteriële samenstelling van spoelingen van de vagina en materiaal dat uit de baarmoederhals was afgenomen. Ook werd geen verband gevonden tussen vaginale droogte bij pSS en de verdeling van de drie meest voorkomende vaginale bacteriële genera. De aanwezigheid van vaginale droogte bij pSS lijkt derhalve de balans van het vaginale ecosysteem niet negatief te beïnvloeden.

Classificatie en stratificatie

In het tweede gedeelte van dit proefschrift werden de nieuwe American College of Rheumatology – European League Against Rheumatism (ACR-EULAR) criteria voor de classificatie van pSS patiënten, en de toegevoegde waarde van echografie van de speekselklieren voor de classificatie en stratificatie van pSS patiënten besproken. De ACR-EULAR criteria bestaan uit 5 onderdelen, welke gewogen worden meegeteld voor de totale

score. Een speekselklierbiopt met een focus score van ≥1 en de aanwezigheid van anti-SSA antilichamen in het bloed leveren elk 3 punten op. Een verminderde speekselproductie, een positieve Schirmer's test (verminderde traanproductie) of verhoogde ocular staining score (sterkere aankleuring van het beschadigde oogoppervlak) leveren elk 1 punt op. De maximale score is 9, en bij een opgetelde score van ≥4 worden patiënten als pSS geclassificeerd.

In **hoofdstuk 5** werd de waarde van de ACR-EULAR criteria onderzocht in een prospectief multidisciplinair cohort van 114 patiënten met een klinische verdenking op pSS. Het klinische diagnostische oordeel van een groep van 3 medische experts werd als gouden standaard gehanteerd. Onze studie bevestigde dat de ACR-EULAR criteria nauwkeurig onderscheid kunnen maken tussen patiënten met en zonder pSS. Voor de classificatie werd gebruik gemaakt van speekselklierbiopten uit de parotisklier of uit speekselkliertjes gelegen in de lip. Bij beide methodes voor het nemen van speekselklierbiopten kon met de ACR-EULAR criteria goed onderscheid gemaakt worden tussen patiënten met en zonder pSS. Wanneer er speekselklierbiopten uit de lip werden gebruikt, hadden de ACR-EULAR classificatie criteria een sensitiviteit van 97% (percentage terecht positieve uitslagen onder de zieke personen) en een specificiteit van 83% (percentage terecht negatieve testuitslagen onder de niet-zieke personen). Wanneer er biopten uit de parotisklier werden gebruikt, was de sensitiviteit van de ACR-EULAR criteria 91% en de specificiteit 92%. Sommige patiënten zonder pSS werden door de ACR-EULAR criteria ten onrechte als pSS patiënt geclassificeerd, met name wanneer patiënten een ACR-EULAR score hadden van 4 tot en met 6 punten. De validiteit (geldigheid) van de ACR-EULAR criteria was gelijk aan die van de American European Consensus Group (AECG) criteria uit 2002, welke tot nu toe veel gebruikt werden voor studies met pSS patiënten. Vergeleken met de ACR criteria uit 2012 hadden de ACR-EULAR criteria een hogere sensitiviteit maar lagere specificiteit. Tot slot werd gekeken naar de validiteit van de individuele items van de ACR-EULAR criteria, waaruit bleek dat op basis van de secretiesnelheid van ongestimuleerd speeksel en de Schirmer test geen goed onderscheid kon worden gemaakt tussen patiënten met en zonder pSS.

Speekselklierechografie heeft goede diagnostische eigenschappen laten zien bij het vaststellen van pSS. Bij een groot deel van de patiënten met pSS zijn bij echografie van de speekselklieren hypoechogene (donkere) gebieden te zien. We hebben daarom onderzocht of speekselklierechografie als extra item kan worden toegevoegd aan de ACR-EULAR criteria (**hoofdstuk 6**). Daarvoor werd een simpel scoringssysteem gebruikt, welke bestond uit de gemiddelde score voor de aanwezigheid van hypoechogene gebieden in de parotisklier en submandibulaire speekselklier aan één zijde van het lichaam. In een cohort van 243 patiënten bij wie speekselklierechografie werd verricht in het Universitair Medisch Centrum Groningen (UMCG) bleek dat speekselklierechografie het beste kon worden toegevoegd aan de ACR-EULAR criteria met een gewicht van 1 punt. Na toevoeging van speekselklierechografie aan de huidige onderdelen bleek het optimale afkappunt voor de ACR-EULAR score voor

classificatie als pSS nog steeds ≥4 punten te zijn. De validiteit van de ACR-EULAR criteria bleef hoog na toevoeging van speekselklierechografie aan de huidige onderdelen. De nauwkeurigheid van de ACR-EULAR criteria bleef tevens gelijk wanneer de items ocular staining score, Schirmer test of ongestimuleerde speekselsecretie snelheid werden vervangen door speekselklierechografie. De sensitiviteit van de criteria nam echter af wanneer het speekselklierbiopt of aanwezigheid van anti-SSA antistoffen werden vervangen door speekselklierechografie. Speekselklierechografie kan het speekselklierbiopt dus niet volledig vervangen, maar bij een deel van de patiënten een biopt wel overbodig maken voor het vaststellen van de classificatie, bijvoorbeeld wanneer de speekselklierechografie afwijkend is en er tevens anti-SSA antistoffen aanwezig zijn. Wanneer speekselklierechografie wordt toegevoegd aan de bestaande ACR-EULAR criteria worden deze criteria beter toepasbaar in de dagelijkse klinische praktijk, omdat artsen dan uit een breder aanbod van diagnostische onderzoeken kunnen kiezen. Bovendien is een echoapparaat in veel reumatologie klinieken reeds beschikbaar.

Het REgistry of Sjögren syndrome in Umcg – LongiTudinal (RESULT) cohort is een onderzoek waarin patiënten met pSS gedurende 10 jaar in het UMCG gevolgd worden. Het RESULT cohort is opgestart met als doel om biologische en klinische parameters te identificeren welke het ziektebeloop van pSS op de lange termijn kunnen voorspellen. In de analyse die wordt beschreven in hoofdstuk 7 werden uitkomsten van speekselklierechografie vergeleken met klinische kenmerken, laboratorium uitkomsten en de speeksel- en traanklierfunctie van 172 patiënten met pSS die voldoen aan classificatie volgens de ACR-EULAR criteria en die deelnemen aan het RESULT cohort. Patiënten met afwijkende bevindingen bij speekselklierechografie lieten een ander klinisch fenotype zien dan patiënten met normale speekselklierechografie. Patiënten met afwijkende speekselklierechografie hadden hogere EULAR Sjögren Syndrome Disease Activity Index (ESSDAI) scores, meer last van gewrichtsontstekingen, hogere IgG en reumafactor waarden, lagere complement waarden, lagere aantallen witte bloedcellen, vaker anti-SSA antistoffen en vaker een afwijkend speekselklierbiopt. Daarnaast hadden patiënten met afwijkende speekselklierechografie een langere ziekteduur en een slechtere speeksel- en traanklierfunctie. Patiënten met normale bevindingen bij speekselklierechografie daarentegen rapporteerden meer symptomen van vermoeidheid en pijn. Speekselklierechografie kan derhalve worden gebruikt om patiënten met pSS te stratificeren (in groepen met gelijke kenmerken te verdelen).

Systemische behandeling

In het laatste gedeelte van dit proefschrift werden de effectiviteit en veiligheid van behandeling van pSS met abatacept onderzocht, en werd een overzicht gegeven van de effectiviteit en veiligheid van andere systemische behandelopties voor pSS. In **hoofdstuk 8** zijn de resultaten van de gerandomiseerde, dubbel blinde Abatacept Sjögren Active Patients fase III (ASAPIII) studie beschreven. In de ASAPIII studie werden 80 patiënten met

pSS met een afwijkend speekselklierbiopt, korte ziekteduur en actieve ziekte (op basis van een ESSDAI score van ≥5) gedurende 24 weken behandeld middels wekelijkse onderhuidse injecties met abatacept of placebo. Na 24 weken werd tussen patiënten die met abatacept of placebo waren behandeld geen significant verschil gevonden in de primaire uitkomstmaat, de systemische ziekte activiteit gemeten met de ESSDAI score na 24 weken. Het verschil in ESSDAI score tussen de groepen in week 24, gecorrigeerd voor de ESSDAI waardes ten tijde van de start van de studie, was -1,3 (95%CI -4,1 tot 1,6). In zowel de placebo als de abatacept groep werd een verbetering gezien in de systemische ziekteactiviteit.

Er werd geen verschil gezien tussen de groepen in ESSPRI score als continue uitkomstmaat. Wel was er in de abatacept groep een hoger percentage patiënten dat een minimale klinisch relevante verbetering van ten minste 1 punt of 15% in ESSPRI score liet zien (58% in de abatacept groep versus 21% in de placebo groep, p=0,001). Gezien de grote impact van pSS op het seksueel functioneren werd ook het effect van abatacept op de FSFI onderzocht. Het seksueel functioneren bleek significant beter te zijn bij deelnemers die met abatacept waren behandeld vergeleken met deelnemers die met placebo waren behandeld. Er werd echter geen significant effect van abatacept op andere patiënt-gerapporteerde uitkomstmaten gezien, waaronder vermoeidheid en kwaliteit van leven. Ook de speeksel- en traanklierfunctie verbeterden niet significant door behandeling met abatacept. Wel toonde abatacept duidelijke effecten op laboratorium parameters. Reumafactor en IgG werden verlaagd door abatacept. De behandeling met abatacept werd goed verdragen door de deelnemers. Er werden geen ernstige bijwerkingen gezien die gerelateerd waren aan de behandeling en geen van de deelnemers overleed tijdens de behandeling. Bovendien was het totale aantal bijwerkingen vergelijkbaar in de abatacept en placebo groep.

Op basis van deze resultaten kunnen we abatacept niet aanraden als standaard behandeling om de systemische ziekteactiviteit te verlagen bij patiënten met pSS. In nadere analyses moet worden onderzocht of patiënten met specifieke klinische uitingen van pSS of specifieke biologische kenmerken mogelijk meer baat hebben bij behandeling met abatacept.

Aangezien hyperactiviteit van B-cellen een belangrijke rol speelt in de pathogenese van pSS, werd in meerdere open label trials en gerandomiseerde placebo-gecontroleerde trials onderzocht of uitschakelen van de B-cellen door middel van rituximab effect heeft op pSS. In **hoofdstuk 9** wordt een overzicht gegeven van het effect van rituximab op biologische en klinische uitkomstmaten bij patiënten met pSS, en van mogelijke biomarkers die de effectiviteit van rituximab kunnen voorspellen. Uit dit overzicht blijkt dat de klinische effecten van rituximab sterk wisselen tussen de verschillende studies, mogelijk door verschillen in de studie populaties, in de kenmerken van de geïncludeerde patiënten en in de gebruikte uitkomstmaten. In meerdere kleinere trials liet rituximab gunstige effecten zien op symptomen van droogheid en vermoeidheid, extra-glandulaire (systemische) uitingen van

pSS zoals gewrichtsontsteking, afwijkingen in het bloed, longbetrokkenheid en vasculitis, B-cel hyperactiviteit en de morfologie van speekselklieren. Twee grotere gerandomiseerde placebo-gecontroleerde studies hebben echter hun primaire uitkomstmaat niet behaald. Het primaire eindpunt bestond in beide onderzoeken uit samengestelde uitkomstmaten die bestonden uit diverse patiënt-gerapporteerde symptomen. Aanvullende analyses, waarin werd gezocht naar factoren die het effect van rituximab kunnen voorspellen, lieten zien dat behandeling met rituximab bij patiënten met weinig ontsteking in de speekselklieren mogelijk schade aan de klieren kan voorkomen. Anderzijds kan rituximab de systemische ziekteactiviteit verlagen bij patiënten met hoge systemische ziekteactiviteit aan het begin van de behandeling, en met hoge aantallen B-cellen in de speekselklieren. Behandeling met rituximab heeft niet bij alle patiënten effect, maar kan gunstig zijn voor een geselecteerde groep patiënten met pSS.

Hoofdstuk 10 biedt een overzicht van de bijwerkingen van verschillende behandelopties voor pSS. Preparaten die verlichting van symptomen geven, zoals oogdruppels of parasympaticomimetica, geven over het algemeen weinig bijwerkingen. Symptoomgerichte therapie biedt echter onvoldoende verlichting op de lange termijn en kan geen schade aan de organen en traan- en speekselklieren voorkomen. Omdat synthetische immuun-regulerende medicijnen (DMARDs) over het algemeen een beperkt effect tonen bij patiënten met pSS, met uitzondering van patiënten met bepaalde specifieke manifestaties, wegen de voordelen van deze medicijnen niet altijd op tegen de nadelen. De verwachtte effecten moeten daarom goed worden afgewogen tegen de mogelijke bijwerkingen. De meeste biologische DMARDs laten een acceptabel veiligheidsprofiel zien. De manier waarop bijwerkingen zijn gerapporteerd in de verschillende onderzoeken naar nieuwe medicijnen voor pSS varieert echter sterk, waardoor het moeilijk is om de resultaten van de verschillende studies met elkaar te vergelijken.

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CURRICULUM VITAE

Jolien Francisca van Nimwegen was born in Leeuwarden on March 15th, 1989. After finishing high school in 2007 at the Piter Jelles Stedelijk Gymnasium in Leeuwarden, she studied medicine at the University of Groningen. In the final year of her medical education, she followed clinical internships in Internal Medicine and Rheumatology, and wrote a thesis on the impact of primary Sjögren's syndrome on female sexual function. After obtaining her medical degree in 2013, she continued as a PhD candidate under the guidance of prof. dr. H. Bootsma, prof. dr. F.G.M. Kroese, prof. dr. A. Vissink and dr. S. Arends at the department of Rheumatology and Clinical Immunology of the University Medical Center Groningen. Her PhD thesis focused on vaginal dryness in primary Sjögren's syndrome, and the classification, diagnosis, and systemic treatment of primary Sjögren's syndrome. She presented her work at several national and international conferences including the European League Against Rheumatism (EULAR), the American College of Rheumatology (ACR), the International Symposium on Sjögren's Syndrome (ISSS), and the Dutch Society for Rheumatology (NVR) annual meeting. In 2018, she enrolled in the training to become a rheumatologist at the University Medical Center Groningen, starting as a resident in Internal Medicine at the Martini hospital in Groningen. In 2019, she received a university teaching qualification in higher education.

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LIST OF ABBREVIATIONS

ACR	American college of rheumatology
AD	adjusted difference
AE	adverse event
AECG	American European consensus group
ALAT	alanine aminotransferase
ANA	anti-nuclear antibodies
APC	antigen presenting cell
APRIL	a proliferation-inducing ligand
ASAPIII	abatacept Sjögren active patients phase III trial
ASAT	aspartate aminotransferase
AUC	area under the ROC curve
BAFF	B-cell activating factor
bDMARD	biological disease-modifying anti-rheumatic drug
BMI	body mass index
BTK	Bruton's tyrosine kinase
CI	confidence interval
CONSORT	consolidated standardss of reporting trials
CRP	C-reactive protein
cTfh cell	circulating T follicular helper cell
CTLA-4	cytotoxic T-lymphocyte antigen 4
CTS	community state types
CVL	cervicovaginal lavage
D2-40	anti-podoplanin, clone D2-40
DAS-28	28-joint disease activity score
DMARD	disease-modifying anti-rheumatic drug
EQ-5D-5L	5-level EuroQol five dimensions health status questionnaire
ERG	avian V-ets erythroblastosis virus E26 oncogene homolog
ES	endocervical swab
ESR	erythrocyte sedimentation rate
ESSDAI	EULAR Sjögren's syndrome disease activity index
ESSPRI	EULAR Sjögren's syndrome patient reported index
EULAR	European league against rheumatism
FACS	fluorescence-activated cell sorting
FDR	false discovery rate
FLC	free light chain
FS	focus score
FSDS	female sexual distress scale
FSFI	female sexual function index

GDA	global disease activity
GEE	generalized estimating equations
H&E	hematoxylin and eosin
HACA	human anti-chimeric antibodies
HADS	hospital anxiety and depression scale
HCQ	hydroxychloroquine
HR-QoL	health-related quality of life
ICAM1	intercellular adhesion molecule 1
IFN	interferon
IgG	immunoglobulin G
IQR	interquartile range
ITT	intention to treat
IVIG	intravenous immunoglobulin G
JIA	juvenile idiopathic arthritis
LG	lissamine green
LT	lymphotoxin
MALT	mucosa-associated lymphoid tissue
MCS	mental component summary
MFI	multidimensional fatigue inventory
MHC	major histocompatibility complex
MMQ	maudsley marital questionnaire
NA	not available/applicable
NRS	numeric rating scale
OR	odds ratio
OSS	ocular staining score
PAS-D	periodic acid-Schiff diastase
PASS	patient acceptable symptom state
PBS	phosphate buffered saline
PCS	physical component summary
PML	progressive leukoencephalopathy
PRO	patient reported outcome
PROFAD	profile of fatigue and discomfort
PROMIS	patient-reported outcomes measurement information system
pSS	primary Sjögren's syndrome
RA	rheumatoid arthritis
RAND-36	RAND 36-item health survey
RANK-L	receptor activator of nuclear factor kappa-B ligand
RCT	randomised controlled trial
RESULT	registry of Sjögren's syndrome in UMCG – longitudinal
RF	rheumatoid factor

ROC	receiver operating characteristic
SAE	serious adverse event
SCLE	subacute cutaneous lupus erythematosus
SD	standard deviation
SF-36	36-item short form health survey
SGUS	salivary gland ultrasound
SLE	systemic lupus erythematosus
SSA	Sjögren's syndrome-related antigen A (Ro)
SSB	Sjögren's syndrome-related antigen B (La) antigen
SSDDI	Sjögren's syndrome disease damage index
SSRI	Sjögren's syndrome responder index
SUSAR	suspected unexpected serious adverse reaction
SWS	stimulated whole salivary flow rate
TBUT	tear break-up time
tCsA	topical cyclosporine A
Tfh cell	T follicular helper cell
UMCG	university medical center Groningen
UWS	unstimulated whole salivary flow rate
VAS	visual analogue scale
vBv	van Bijsterveld score
VCAM1	vascular cell adhesion protein 1
VHI	vaginal health index
WPAI	work participation and activity impairment questionnaire