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Graz, 18.09.2021

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Graz, am 18.09.2021 *Alexander Sanz eh.*

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List of abbreviations

ACR	American College of Rheumatology
AECG	American-European Consensus Group
ANA	Antinuclear antibodies
ANOVA	Analysis of variance
BE	Base excess
C3	Complement factor 3
C4	Complement factor 4
CCP	Anti-cyclic citrullinated peptide antibodies
CD	Cluster of differentiation
CH50	Total hemolytic complement
CRP	C-reactive protein
DEP	Differentially expressed protein
Diff.	Difference
EGF	Epidermal growth factor
ENO1	Alpha-enolase
ESR	Erythrocytes sedimentation rate
ESSDAI	EULAR Sjögren's syndrome disease activity index
ESSPRI	EULAR Sjögren's Syndrome patient-reported index
EULAR	European League against Rheumatism

FL	Free light chains
FTCD	Formimidoyltransferase-cyclodeaminase
HRQL	Health related quality of life
Ig	Immunoglobulin
max.	Maximum
MGUS	Monoclonal Gammopathy of Undetermined significance
MHC2	Major histocompatibility complex class 2
MDC	Minimal detectable change
min.	Minutes, minimum
mmol	Millimol
mU	Milliunits
NK	Natural killer cell
NKT	Natural killer T-cell
NRS	Numeric rating scale
n.s.	Non-significant
OSDI©	Ocular surface disease index
PhGA	Physician's Global Assessment
ProFaD-SSI	Profile of Fatigue and Discomfort – Sicca Symptoms Inventory
PSS	Primary Sjögren's Syndrome
QoL	Quality of Life

RF	Rheumatoid factor
RTA	Renal tubular acidosis
SD	Standard deviation
SEM	Standard error of measurement
SNAP-25	Synaptosomal-associated protein 25
SS	Sjögren's syndrome
SSA	Sjögren's Syndrome-A antibodies (Ro)
SSB	Sjögren's Syndrome-B antibodies (La)
TSH	basal Thyroid-stimulating hormone
U	Unit
UCHL1	Ubiquitin carboxyl-terminal hydrolase isozyme L1
USFR	Unstimulated salivary flow rate
VAS	Visual analogue scale
XI	Xerostomia Inventory

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Zusammenfassung

Einleitung/Ziele: PatientInnen mit primärem Sjögren-Syndrom (PSS) sind nicht nur mit körperlichen, sondern auch mit psychosozialen Herausforderungen konfrontiert, die zu einer Beeinträchtigung der gesundheitsbezogenen Lebensqualität (health-related quality of life - HRQL) führen können. Der Fragebogen "Primary Sjögren's Syndrome – Quality of life (PSS-QoL)" ist ein neues Instrument zur Erfassung körperlicher und psychosozialer Aspekte bei PSS-Patienten. Ziel dieser Studie war es zu untersuchen, welche Faktoren den Krankheitsverlauf beeinflussen und ob es Biomarker gibt, die das subjektive Wohlbefinden der PatientInnen widerspiegeln.

Methoden: Daten von PatientInnen, die an der rheumatologischen Ambulanz der Medizinischen Universität Graz behandelt werden und welche die Klassifikationskriterien der „American College of Rheumatology/European League Against Rheumatism (ACR/EULAR)" von 2016 für PSS erfüllen, wurden erhoben. Die von PatientInnen angegebenen persönlichen Einschätzungen wurden mit Hilfe folgender Fragebögen erhoben: „PSS-QoL", „EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI)", „Xerostomia-Inventory (XI)", „Ocular Surface Disease Index (OSDI©)", „Profile of Fatigue and Discomfort – Sicca Symptoms Inventory (ProFaD-SSI)". Sicca-Symptome wurden mit dem Schirmer-Test, dem Saxon-Test und der Rate des unstimulierten Speichelflusses (unstimulated saliva flow rate) objektiviert. Außerdem wurde der „EULAR Sjögren's Syndrome disease activity index (ESSDAI)" ermittelt. Serum IgG-Spiegel, Rheumafaktoren (RF) IgA und IgM und multiple andere Laborparameter wurden ebenfalls bestimmt.

Ergebnisse: Daten von 107 PatientInnen wurden analysiert. 93,5% der PatientInnen waren weiblich (n=100). Das Durchschnittsalter betrug 60,31 (±12,23) Jahre und die mediane Krankheitsdauer betrug 6 [0-23] Jahre. Der „PSS-QoL/physical" zeigte den höchsten Median in der Altersgruppe „50-59" (17 [8-32]), während der „PSS-QoL/psychosocial" den höchsten Median in der Altersgruppe „älter als 70" (25,5 [6-43]) aufwies. Von 52 PatientInnen lagen Longitudinaldaten des „PSS-QoL", des „ESSPRI" und des „ESSDAI" über einen Zeitraum von zwei Jahren vor. Lediglich die „ESSPRI/fatigue"-Domäne wies eine signifikante Veränderung über einen Zeitraum von zwei Jahren (von 3,4 auf 4,5, p=0,013) auf. 69,7% der PatientInnen zeigten keine Veränderung von klinischer Bedeutung in Bezug auf ihre „PSS-QoL"-Gesamtscores. 8,3% der PatientInnen fühlten sich körperlich

schlechter, während 20,6% über mehr psychosoziale Schwierigkeiten klagten als zwei Jahre zuvor.

Der „PSS-QoL“-Gesamtscore korrelierte negativ mit IgG (ρ =-0,261, p<0,01) und RF-IgA (ρ =-0,408, p<0,01). RF IgA korrelierte auch negativ mit der „PSS-QoL/physical“-Domäne (ρ =-0,307, p<0,01) und dem ESSPRI (ρ =-0,260, p<0,05), während IgG negativ mit der „PSS- QoL/psychosocial“-Domäne (ρ = -0,245, p < 0,05) korrelierte. Die „ProFaD-SSI/mental fatigue“-Domäne korrelierte negativ mit IgG (ρ =-0,267, p<0,01) und RF-IgA (ρ =-0,358, p<0,01). Der Saxon-Test korrelierte negativ mit allen drei immunologischen Parametern, insbesondere aber mit RF IgM (ρ =-0,607, p<0,01).

Diskussion: Es scheint, dass im Verlauf der Erkrankung die psychosoziale Belastung schneller zunimmt als die körperliche, daher sollte eine psychologische Betreuung in einem ganzheitlicheren Therapieansatz eingebunden sein. Zukünftige Studien mit gesunden Kontrollgruppen sind notwendig, um mögliche Cofaktoren wie Komorbiditäten, die in bestimmten Altersgruppen häufiger vorkommen, zu eliminieren. Die Menge an Serum-Rheumafaktoren und IgG korrelierte positiv und negativ mit verschiedenen objektiven und subjektiven Messgrößen und ihre Verwendung als Biomarker sollte in zukünftigen Studien untersucht werden.

Abstract

Introduction/Objectives: Patients suffering from primary Sjögren's Syndrome (PSS) are not only facing physical but also psychosocial challenges that can result in a decrease in health-related quality of life (HRQL). The aim of this study was to investigate possible factors that influence the course of the disease and to discover if there are any biomarkers that reflect the patient's subjective well-being. In addition an attempt was made to find out what objective and subjective tests for sicca symptoms correlate the most.

Methods: Data of patients from the rheumatology outpatient clinic of the Medical University of Graz fulfilling the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for PSS were obtained. Patient-reported outcomes were quantified using the PSS-QoL, EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), Xerostomia Inventory (XI), Ocular Surface Disease Index (OSDI©), and the Profile of Fatigue and Discomfort – Sicca Symptoms Inventory (ProFaD-SSI). Sicca signs were measured using the Schirmer's test, Saxon test and the unstimulated salivary flow rate. Also, the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) was obtained. Serum IgG levels, rheumatoid factors (RF) IgA and IgM and various other labaratory parameters were determined.

Results: Data of 107 patients were analyzed. 93.5% of the patients were female (n=100), the mean age was 60.31 (±12.23) years and the median disease duration was 6 [0-23] years. The PSS-Qol/physical showed the highest median in the age group "50-59" (17 [8-32]), whereas the PSS-QoL/psychosocial showed the highest median in the age group "older than 70" (25.5 [6-43]). Longitudinal data of PSS-QoL, ESSPRI and ESSDAI over two years were available from 52 patients. Only the ESSPRI/fatigue domain showed a significant change over a period of two years (from 3.4 to 4.5, p=0.013). 69.7% of the patients experienced no change of clinical importance regarding their PSS-QoL total scores. 8.3% of the patients felt physically worse, while 20.6% experienced more psychosocial difficulties than two years before.

The PSS-QoL total score negatively correlated with IgG (ρ =-0.261, p<0.01) and RF IgA (ρ =-0.408, p<0.01). RF IgA also negatively correlated with the physical domain of the PSS-QoL (ρ =-0.307, p<0.01) and the ESSPRI (ρ =-0.260, p<0.05), while IgG negatively

correlated with the psychosocial domain of the PSS-QoL (ρ =-0.245, p<0.05). The ProFaD-SSI/mental fatigue domain negatively correlated with IgG (ρ =-0.267, p<0.01) and RF IgA (ρ =-0.358, p<0.01). The Saxon test negatively correlated with all three immunological parameters, but especially with RF IgM (ρ =-0.607, p<0.01).

Discussion: It appears that throughout the course of the disease, the psychosocial burden increases more rapidly than the physical one, so psychological care should be part of a more holistic therapy approach. Future studies involving healthy control groups are necessary to eliminate possible cofactors like comorbidities, which have a higher prevalence in certain age groups. The quantity of serum rheumatoid factors and IgG positively and negatively correlated with different objective and subjective measures and their use as biomarkers should be investigated in future studies.

1. Introduction

The Sjögren's syndrome (SS) was first described by the Swedish physician Henrik Sjögren in the early 1900s. He described a group of women whose chronic arthritis was accompanied by dry eyes and dry mouth [1]. This disease can occur alone in which case it is known as primary Sjögren's syndrome (PSS). When Sjögren's syndrome occurs in association with another systemic autoimmune disease, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), scleroderma, or dermatomyositis, the term secondary Sjögren's syndrome is used [2].

1.1 Epidemiology

Primary Sjögren's syndrome mostly affects women (female:male ratio 9:1) with an onset age at approximately 45 years. According to the classification criteria of the American-European Consensus Group (AECG) the worldwide prevalence of PSS is approximately 0.2% with a yearly incidence of 4/100 000 [3]. According to Cornec and Chiche the calculated prevalence in Europe is about 39/100 000 (0.039%) based on a population of 2.5 million people, affecting one out of 2564 people. There is a large heterogeneity of single prevalence studies whose results range from 11.34 to 3790.09 per 100 000 people [4]. There are also over 200 well documented cases of childhood PSS in the pediatric medical literature [5].

1.2 Pathophysiology

Primary Sjögren's syndrome is a systemic autoimmune disease, characterized by lymphocytic infiltration of the secretory glands (mainly sialadenitis), which leads to cell death and hyposecretion. The pathophysiology is not completely understood, but it is assumed that T-cells and B-cells play an equally important role [6]. The presence and often predominance of $CD4^+$ cells (T helper cells) in salivary gland infiltrates indicate their important role in the pathogenesis of PSS [7]. T helper are hypothesized to be the main

subtype, since their co-receptor CD4 binds to major histocompatibility complex class 2 (MHC2) molecules, which are associated with PSS [6,8]. In addition increased levels of pro inflammatory Th 1 cytokines (e.g. interleukines 1b, 2 and 6, interferon-γ and tumor necrosis factor-α) were detected in the saliva of patients suffering from PSS [9].

The hypothesis that also B-cells play a role in the pathogenesis of PSS is based on elevated circulating and salivary gland tissue levels of the B-cell activating factor (BAFF), which is associated with a higher disease activity and a higher risk for non-Hodgkin B-cell lymphoma [10–12]. BAFF is responsible for the proliferation, maturation and survival of B-cells and is primarily induced by type I and type II interferons [13,14]. The production of these interferons is induced by Toll like receptors (TLRs), which can be activated by viruses (e.g. Epstein-Barr virus). Patients with PSS show increased activity of TLRs, but a specific cause has not yet been identified [15,16]. So it remains unclear how those changes in the adaptive immune system lead to the clinical manifestations of the disease.

1.3 Clinical features

Three main symptoms are present in more than 80% of the patients: Sicca syndrome (dryness of the mouth, nose, eyes, skin and vagina), fatigue and joint pain [2]. The clinical features can be divided in glandular and extra-glandular manifestations.

1.3.1 Glandular manifestations

Due to lymphocytic infiltration of the secretory glands, chronic or reoccurring parotitis paired with swelling is one of the most common early signs of PSS, especially in children [17]. Almost 20% of the patients suffer from parotid swelling at the time of diagnosis [18]. Patients with continual salivary gland swelling must be assessed for the presence of lymphoma (14-fold increased risk of MALT lymphoma in PSS) and a biopsy may be necessary [19].

Despite adequate oral hygiene, mouth dryness (xerostomia) can cause clinical symptoms such as difficulties swallowing, chewing, and speaking, as well as burning mouth, halitosis,

altered taste, dry buccal mucosa, glossitis, cracked and peeled lips, oral candidiasis, and dental caries [20].

Clinical manifestations of eye dryness include a stinging, burning or scratchy sensation in the eyes, redness of the eyes, sensitivity to light, a sensation of having something in your eyes, difficulties with nighttime driving, blurred vision or eye fatigue which can lead to enormous difficulties with reading [21]. This condition can result in increased tear osmolarity and levels of inflammatory mediators which can lead to keratoconjunctivitis sicca [22].

1.3.2 Extra-glandular manifestations

Inflammation of the smaller joints (hands and upper limbs) occurs in about 50% of all PSS-patients and is generally moderate (<5 joints). Patients may have arthralgia with inflammatory characteristics (morning stiffness > 30min) or sometimes symmetric polysynovitis [23].

Pulmonary manifestations are present in 9-20% of all PSS-patients. The most common manifestations are nonspecific interstitial pneumonia (45%) and usual interstitial pneumonia (16%). Also the lower airways can be affected, causing tracheobronchitis sicca and bronchiolitis [24].

Cutaneous involvement is relatively common among PSS-patients. About 9% suffer from annular erythema (AE), a rash characterized by a wide evaluated border and central pallor that may lead to hyperpigmentation. Purpura (cutaneous vasculitis) is the most common type of vasculitis in PSS-patients (88% of vasculitides). A rare, but severe type of vasculitis is cryoglobulinemia, which is also linked to an increased mortality rate and more likely occurs in patients with antibodies against the hepatitis C virus. The second most common cutaneous manifestation is ulcers (8%) [24–26].

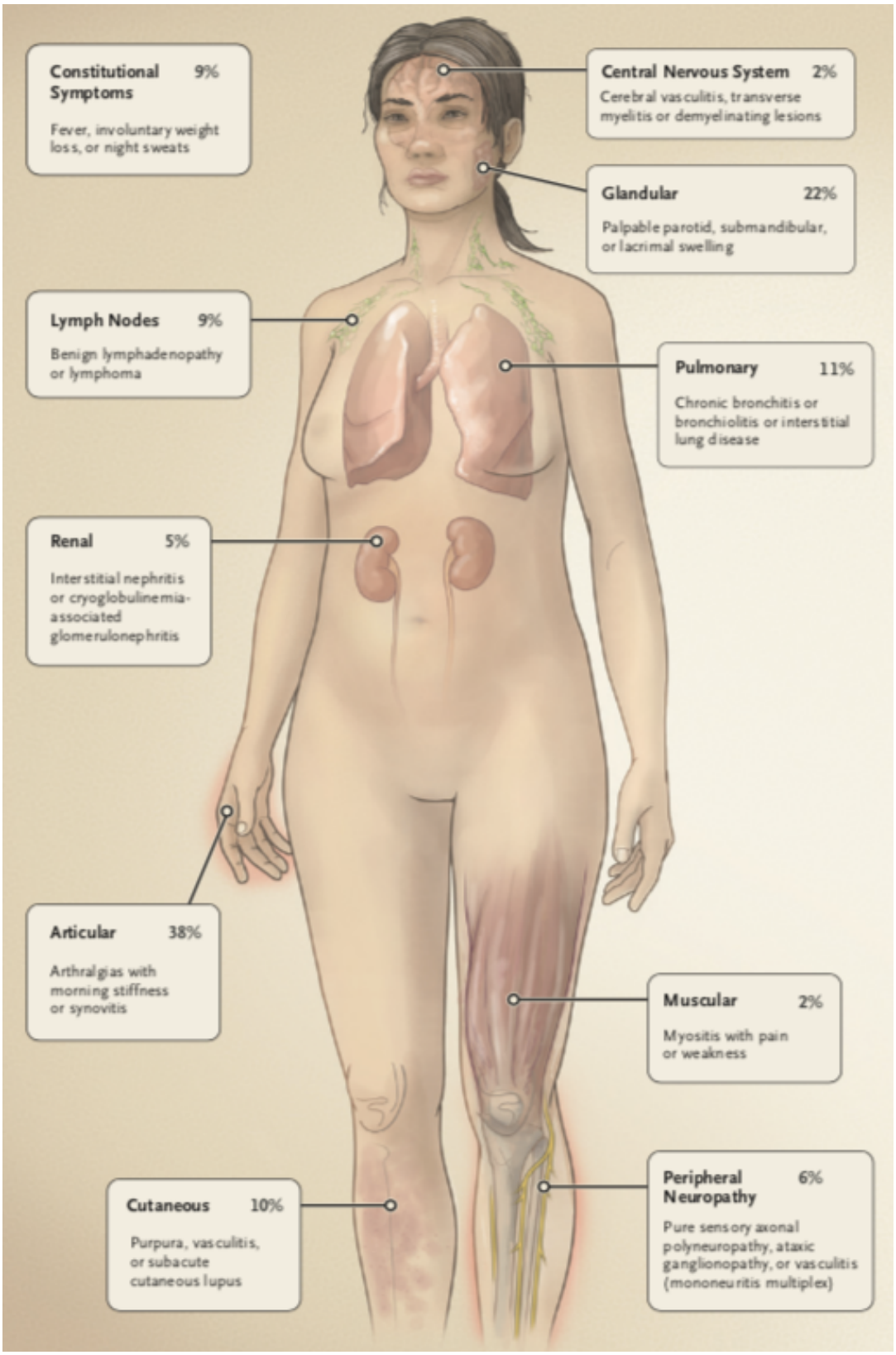
The major renal involvement associated with PSS is chronic tubulointerstitial nephritis; renal tubular acidosis (RTA) is the most common clinical manifestation and is caused by generalized renal tubule dysfunction, resulting in renal acid retention or bicarbonate loss. RTA is present in about 9% of PSS-patients [25].

Neurological manifestations of PSS are common (18–45% of patients) and affect both the central and peripheral nervous systems (including the sensitivomotor and autonomic nervous systems), with peripheral manifestations having a higher prevalence [24]. Some of the more common peripheral manifestations include neuropathies such as small fiber neuropathy and autonomic neuropathy.

Other extra-glandular manifestations include: constitutional symptoms like night sweat or weight loss, lymphadenopathy including lymphoma, PSS-associated celiac disease, hematological and immunological manifestations. [5].

Figure 1 gives an overview of the prevalence of different manifestations of PSS according to a paper from Mariette et al. from 2018 [2].

Figure 1: Prevalence of different manifestations of PSS [2]



Reproduced with permission from Mariette X, Criswell LA. Primary Sjögren's Syndrome. N Engl J Med [Internet]. 2018;378(10):931–9, Copyright Massachusetts Medical Society.

1.3.3 Fatigue

60-70% of PSS-patients suffer from fatigue, compared to approximately 20% in the general population [27]. Many patients describe it as „an ever-present, fluctuating, and uncontrollable lack of energy“ [28]. Fatigue can have many facets but can be divided into two main types: Mental fatigue and somatic fatigue. Mental fatigue mainly presents itself with a difficulty to concentrate and to memorize things, while somatic fatigue goes along with a lack of physical energy [29]. The biological basis for fatigue in PSS is widely unknown and proinflammatory mechanisms have been thought to play a role, but a connection between serum and tissue markers and fatigue has not been identified yet [30]. Anti-Sjögren's Syndrome-A/Ro antibody (Anti-Ro/SSA)-positive patients are not more likely to suffer from fatigue than seronegative patients [27]. A study from Bodewes et al. from 2019 compared over 1300 serum proteins between fatigued and non-fatigued patients and identified 16 differentially expressed proteins (DEPs). 14 of those were upregulated such as neuroactive synaptosomal-associated protein 25 (SNAP-25), alpha-enolase (ENO1) and ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1). A member of the epidermal growth factor superfamily (EGF) and formimidoyltransferase-cyclodeaminase (FTCD) were downregulated. This set of proteins forms a „fatigue-signature“ and can be useful to distinguish fatigued from non-fatigued patients [30].

1.4 Measurement of disease activity

There are two facets of clinical features for which two disease activity indexes have been developed by the European League Against Rheumatism (EULAR) Sjögren's Syndrome (SS) task force: the EULAR SS disease activity index (ESSDAI) for systemic features and the EULAR SS patient-reported index (ESSPRI) for patients' symptoms. "These indexes have been developed to be used as outcome measures in clinical trials and improve clinical research in the field of primary SS" [31]. The ESSDAI and the ESSPRI are explained in detail in the *Patients and Methods* section.

1.5 Measurement of health-related quality of life

The clinical manifestations mentioned above have a heavy impact on the psychosocial health and the health-related quality of life (HRQL). HRQL may be defined as the total burden of disease and therapy on a patient's daily individual and social life in the context of a chronic disease like PSS. The components of quality of life (physical, mental, and social aspects) that are related to an individual's health are referred to as HRQL [32]. A study pointed out the big impact of the disease on psychosocial health by showing that 33.8% of patients with PSS suffer from anxiety and 36.9% suffer from depression [33]. A helpful tool to assess the HRQL in patients with PSS is the PSS-Quality of life (PSS-QoL). The PSS-QoL is a questionnaire containing 25 questions about the patients' subjective well-being and can be divided into two main categories: physical (discomfort and dryness) and psychosocial. The PSS-QoL strongly correlates with the ESSPRI and moderately correlates with the Euro-QoL-5D (EQ-5D), a questionnaire about general HRQL and not specific to any disease [34].

1.6 Diagnosis

Often the diagnosis of primary Sjögren's syndrome is based on the presence of the classic symptoms of mouth and eye dryness, fatigue, and pain, that are present for a period of up to ten years before diagnosis. Although, systemic complications sometimes are the key to diagnosis. All patients presenting with the mentioned symptoms should be queried about manifestations of PSS and about the presence of other autoimmune diseases among family members [2].

1.6.1 Laboratory testing

Anti-Sjögren's Syndrome-A/Ro (SSA) and Anti-Sjögren's Syndrome-B/La (SSB) antibodies are present in two thirds of patients and the rheumatoid factor (RF) is present in approximately half of the patients. Those parameters should be routinely assessed when there is a suspicion of PSS. When there is as an absence of anti-SSA/SSB antibodies, a biopsy of minor salivary glands is recommended for establishing the diagnosis and is also an important tool for the estimation of prognosis [2].

Anti-SSA/Ro and Anti-SSB/La are directed against the Ro/La-ribonucleoprotein complexes and have been correlated with younger age, more severe exocrine gland dysfunction and a higher prevalence of extra-glandular manifestations. But there are more antibodies that are linked to certain clinical manifestations. Anti-nuclear antibodies (ANAs) and rheumatoid factors have also been associated to extra-glandular manifestations, while cryoglobulins are linked to a more severe disease progression, lymphoma development and death. Antibodies to cyclic citrullinated peptides (CCP) are rarely found in PSS-patients and have been linked in some cases to the development of non-erosive arthritis [35].

There are multiple studies exploring the roles of rheumatoid factors and immunoglobulin G (IgG) for prognosis and disease activity:

IgG: A study with 46 patients published in 2018 showed that there are no correlations between IgG and the ESSDAI and ESSPRI [36]. On the other hand, a longitudinal study from 2018 including 91 patients found out that after a ten-year period, patients with a high IgG titer (>1600 mg/dl) were more likely to have extra-glandular manifestations than patients with normal IgG serum levels. The study referred to extra-glandular manifestations included in the ESSDAI [37].

RF IgA: A study from Lee et al. from 2017 showed that high RF IgA levels have been linked to renal manifestations, but not to other extra-glandular manifestations. The study also indicated that RF IgA is associated with dry mouth and a lower unstimulated salivary flow rate (USFR). Furthermore, high RF IgA showed correlations with high RF IgM levels, high RF IgG levels, serum IgG levels and ANA levels. There were no associations found with age, duration of disease and the ESSDAI [38]. A different study showed a positive correlation between RF IgA and hypergammaglobulinemia [39].

1.6.2 Objectification of sicca symptoms

The Schirmer's test for determination of ocular dryness and the unstimulated salivary flow rate to assess oral dryness are the most common tools [2]. Another useful test for the objectification of oral dryness is the Saxon test. These three tests are explained in detail in the *Patients and Methods* section.

1.6.3 ACR-EULAR criteria

The American College of Rheumatology (ACR) – European League against Rheumatism (EULAR) guidelines were created for classification purposes, but they can also be helpful in determining a diagnosis of primary Sjögren's syndrome in the presence of dryness symptoms [2].

2016 ACR-EULAR criteria: "These classification criteria are based on the weighted sum of five items: anti-SSA/Ro antibody positivity and focal lymphocytic sialadenitis with a focus score of ≥ 1 foci/4 mm^2, each scoring 3; an ocular staining score of ≥ 5 (or van Bijsterveld score of ≥ 4), a Schirmer's test result of ≤ 5 mm/5 minutes, and an unstimulated salivary flow rate of ≤ 0.1 ml/minute, each scoring 1. Individuals with signs and/or symptoms suggestive of Sjögren's syndrome who have a total score of ≥ 4 for the above items meet the criteria for PSS." [40]

1.7 Treatment

1.7.1 Management of sicca symptoms and fatigue

For most patients the therapy objective is to improve quality of life by treating sicca and fatigue symptoms. This can be very challenging for physicians since evidence-based treatment options are scarce and focus only on symptomatic therapy [41].

The first step for the treatment of dry eyes is educating the patient about harmful environmental factors (e.g. air-conditioning systems). Pharmacological treatment options for dry eyes include the topical application of tear substitutes, cyclosporine A, or glucocorticoids. Another method to increase the tear and saliva production is the oral application of muscarinic agonists such as pilocarpine and cevimeline [41]. In severe cases of eye dryness caused by an occlusion of the tear duct, the application of punctal plugs or cauterization has to be considered [1].

The treatment of xerostomia includes the application of oral muscarinic agonists, as well as education of the patient especially about drugs that can induce or worsen xerostomia (e.g.

anticholinergics) [41]. In addition different saliva substitutes or chewing gums containing xylitol can be used to improve the symptoms [42].

The treatment options for fatigue are very scarce and focus around maintaining good sleep hygiene and aerobic endurance training. The benefit of a therapy with hydroxychlorine or rituximab is still not proven and the therapy approach should focus on non-pharmaceutical treatment options. Further research on the pathogenesis of fatigue in PSS-patients is needed to find more target-orientated approaches [43].

1.7.2 Management of systemic manifestations

While its efficacy is highly debated due to a lack of well-designed clinical trials, hydroxychloroquine is the first-line treatment for a variety of mild to moderate systemic manifestations such as arthralgia, arthritis and cutaneous lesions. For severe organ manifestations, high-dose methylprednisolone and cyclophosphamide have been shown to be efficient. The recommended treatment options for severe vasculitis are rituximab or plasmapheresis [41]. Rituximab is also recommended for patients with serious organ manifestations where more conservative and less costly therapies fail, but should be considered as a third-line therapy, since it still has no proven effect on the outcome, except for PSS-patients with cryoglobulinemia where it should be considered as a first-line therapy [24,44].

1.7.3 EULAR recommendations

In 2020 the EULAR published new recommendations for the management of PSS-patients [45]. Table 1 below gives an overview of all treatment options and their level of evidence.

Table 1: Treatment recommendations by the EULAR [45]

	LoE	GoR	Vote (%)	LoA (0–10)
A.Patients with PSS should be managed at, or in close collaboration with, centres of expertise following a multidisciplinary approach	NA	NA	90	9.2
B.The first therapeutic approach for dryness should be symptomatic relief using topical therapies	NA	NA	93	8.9
C.Systemic therapies may be considered for the treatment of active systemic disease	NA	NA	90	9.1
1.Baseline evaluation of salivary gland function is recommended before starting treatment for oral dryness	5	D	81	8.7
2.The preferred first therapeutic approach for oral dryness according to salivary gland function may be:	1a/*1b	B	88	8.7
2.1. Non-pharmacological stimulation for mild dysfunction;				
2.2. Pharmacological stimulation* for moderate dysfunction;				
2.3. Saliva substitution for severe dysfunction				
3.The first-line therapeutic approach to ocular dryness includes the use of artificial tears and ocular gels/ointments	1a	B	98	9.5
4.Refractory/severe ocular dryness may be managed using topical immunosuppressive-containing drops* and autologous serum eye drops	1a/*1b	B/D	94	9.1
5.Concomitant diseases should be evaluated in patients presenting with fatigue/pain, whose severity should be scored using specific tools	5	D	93	9.0
6.Consider analgesics or other pain-modifying agents for musculoskeletal pain, considering the balance between potential benefits and side-effects	4	C	89	8.9
7.Treatment of systemic disease should be tailored to organ-specific severity using the ESSDAI definitions	4	C	89	9.0
8.Glucocorticoids should be used at the minimum dose and length of time necessary to control active systemic disease	4	C	85	9.6
9.Immunosuppressive agents should be mainly used as GC-sparing agents, with no evidence supporting the choice of one agent over another	4	C	82	8.9
10.B-cell targeted therapies may be considered in patients with severe, refractory systemic disease	1b	B	98	8.6
11.The systemic organ-specific therapeutic approach may follow, as a general rule, the sequential (or combined) use of GCs, immunosuppressive agents and biologics	5	D	98	8.6
12.Treatment of B-cell lymphoma should be individualised according to the specific histological subtype and disease stage	4	C	88	9.7
LoE and GoR according to the Oxford Centre for Evidence-based Medicine—LoE (March 2009). Vote (%): % of participants scoring the recommendation as at least ‘important’ (score of ≥4 on 5-point scale). LoA: mean score (scale of ‘0’ as no agreement, ‘10’ full agreement).				
ESSDAI, EULAR Sjögren's syndrome disease activity index; EULAR, European League Against Rheumatism; GC, glucocorticoid; GoR, grade of recommendation; LoA, levels of agreement; LoE, levels of evidence; NA, not applicable.				

To sum up the situation regarding treatment of PSS, Mariette stated in 2018 that the heterogeneity of the disease and the varying results of therapeutic trials require a more individualized approach to therapy to achieve improved long-term outcomes in PSS-patients [2].

1.8 Research objectives

1.8.1 Aim I: Detection of possible factors that are linked to a change of patient-reported outcomes and quality of life

There are multiple studies about the course of the disease on the basis of objective measures. But little is known how the patient-reported outcomes and the subjective feeling of quality of life change over time and what factors go along with a worsening or improvement in the patient's subjective well-being. Therefore, one aim of this study was to verify or falsify the following hypothesis: *There is a connection between patient-reported outcomes and a variety of objective measures that may be influencing factors.*

1.8.2 Aim II: Detection of differences in the course of the disease in different age groups

It is known that with increasing age the prevalence of sicca symptoms also increases. That can be due to side effects of different medications or an age-based decrease of exocrine gland function, meaning higher age may be linked to a more rapid progression of sicca symptoms in PSS-patients [46]. So, a plausible hypothesis is that *there are multiple factors that affect the course of the disease differently in each age group.* The findings obtained in this work may help to get a better understanding of influencing factors in different age groups and may help to find the right treatment options for different age groups.

1.8.3 Aim III: Exploring possible impacts of immunological parameters on patient-reported outcomes and sicca symptoms

Correlations between increased levels of rheumatoid factors or IgG and patient-reported outcomes have not been broadly investigated yet. Although one study was performed indicating that there is a negative correlation between IgG levels and fatigue [27]. So another aim of this study was to verify the hypothesis that *the quantity of serum IgG and rheumatoid factors IgA and IgM correlates with subjective well-being and also objective tests for sicca symptoms.* The obtained findings may be helpful for the establishment of new biomarkers.

1.8.4 Aim IV: Detection of correlations between subjective and objective tests for sicca symptoms

Correlations between objective tests like the Saxon test and the unstimulated salivary flow rate for xerostomia as well as the Schirmer's test for eye dryness and PSS-QoL/mouth and the PSS-QoL/eye domains have not been investigated yet. Which makes it important to verify the hypothesis that *the PSS-QoL sicca domains correlate with objective tests for sicca symptoms.* Therefore, the findings may be important for establishing the PSS-QoL as a measurement tool in future studies and its use for the evaluation of the success of therapies.

2 Patients and Methods

Patients who suffer from PSS treated at the rheumatology outpatient clinic of the Medical University of Graz were asked to participate in a prospective cohort study.

Patient data were collected between February 2018 and October 2019. This project was approved by the institutional review board of the Medical University of Graz and written informed consent was obtained of each patient. All patients fulfilled the 2016 ACR-EULAR criteria [40] and were over 18 years of age.

2.1 Patient reported outcomes

PSS-QoL (Primary Sjögren's syndrome – Quality of Life): The questionnaire contains 25 questions and consists of a physical (page 1 and 2) and a psychosocial dimension (page 3) and collects information about the patients' reported HRQL within the last four weeks. The possible score ranges from 0 to 96 points for women and from 0 to 92 points for men, due to questions regarding vaginal dryness. To detect the severity of pain the first question of the physical part is scored on a numeric rating scale (NRS), ranging from 0 to 10 (the marked number is added to the score). 0 stands for "no pain" and 10 stands for "unbearable pain". The remaining questions regarding physical symptoms ask whether certain symptoms are present or not. A marked checkbox stands for a "yes" and counts as one point. The psychosocial part consists of 14 questions/statements and can be scored on a five-point Likert scale where points range from 0 (=never) to 4 (=always) [34].
The German version of the *PSS-QoL* is stated in Figure 2.

The *PSS-QoL* was organized into the following domains for this study:

- PSS-QoL/total: Questions 1-25 (0-96 points for women and 0-92 points for men)
    - PSS-QoL/physical: Questions 1-11 (0-40 points for women and 0-36 points for men)
    - PSS-QoL/psychosocial: Questions 12-25 (0-56 points)

- PSS-QoL/discomfort: Questions 1-6 (0-15 points)
- PSS-QoL/dryness: Questions 7-11 (0-25 points for women and 0-21 points for men)
    - PSS-QoL/mouth: Question 7 (0-7 points)
    - PSS-QoL/eye: Question 8 (0-8 points)
    - PSS-QoL/vagina: Question 11 (0-4 points)

Figure 2: German version of the PSS-QoL [34]

Fragebogen zur Einschätzung der Lebensqualität bei PatientInnen mit dem primären Sjögren$ Syndrom

Die nachfolgenden Fragen beziehen sich auf Ihre Beschwerden innerhalb der letzten vier Wochen.

Wie stark waren Ihre Schmerzen?

0	1	2	3	4	5	6	7	8	9	10

Keine Schmerzen **unerträgliche Schmerzen**

Ich hatte Schmerzen in den Gelenken

o Nein o Ja

Ich hatte immer wiederkehrende, wandernde Schmerzen

o Nein o Ja

Ich hatte Verdauungsprobleme

Verstopfung: o Nein o Ja

Magen/Bauchschmerzen: o Nein o Ja

Ich hatte Probleme beim Schlafen

o Nein o Ja

Spüren Sie eine Trockenheit im Mund?

o Nein o Ja

Wenn ja: Hatten Sie folgende zusätzliche Beschwerden? (Mehrfachantworten möglich)

- o Brennen im Mund
- o Schwierigkeiten beim Sprechen
- o Zahnprobleme
- o Veränderter Geschmacksinn
- o Schwierigkeiten beim Essen trockener Speisen
- o Zwang, in der Nacht etwas trinken zu müssen

Spüren Sie eine Trockenheit in den Augen?

o Nein o Ja

Wenn ja: Hatten Sie folgende zusätzliche Beschwerden? (Mehrfachantworten möglich)

- o Wiederkehrende Entzündungen
- o Schmerzen
- o Sandkorngefühl
- o Verklebte Augen
- o Verschlechterte Sehkraft
- o Keine Tränenflüssigkeit (weinen ist nicht möglich)
- o Alltagsaktivitäten wie Autofahren, lesen und fernsehen sind eingeschränkt bis gar nicht möglich

Spüren Sie eine Trockenheit Ihrer Haut?

o Nein o Ja

Wenn ja: Hatten Sie folgende zusätzliche Beschwerden? (Mehrfachantworten möglich)

- o Rötungen der Haut
- o Die Haut spannt

Spüren Sie eine Trockenheit im Nasenbereich?

o Nein o Ja

Wenn ja: Hatten Sie folgende zusätzliche Beschwerden? (Mehrfachantworten möglich)

- o Veränderung des Geruchsinns
- o Nasenbluten

Folgende Frage ist nur von Frauen zu beantworten:

Spüren Sie, dass Ihre Scheide trocken ist?

o Nein o Ja

Wenn ja: Hatten Sie folgende zusätzliche Beschwerden? (Mehrfachantworten möglich)

- o Allgemeine Schmerzen
- o Juckreiz
- o Schmerzen beim Geschlechtsverkehr

Bitte kreuzen Sie an, inwieweit folgende Aussagen auf Sie zutreffen:

	Nie	Selten	Manchmal	Oft	Immer
Ich habe das Gefühl, dass - ich die einzige mit diesen Beschwerden bin - meine Beschwerden nicht ernst genommen werden - ich mit meinen Beschwerden überfordert bin - meine Familie/Freunde Verständnis für mich zeigen	☐	☐	☐	☐	☐
Ich bin zu müde um Verabredungen mit der Familie/Freunden einzuhalten	☐	☐	☐	☐	☐
Ich ziehe mich zurück	☐	☐	☐	☐	☐
Ich habe Angst vor Nebenwirkungen	☐	☐	☐	☐	☐
Ich habe Angst vor dem weiteren Verlauf meiner Erkrankung	☐	☐	☐	☐	☐
Ich fühle mich wohl in meinem Körper	☐	☐	☐	☐	☐
Ich schaffe in meinem Alltag weniger, als vor Krankheitsbeginn	☐	☐	☐	☐	☐
Ich werde schnell müde	☐	☐	☐	☐	☐
Alltagsaktivitäten, wie Auto fahren, Arbeiten, Haushalt, sportliche Aktivität sind eine Herausforderung	☐	☐	☐	☐	☐
Hilfsmittel, wie Augentropfen, Cremes und Physiotherapie stellen für mich eine finanzielle Belastung dar	☐	☐	☐	☐	☐
Meine Lebensqualität ist durch die Erkrankung eingeschränkt	☐	☐	☐	☐	☐

EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI): The *ESSPRI* is a very simple tool to measure the patients' subjective symptoms. It consists of three questions asking about how severe the symptoms pain, fatigue and dryness have been within the last two weeks. The questions are answered by marking a number on a NRS ranging from 0 to 10, where 0 means "no symptoms" and 10 means "maximum imaginable symptoms" [47]. The total ESSPRI score is defined as the mean value of all three answers and therefore can range from 0 to 10. The patient acceptable symptom state (PASS) is defined as an ESSPRI<5 [31].

Ocular Surface Disease Index (OSDI)©: The *OSDI©*, developed by the Outcomes Research Group at Allergan, is a questionnaire to obtain information about dealing with dry eye syndrome. The twelve questions relate to experiences within the last week. The questionnaire is graded on a five-point Likert scale from 0 to 4, where 0 means "none of the time" and 4 means "all of the time". The total score is calculated on the basis of the following formula:

OSDI© = [(sum of scores for all questions answered) × 100] / [(total number of questions answered) × 4].

Therefore the score can range from 0 to 100 [48].

Xerostomia Inventory: The *Xerostomia Inventory (XI)* is an eleven-question tool developed to evaluate the symptoms of xerostomia. This test is scored on a five-point Likert scale that rates the frequency of experiencing xerostomia symptoms from 1 = "Never" to 5 = "Very often". The score ranges from 11 (no xerostomia) to 55 (severe xerostomia) [49].

Profile of Fatigue and Discomfort – Sicca Symptoms Inventory (ProFaD-SSI): The *ProFaD-SSI* was developed as a patient-reported assessment tool for use in PSS and other rheumatic diseases [29]. For this study the short form (19 questions) was used. The test is scored on a numeric scale ranging from 0 to 7, where 0 represents not having the mentioned condition at all and 7 represents "as bad as imaginable" and is asking the patients about their experiences within the last two weeks. It is divided into the following parts which should be calculated separately [50]:

- Somatic fatigue: Questions about feeling physically tired. The score is the mean value of question 1 to 4.

- Mental fatigue: Questions about the ability to concentrate and memorize things. The score is the mean value of question 5 and 6.
- Profile of discomfort and sicca symptomology: questions about general discomfort (question 7-14)

The "Sicca questionnaire": This questionnaire was developed by the Division of Rheumatology and Immunology, Department of Internal Medicine, Medical University of Graz, and is used for patients at the outpatient clinic. It contains ten questions about the severity of sicca symptoms and can be scored on a 100 mm visual analogue scale (VAS). It ranges from "no symptoms" to "as bad as imaginable". The mean value of all ten questions forms the "global sicca" score and the mean value of the first six questions forms the "eye sicca" score.

2.2 Clinical parameters

The following demographic data were collected: Age, sex, smoking habit, height, weight, date of diagnosis, time from onset of symptoms to diagnosis, duration of disease (time from diagnosis to evaluation date), age when diagnosed with PSS, age at onset of symptoms, co-existing diseases, medication.

The following laboratory parameters were assessed:

- *Leucocytes in 10^9/l*
- *Erythrocytes in 10^6/µl*
- *Haemoglobin in g/dl*
- *Thrombocytes in 10^9/l*
- *Neutrophil granulocytes in 10^9/l*
- *Lymphocytes 10^9/l*
- *C-reactive proteine (CRP) in mg/l*
- *Erythrocyte sedimentation rate (ESR) in mm (after first hour)*
- *Lactate in mmol/l*

- *pH-value*
- *Standard bicarbonate (HCO3-) in mmol/l*
- *Base excess (BE) in mmol/l*
- *Immunoglobulin A (IgA) in U/ml*
- *Immunoglobulin M (IgM) in U/ml*
- *Immunoglobulin G (IgG) in U/ml*
- *Immunoglobulin G1-G4 (Ig G1-G4) in U/ml*
- *Kappa free light chains (FL Kappa) in mg/l*
- *Lambda free light chains (FL Lambda) in mg/l*
- *Rheumatoid factor Immunoglobulin A (RF IgA) in U/ml**
- *Rheumatoid factor Immunoglobulin M (RF IgM) in U/ml*
- *Protein/Creatinine ratio in urine in µg/mmol*
- *Beta-2 microglobuline in µg/l*
- *Beta-2 microglobuline in urine in µg/l*
- *N-acetyl-beta-glucosaminidase in urine (Beta-NAG in urine) in U/l*
- *Albumine in percent of total protein*
- *α_1-Globuline in percent of total protein*
- *α_2-Globuline in percent of total protein*
- *β-Globuline in percent of total protein*
- *γ-Globuline in percent of total protein*
- *Complement factor 3 (C3) in g/l*
- *Complement factor 4 (C4) in g/l*
- *Complement total (CH50) in U/ml*
- *Antinuclear antibodies (ANA) in dilution ratio (1:x)*
- *Anti-Ro52/SSA antibodies in U/ml*
- *Anti-Ro60/SSA antibodies in U/ml*
- *Anti-La/SSB antibodies in U/ml*
- *Anti-cyclic citrullinated peptide antibodies (CCP) in U/ml*
- *Basal thyroid-stimulating hormone (TSH) in mU/l*
- *Cryoglobulins in positive/negative*

*****: Values over 500 U/ml were not quantifiable and are indicated as 500 U/ml.

Focus score: The focus score is a tool for the objectification of chronic lymphocytic sialadenitis. It equals the number of foci with lymphocyte infiltration (>50 lymphocytes) per 4 mm^2 of Labial salivary gland tissue acquisitioned by biopsy [51]. A focus score ≥1 foci/4 mm^2 is one of five disease classifying criteria for PSS according to the 2016 ACR-EULAR criteria [40].

Schirmer's test: For the Schirmer's test a strip of 35 mm x 5 mm filter paper is placed in the lower eyelid pouch of each eye and the patient is asked to gently close his eyes. After five minutes the strips are removed and the distance the moisture has traveled on the strip is measured. Less than 10 mm of moisture on the filter paper is considered abnormal, although many practitioners only consider values of less than 5 mm to be significant, which is also the cut-off value for the 2016 ACR-EULAR criteria [40,52].

Saxon test: The Saxon test is a simple, reproducible, and low-cost test for xerostomia, where patients are asked to chew on a sterile gauze ball for exactly two minutes. The amount of saliva produced is quantitated by weighing the ball before and after chewing. Healthy individuals should produce ≥ 2.75g/2min [53].

Unstimulated salivary flow rate: For this simple test patients are asked to spit any saliva that accumulates in the floor of their mouth into a pre-weighed tube. This test is performed for five minutes and the amount of produced saliva is expressed as milliliters per minute. Healthy individuals produce between 0.3 and 0.5 ml/min. Rates between 0.10 and 0.01 ml/min are considered as hyposalivation [54], which is also a criterion for PSS according to the 2016 ACR-EULAR criteria [40].

Physician's global assessment (PhGA): The PhGA is the subjective disease activity graded by a physician on a scale from 0 (no disease activity) to 10 (high disease activity).

EULAR Sjögren's syndrome disease activity index (ESSDAI): The ESSDAI is a clinical index designed to measure disease activity in patients with PSS. This index consists of twelve domains, ten related to extra-glandular manifestations, one hematological and one biological domain reflecting B-cell activity [55]. None of these domains include any aspects of dryness. The levels of activity of each domain range from 0 to 3 points (0 = „no activity", 1 = „low activity", 2 = „moderate activity", 3 = „high activity") and are multiplied by their respective weights (ranging from 1 to 7 points) to obtain the total score [56]. The score can

range from 0 to 123 points and disease activity can be defined as low-activity (ESSDAI < 5), moderate-activity (5 ≤ ESSDAI ≤ 13) and high-activity (ESSDAI ≥ 14) [31].

2.3 Statistical analysis

All test evaluations were performed using the program *SPSS*© V25.0 (IBM, Chicago, IL, USA). The level of significance was chosen at 5 %.

The unpaired t-test was used to compare two normally distributed (parametric) groups ($p<0.05$). The Mann-Whitney U test was performed to compare not normally distributed (non-parametric) parameters. Normal distribution was examined by Shapiro Wilk test.

For comparison of three or more groups with normally distributed data, a one-way analysis of variance (one-way ANOVA) was performed. Homogeneity of variances was asserted using the Levene's Test. If the Levene's test was significant ($p<0.05$), the homogeneity of variances was not given and the Welch-ANOVA was interpreted. If the Levene's test yielded $p>0.05$, equal variances could be assumed and the one-way ANOVA was interpreted. When there was a significant difference between groups, post-hoc tests were performed to find out which of the groups differed. If homogeneity of variances was given, the Tukey post-hoc test was interpreted, otherwise if the homogeneity of variances was not given, the Games-Howell post-hoc test was interpreted. If groups were not normally distributed, the Kruskal-Wallis test was performed. If this test was significant, the Mann-Whitney-U test was performed on any possible combination of two groups to discover which groups differed.

Clinical and immunological parameters were correlated by using the Spearman's rho rank correlation coefficient (ρ).

The results were interpreted the following way:

- ρ <0.1: No correlation
- 0.1≤ ρ <0.3: Weak correlation
- 0.3≤ ρ <0.5: Moderate correlation
- ρ >0.5: Strong correlation
- Negative values for ρ were considered as negative correlations.
- The significancy level was at $p<0.05$.

Furthermore, the correlation between subjective and objective tests for mouth and eye dryness was determined.

2.3.1 Longitudinal analysis

The PSS-QoL was completed from 52 patients of this cohort in 2016 for the first time. Additionally, data from the ESSPRI, the "sicca questionnaire" and the ESSDAI were available.

These data were compared with recent parameters by using the paired t-test (parametric) or the Wilcoxon signed-rank test (non-parametric). The following parameters were compared that way: PSS-QoL/total, PSS-QoL/physical, PSS-QoL/psychosocial, PSS-QoL/dryness, PSS-QoL/discomfort, PSS-QoL/mouth, PSS-QoL/eyes, ESSPRI, ESSPRI/fatigue, ESSPRI/pain, ESSPRI/dryness, Global sicca, Eye sicca and the ESSDAI.

The PSS-QoL-change score was determined (difference between PSS-QoL (from 2016) and recent PSS-QoL): An increase of PSS-QoL score was considered as "worse" and a decrease was considered as "better". Further, the minimal detectable change (MDC) was identified to define a cut-off for clinically important change. "The MDC that would be found to be statistically significantly different from zero (no change) at a given level of confidence. This is calculated using the formula: MDC (95% confidence level) = 1.96 * $\sqrt{}$2 * SEM (standard error of measurement). The SEM can be estimated by the standard deviation of baseline scores multiplied by the square root of one minus the reliability coefficient" [57]. The MDC was then applied to define the groups: "Same" = − MDC < Diff. (difference between test scores) < MDC, "Better" = Diff. ≤ − MDC, "Worse" = Diff. ≥ MDC. The MDC was calculated for the PSS-QoL/total, the PSS-QoL/physical, the PSS-QoL/psychosocial and the PSS-QoL/dryness. Different clinical parameters from the follow-up time where then compared between the groups.

2.3.2 Immunological parameters

Patients were divided into two groups (normal, high/positive) according to IgG (normal = <16 U/ml, high = ≥16 U/ml), RF IgA (positive = >20U/mL) and RF IgM (positive = >14 U/mL). The unpaired t-test and the Mann-Whitney-U test were performed to detect any significant differences ($p<0.05$) between the groups. The following variables were compared across the groups: ESSPRI, PSS-QoL/total, PSS-QoL/physical, PSS-QoL/discomfort, PSS-

QoL/dryness, PSS-QoL/psychosocial, PSS-QoL/mouth, PSS QoL/eye, PSS-QoL/vagina, duration of disease, Saxon test, unstimulated salivary flow rate, Schirmer's test, OSDI©, Xerostomia Inventory, ProFaD/somatic fatigue, ProFaD/mental fatigue, and ProFaD/general discomfort.

2.3.3 The ESSDAI score

According to their ESSDAI score the patients were divided into two groups (ESSDAI<5=low disease activity, ESSDAI score ≥5= moderate to high disease activity). The unpaired t-test and the Mann-Whitney-U test were performed to detect any significant differences ($p<0.05$) between the groups.

2.4 Tables

Normally distributed data are indicated with their mean value and their standard deviation (SD) and not normally distributed data are indicated with their median and range.

Tables and graphs shown in this work were created with *Microsoft Excel 2011*© (Microsoft, Redmond, WA, USA) and *SPSS*© V25.0 (IBM, Chicago, IL, USA)

3 Results

3.1 Demographics

Two patients had to be excluded, since their PSS diagnosis was changed. Data of 109 PSS-patients were available: 100 patients were female (93.5%). The mean age was 60.31 (±12.23) years and the median disease duration was 6 (0-23) years. The median PSS-QoL/total score was 33 (8-72). Demographic data are depicted in Table 2.

Table 2: Demographic table

		Count (%)	Mean ± SD	Median [range]
Sex	Female	100 (93.5)		
	Male	7 (6.5)		
Age (years)			60.31 ± 12.23	
Duration of disease (years)				6 [0-23]
Age at diagnosis (years)			53.29 ± 12.78	
Age at fist onset (years)			47.65 ± 13.56	
Time from onset to diagnosis (years)				3 [0-21]
ESSDAI				2 [0-14]
Low activity (ESSDAI < 5)		73 (68.2)		
Moderate activity (5 ≤ ESSDAI ≤ 13)		29 (27.1)		
High activity (ESSDAI ≥ 14)		2 (1.9)		
ESSPRI				4.33 [0.33-9.33]
PASS (ESSPRI <5)		60 (56.1)		
PhGA				2 [0-7]
PSS-QoL/Total				33 [8-72]
PSS-QoL/Discomfort				6 [0-15]
PSS-QoL/Dryness			9.49 ± 4.01	
PSS-QoL/Physical				15 [4-32]
PSS-QoL/Psychosocial				18 [0-43]
PSS-QoL/mouth				3 [0-7]
PSS-QoL/eye				3 [0-8]
PSS-QoL/vagina				2 [0-3]
Xerostomia Inventory			24.94 ± 9.15	
ProFaD/Somatic fatigue				2.25 [0-7]
ProFaD/Mental fatigue				2.5 [0-7]
ProFaD/General discomfort				13.2 [0-38]
OSDI®			42.62 ± 20.94	
Global sicca				37.85 [1.3-78.7]
Eye sicca				33.25 [0.67-85]
IgG (U/ml)				14.3 [6.51-37.2]
Schirmer's test (mm)				2 [0-35]
Saxon test (g/2min)				1.5 [0-4.5]
Unstim. salivary flow rate (ml/min)				0.1 [0-1]

SD: Standard deviation; ESSDAI: EULAR Sjögren's syndrome disease activity index; ESSPRI: EULAR Sjögren's Syndrome patient-reported index; PASS: patient acceptable symptom state; PhGA: Physician's global assessment; PSS-QoL: Primary Sjögren's syndrome – Quality of Life; ProFaD: Profile of fatigue and discomfort; OSDI©: Ocular surface disease index; IgG: Immunoglobulin G

3.2 Drug therapy

Two thirds of the PSS-patients (65.5%) were drug-treated. No patients took more than three different drugs. Pilocarpine hydrochloride (Salagen®), a muscarinic agonist, was the most subscribed substance (43.9%). Treatment details are shown in table 3.

Table 3: Drug therapy

Drug Therapy	
Number of Drugs	**Count (%)**
0	38 (35.5)
1	51 (47.7)
2	15 (14)
3	3 (2.8)
Drugs	
Pilocarpine hydrochloride	47 (43.9)
Antimalarials	23 (21.5)
Other Immunosuppressants	13 (12.1)
Glucocorticoids	7 (6.5)

Table 4 shows the difference between drug groups (none, one, two or three different drugs) and clinical parameters. Especially the PSS-QoL/dryness score was significantly (p=0.009) higher in the group that took two or three drugs (11.89 ±4.79) compared to the group that took no drugs (8.54 ±3.65).

Table 4: Differences by the number of drugs

						Difference between groups		Post-hoc test	Diff. n.p. groups
Variable (✓= normally distributed)	Number of drugs	Mean/ Median	SD/Min.-Max.		N	ANOVA	Kruskal-Wallis	Tukey/Games-Howell	Mann-Whitney-U
PhGA	1) none	2.00	0.00	7.00	29		0.033		1/3=0.009 2/3=0.036
	2) one	2.00	0.00	5.00	46				
	3) two or three	3.00	1.00	5.00	15				
Lymphocytes ✓	1) none	1.39	0.44		33	0.022		2/3=0.02	
	2) one	1.56	0.58		49				
	3) two or three	1.18	0.45		18				
PSS-QoL/dryness ✓	1) none	8.54	3.65		37	0.012		1/3=0.009	
	2) one	9.33	3.70		51				
	3) two or three	11.89	4.79		18				

Mean/Median, SD/Min.-Max.: For variables marked with a "✓" (normally distributed), the mean value and the standard deviation (SD) are shown. For other variables the median and the range are shown. N: The number of patients falling into that group; Diff: Difference; n.p.: non-parametric; x/y: This shows the number of the groups that differ.

3.3 Age

Patients were classified to the following age groups: younger than 50, 50-59, 60-69, older than 70.

The significant differences between clinical variables and age groups are shown in table 5.
The PSS-QoL/total showed the lowest median value in the age group “younger than 50” (21 [8-61]) and the highest level in the age group “50-59” (35.5 [11-68]).
The PSS-Qol/physical showed the highest level in the age group “50-59” (17 [8-32]). The Global sicca score also showed the highest value in that age group (44.8 [4.8-78.7]). The PSS-QoL/psychosocial showed the highest level in the age group “older than 70” (25.5 [6-43]). The ProFaD/mental fatigue was also the highest in the oldest group (2.75 [0-7])
The OSDI© and the Eye sicca score both showed the highest value in the group ”50-59” (OSDI©: 24.23 ±22.86; Eye sicca: 44.37 ±22.63).
The median RF IgA level showed a decline with increasing age (Kruskal-Wallis: $p<0.001$).

Table 5: Differences by age

						Difference between groups		Post-hoc tests for ANOVA		Diff. n.p. groups
Variable (✓= normally distributed)	Age Croups	Mean/Median	SD/Min.	-Max.	N	ANOVA	Kruskal-Wallis	Tukey/Games-Howell		Mann-Whitney-U
CRP	1) younger than 50	0.70	0.40	4.70	21		0.003			1/2=0.003 1/3=0.004 1/4=0.001
	2) 50-59	2.27	0.10	9.70	26					
	3) 60-69	1.20	0.60	6.90	30					
	4) older than 70	1.80	0.60	19.90	27					
Leucocytes ✓	1) younger than 50	4.86	1.44		21	0.031		n.s.		
	2) 50-59	4.82	1.21		26					
	3) 60-69	5.49	1.46		32					
	4) older than 70	5.84	1.62		27					
α2-Globuline ✓	1) younger than 50	8.87	1.44		20	0.011		1/3=0.011 1/4=0.033		
	2) 50-59	9.54	1.15		23					
	3) 60-69	10.15	1.59		32					
	4) older than 70	10.06	1.42		25					
Eye sicca ✓	1) younger than 50	30.25	23.01		21	0.032		2/3=0.039		
	2) 50-59	44.37	22.63		26					
	3) 60-69	29.89	14.8		32					
	4) older than 70	37.56	21.11		23					
PSS-QoL/physical	1) younger than 50	10.00	4.00	27.00	21		0.020			1/2=0.003 1/3=0.008
	2) 50-59	17.00	8.00	32.00	25					
	3) 60-69	16.00	7.00	27.00	31					
	4) older than 70	15.50	6.00	29.00	26					
PSS-QoL/psychosocial	1) younger than 50	11.00	0.00	35.00	20		0.009			1/2=0.006 1/3=0.018 1/4=0.003
	2) 50-59	19.50	7.00	38.00	26					
	3) 60-69	17.50	4.00	40.00	32					
	4) older than 70	25.50	6.00	43.00	26					
PSS-QoL/total	1) younger than 50	21.00	8.00	61.00	21		0.015			1/2=0.005 1/3=0.010 1/4=0.006
	2) 50-59	35.50	11.00	68.00	26					
	3) 60-69	32.50	12.00	62.00	32					
	4) older than 70	35.00	12.00	72.00	27					
OSDI® ✓	1) younger than 50	15.33	19.57		21	0.010			1/2=0.009	
	2) 50-59	24.23	22.86		26					
	3) 60-69	17.87	17.63		30					
	4) older than 70	19.57	20.04		23					
β-NAG-Urine	1) younger than 50	0.29	0.12	0.75	19		0.001			1/2=0.004 1/3=0.022 1/4=<0.001 3/4=0.025
	2) 50-59	0.51	0.27	1.20	21					
	3) 60-69	0.44	0.13	1.11	27					
	4) older than 70	0.56	0.29	10.90	22					
RO60	1) younger than 50	282.00	0.00	282.00	20		0.021			1/4=0.004 2/4=0.014 3/4=0.044
	2) 50-59	282.00	0.00	308.00	21					
	3) 60-69	282.00	0.00	331.00	28					
	4) older than 70	211.00	0.00	282.00	24					
RFIgA	1) younger than 50	500.00	1.00	500.00	16		<0.001			1/2=0.005 1/3=0.006 1/4=<0.001 2/4=0.011
	2) 50-59	61.50	1.00	500.00	18					
	3) 60-69	50.50	1.00	500.00	26					
	4) older than 70	8.00	1.00	423.00	19					
Global sicca	1) younger than 50	29.85	1.30	68.1	20		0.006			1/2=0.007 2/3=0.002
	2) 50-59	44.80	4.80	78.7	25					
	3) 60-69	32.40	11.40	76.7	31					
	4) older than 70	41.35	5.60	70.5	22					
IgG	1) younger than 50	17.35	8.65	33.80	20		0.024			1/3=0.010 1/4=0.013
	2) 50-59	14.85	7.50	32.70	26					
	3) 60-69	11.90	7.44	33.60	31					
	4) older than 70	12.45	6.51	37.20	26					
IgG1	1) younger than 50	12.95	6.04	30.00	18		0.016			1/3=0.010 1/4=0.006
	2) 50-59	10.10	5.30	29.20	19					
	3) 60-69	8.54	3.98	28.70	30					
	4) older than 70	8.14	4.12	34.00	24					
ProFaD/mental fatigue	1) younger than 50	1.00	0.00	6.50	20		0.039			1/2=0.010 1/3=0.018 1/4=0.026
	2) 50-59	2.50	0.00	6.50	26					
	3) 60-69	2.50	0.00	6.50	32					
	4) older than 70	2.75	0.00	7.00	22					

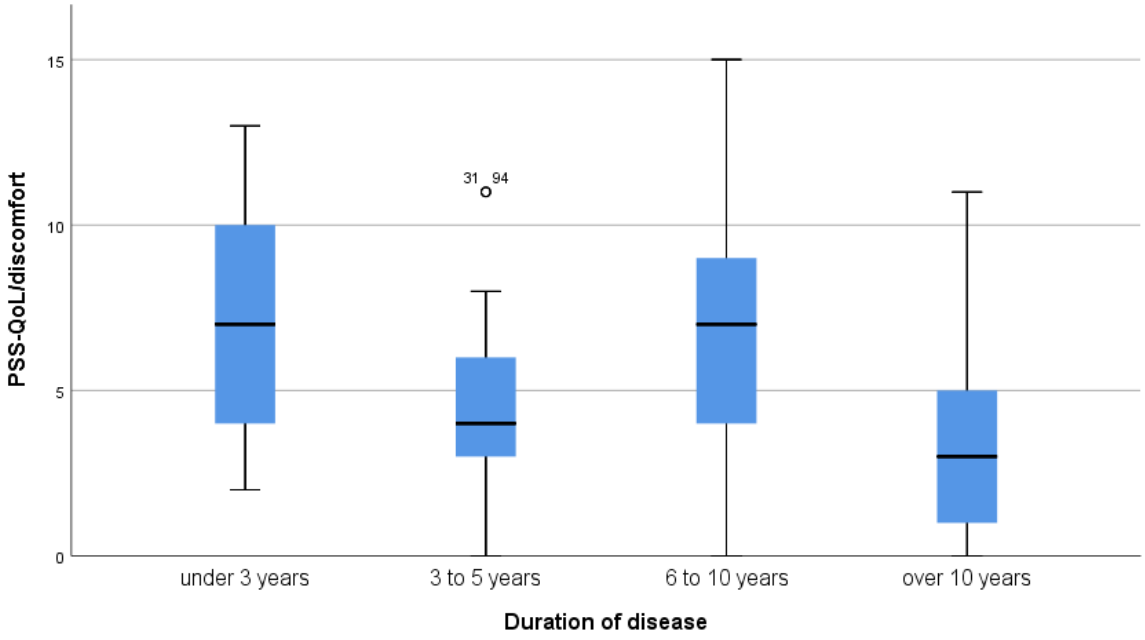
Mean/Median, SD/Min.-Max.: For variables marked with a "✓" (normally distributed), the mean value and the standard deviation (SD) are shown. For other variables the median and the range are shown. N: The number of patients falling into that group; Diff: Difference; n.p.: non-parametric; x/y: This shows the numbers of the groups that differ. n.s.: non-significant.

3.4 Disease duration

The following groups were classified regarding disease duration: Disease duration under 3 years, 3-5 years, 6-10 years, over 10 years. Group comparisons are shown in table 6. ProFaD domains showed the highest score in the group "under 3 years" (somatic fatigue: 3.13 [0-7]; mental fatigue: 3 [0-7]; general discomfort 15.62 [0.2-36.87]). The median IgG level showed the highest value in the group "over 10 years" (15.95 [8.05-26.06]).

Figure 3 shows the feeling of discomfort (PSS-QoL/discomfort) according to the disease duration. Patients at the beginning of the disease experienced the highest level of subjective discomfort (PSS-QoL/discomfort: 7.41 ±3.58).

Figure 3: Discomfort levels by duration of disease



Patient 31 and patient 94 were outliers with a score of 11.

Table 6: Differences by duration of disease

						Difference between groups		Post-hoc tests for ANOVA	Diff. n.p. groups
Variable (✓= normally distributed)	Duration of disease	Mean/Median	SD/Min.-Max.		N	ANOVA	Kruskal-Wallis	Tukey/Games-Howell	Mann-Whitney-U
PSS-QoL/discomfort ✓	1) under 3 years	7.41	3.58		32	0.010		1/4 = 0.015	
	2) 3-5 years	4.82	3.64		17				
	3) 6-10 years	6.40	3.73		35				
	4) over 10 years	4.18	3.45		17				
Lymphocytes	1) under 3 years	1.50	0.50	3.60	29		0.039		1/4=0.013 3/4=0.009
	2) 3-5 years	1.40	0.80	2.20	17				
	3) 6-10 years	1.50	0.00	2.90	34				
	4) over 10 years	1.15	0.30	2.00	18				
ESSPRI/pain	1) under 3 years	4.00	0.00	9.00	33		0.045		1/4=0.013 3/4=0.032
	2) 3-5 years	2.00	0.00	8.00	17				
	3) 6-10 years	4.00	0.00	9.00	36				
	4) over 10 years	1.50	0.00	7.00	18				
ProFaD/somatic fatigue	1) under 3 years	3.13	0.00	7.00	30		0.011		1/2=0.012 1/4=0.034 2/3=0.041
	2) 3-5 years	1.00	0.00	5.50	17				
	3) 6-10 years	2.75	0.00	6.00	35				
	4) over 10 years	1.25	0.25	6.00	18				
ProFaD/mental fatigue	1) under 3 years	3.00	0.00	7.00	30		0.016		1/2=0.002 2/3=0.037
	2) 3-5 years	1.50	0.00	6.50	17				
	3) 6-10 years	2.75	0.00	6.50	35				
	4) over 10 years	1.75	0.00	5.00	18				
ProFaD/general discomfort	1) under 3 years	15.62	0.20	36.87	30		0.037		1/2=0.017 2/3=0.037
	2) 3-5 years	5.00	0.00	31.10	17				
	3) 6-10 years	15.00	0.00	34.93	33				
	4) over 10 years	5.77	0.00	38.00	16				
IgG	1) under 3 years	15.12	7.50	37.20	32		0.048		1/3=0.007
	2) 3-5 years	14.90	7.44	33.80	17				
	3) 6-10 years	11.70	6.51	28.40	35				
	4) over 10 years	15.95	8.05	26.06	18				
C4	1) under 3 years	0.10	0.00	0.30	30		0.040		1/2=0.041 2/4=0.022 3/4=0.048
	2) 3-5 years	0.20	0.00	1.00	17				
	3) 6-10 years	0.20	0.00	0.30	35				
	4) over 10 years	0.10	0.00	0.20	17				

Mean/Median, SD/Min.-Max.: For variables marked with a "✓" (normally distributed), the mean value and the standard deviation (SD) are shown. For other variables the median and the range are shown. N: The number of patients falling into that group; Diff: Difference; n.p.: non-parametric; x/y: This shows the numbers of the groups that differ. n.s.: non-significant.

3.5 Longitudinal analysis

3.5.1 Comparison of baseline scores versus follow-up scores

14 baseline scores and follow-up scores were compared. Only the ESSPRI/fatigue scores were significantly different (p=0.013). The mean value of the baseline score was 3.4 (± 2.4) and the mean value of the follow-up score was 4.5 (± 2.9).

3.5.2 MDC

The minimal detectable change (MDC) was calculated: PSS-QoL/total = 12.2; PSS-QoL/physical = 7.6; PSS-QoL/psychosocial = 8.5; PSS-QoL/dryness = 3.5.

Patients were assigned to three groups: “same”, “better”, “worse”; according to the MDC.

3.5.3 Change of the PSS-QoL/total

Only 33 of the 52 patients completed the entire PSS-QoL twice: 23 patients (69.7%) were in group “same”. 6 patients (8.2%) were considered as “worse” and 4 patients (12.1%) were feeling “better”. Over those three groups only the TSH value was significantly different (Kruskal-Wallis test: p=0.01). The median TSH value in the group “same” (n=13) was 1.67 [0.15-2.94] mU/l. For the group “better” (n=2) it was 0.14 [0.00-0.25] mU/l, and for the group “worse” (n=3) it was 3.23 [1.88-4.57] mU/l. The difference between the groups “same” and “better” was significant (p=0.007) and also the difference between the groups “better” and “worse” (p=0.012).

3.5.4 Change of the PSS-QoL/physical

There were 48 patients who completed the entire PSS-QoL/physical at both times. 40 patients (83.3%) were in the group “same”, 4 patients (8.3%) were considered as “worse” and 4 patients (8.3%) were in the “better” group.

Table 7 shows a highly significant difference (p=0.004) between the ESSDAI-scores in the groups “same” and “better”. The level of Anti La-Antibodies was also significantly different (p=0.002) between the groups “same” and “better”.

Table 7: Differences over the PSS-QoL/physical

						Difference between groups		Post-hoc tests for ANOVA	Diff. n.p. groups
Variable (✓= normally distributed)	Development	Mean/Median	SD/Min.	-Max.	N	ANOVA	Kruskal-Wallis	Tukey/Games-Howell	Mann-Whitney-U
ESSDAI	1) same	1.00	0.00	14.00	39		0.045		1/2=0.004
	2) better	7.00	5.00	10.00	4				
	3) worse	1.00	0.00	8.00	4				
Lymphocytes ✓	1) same	1.48	0.46		40	0.033		1/2=0.02	
	2) better	0.83	0.50		4				
	3) worse	1.57	0.54		4				
Unstim. salivary flow rate	1) same	0.10	0.00	0.60	13		0.046		n.s.
	2) better	0.00	0.00	0.04	3				
	3) worse	0.33	0.04	0.60	4				
Protein/Creatinine ratio	1) same	82.00	55.00	200.00	36		0.016		1/2=0.040 1/3=0.041
	2) better	132.00	104.00	186.00	3				
	3) worse	60.00	47.00	74.00	4				
Anti-La Antibodies	1) same	42.50	0.00	320.00	38		0.021		1/2=0.002
	2) better	320.00	304.00	320.00	4				
	3) worse	240.00	0.20	320.00	3				

Mean/Median, SD/Min.-Max.: For variables marked with a “✓” (normally distributed), the mean value and the standard deviation (SD) are shown. For other variables the median and the range are shown. N: The number of patients falling into that group; Diff: Difference; n.p.: non-parametric; x/y: This shows the number of the groups that differ. ESSDAI: EULAR Sjögren’s Syndrome disease activity index.

3.5.5 Change of the PSS-QoL/psychosocial

34 patients completed the entire PSS-QoL/psychosocial at both times: 24 patients (70.6%) were in the group “same”. 7 patients (20.6%) were considered as “worse” and 3 patients (8.8%) fell into the “better” group.

Especially patients with a worsening of the PSS-Qol/psychosocial domain were significantly (p=0.014) older (65.7 ±5.47) than patients who felt an improvement (44.3 ±11.37 years).

Patients who felt better had a significantly lower OSDI© score (10.42 [6.25-10.42]) than patients in the group “same” (50 [8.33-87.5], p=0.011).

Table 8 shows all significant differences between the groups.

Table 8: Differences over the PSS-QoL/psychosocial

						Difference between groups		Post-hoc tests for ANOVA	Diff. n.p. groups
Variable (✓= normally distributed)	Development	Mean/Median	SD/Min.-Max.		N	ANOVA	Kruskal-Wallis	Tukey/Games Howell	Mann-Whitney-U
Age (years) ✓	1) same	58.50	11.09		24	0.018		2/3=0.014	
	2) better	44.30	11.37		3				
	3) worse	65.70	5.47		7				
TSH ✓	1) same	1.5	0.79		13	0.023		1/3=0.049 2/3=0.033	
	2) better	0.65	0.52		2				
	3) worse	2.85	1.34		4				
OSDI®	1) same	50.00	8.33	87.50	23		0.016		1/2=0.011 2/3=0.036
	2) better	10.42	6.25	10.42	3				
	3) worse	30.42	10.42	70.00	6				
Eye-Sicca	1) same	39.92	0.67	85.00	24		0.031		1/2=0.037
	2) better	18.83	4.33	22.00	3				
	3) worse	17.83	13.50	53.83	7				
ProFaD/general discomfort ✓	1) same	14.80	11.12		24	0.049		2/3=0.039	
	2) better	3.02	1.15		3				
	3) worse	21.83	8.45		6				

Mean/Median, SD/Min.-Max.: For variables marked with a “✓” (normally distributed), the mean value and the standard deviation (SD) are shown. For other variables the median and the range are shown. N: The number of patients falling into that group; Diff: Difference; n.p.: non-parametric; x/y: This shows the number of the groups that differ.

3.5.6 Change of the PSS-QoL/dryness

49 patients completed the entire dryness domain of the PSS-QoL twice. 32 patients (65.3%) were in the group "same". 5 patients (10.2%) were considered as "worse" and 12 patients (24.5%) were in the "better" group. Group comparisons are depicted in table 9. The unstimulated salivary flow rate was significantly lower in the "better" group (0 [0-0.04] ml/min) compared to the "same" group (0.1 [0-0.6] ml/min; p=0.04). The Saxon test in the "worse" group was significantly higher (2.25 [2-3.6] g/2 min.) compared to the "better" group (1.15 [0-2.5] g/2 min.; p=0.018) and also significantly higher compared to the "same" group (1.52 [0.32-2.5] g/2 min.; p=0.028)

Table 9: Differences over the PSS-QoL/dryness

Variable (✓= normally distributed)	Development	Median	Min.	Max.	N	Kruskal-Wallis	Mann-Whitney-U
Unstim. salivary flow rate	1) same	0.10	0.00	0.60	20	0.020	1/2=0.04
	2) better	0.00	0.00	0.04	4		
	3) worse	0.33	0.04	0.60	4		
Saxon's test	1) same	1.52	0.32	2.50	20	0.042	1/3=0.028 2/3=0.018
	2) better	1.15	0.00	2.50	8		
	3) worse	2.25	2.00	3.60	4		
Focus score	1) same	2.00	0.00	3.00	20	0.033	1/2=0.014
	2) better	3.00	2.00	3.00	10		
	3) worse	2.00	0.00	3.00	4		
β2-Microglobuline	1) same	200.00	188.00	3080.00	29	0.003	1/2=0.002 2/3=0.011
	2) better	206.00	200.00	538.00	11		
	3) worse	200.00	189.00	200.00	5		
Anti-La Antibodies	1) same	38.00	0.00	320.00	31	0.044	1/2=0.018
	2) better	320.00	0.00	320.00	11		
	3) worse	215.00	0.20	320.00	4		

Min.-Max.: Shows the range; N: The number of patients falling into that group; x/y: This shows the number of the groups that differ.

3.5.7 Change of life quality (PSS-QoL/Question 25)

47 patients answered that question at both times. 24 patients (51.1%) fell into the group "same". 16 patients (34%) were considered as "worse" and 7 patients (14.9%) fell into the "better" group. Over those three groups only the duration of the disease was significantly different (Kruskal-Wallis test: p=0.023). The median duration of the disease in the group "same" was 7 [2-23] years. For the "better" group it was 3.5 [5-21] years, and for the group "worse" it was 6 [2-13] years. The Mann-Whitney-U test showed a significant difference between the groups "same" and "better" (p=0.007) and between the groups "worse" and "better" (p=0.012).

3.6 IgG and rheumatoid factors

The PSS-QoL/total, the PSS-QoL/physical, the PSS-QoL/discomfort, the PSS-QoL/vagina and the ESSPRI showed significantly higher scores when IgG and RF IgA were within normal range. The PSS-QoL/psychosocial was significantly higher when IgG and both RFs were within normal range. The PSS-QoL/dryness only showed a significantly higher score when IgG was within normal range. The normal range is detailed in the *Patients and Methods* section.

The Saxon test also showed significantly higher values when all three immunological parameters were under their cut-off levels. The other variables regarding mouth dryness (unstimulated salivary flow rate, PSS-QoL/mouth, Xerostomia Inventory) showed no significant differences between the groups across all three immunological parameters.

The p-values and the detailed scores can be seen in Table 10.

Table 10: Differences between variables depending on the level of patients' IgG and rheumatoid factors

t-test/Mann-Whitney U	IgG						RF IgA						RF IgM					
Variable	Level	N	M	SD/Range		p-value	Level	N	M	SD/Range		p-value	Level	N	M	SD/Range		p-value
ESSPRI	normal	64	15.20	2.04		**0.005**	negative	29	15.90	2.06		**0.038**	negative	25	13.96	2.36		0.186
	high	39	11.44	2.34			positive	50	12.80	2.11			positive	36	11.64	2.12		
PSS-QoL/total	normal	64	39.52	15.16		**0.002**	negative	29	42.79	13.41		**0.002**	negative	25	38.28	16.85		0.106
	high	39	29.36	15.59			positive	50	31.84	14.92			positive	36	31.44	15.40		
PSS-QoL/physical	normal	64	16.50	6.00	32.00	**0.006**	negative	29	16.00	7.00	29.00	**0.043**	negative	25	15.00	6.00	27.00	0.833
	high	36	12.00	4.00	30.00		positive	48	13.50	6.00	32.00		positive	35	14.00	4.00	32.00	
PSS-QoL/discomfort	normal	64	6.89	3.54		**0.007**	negative	29	7.41	3.43		**0.012**	negative	25	6.28	3.46		0.197
	high	36	4.86	3.80			positive	48	5.35	3.41			positive	35	5.09	3.68		
PSS-QoL/dryness	normal	64	10.20	3.84		**0.031**	negative	29	10.00	4.00	15.00	0.275	negative	25	10.00	1.00	15.00	0.713
	high	39	8.46	4.06			positive	50	9.00	1.00	22.00		positive	36	9.00	3.00	22.00	
PSS-QoL/psychosocial	normal	63	22.78	10.42		**0.006**	negative	29	25.34	9.85		**0.002**	negative	25	23.48	11.71		**0.041**
	high	38	16.84	9.86			positive	48	18.06	9.22			positive	35	17.54	9.32		
PSS-QoL/mouth	normal	64	3.00	0.00	6.00	0.552	negative	29	3.00	1.00	6.00	0.457	negative	25	2.72	1.46		0.145
	high	36	3.00	0.00	7.00		positive	48	3.00	0.00	7.00		positive	35	3.29	1.47		
PSS-QoL/eye	normal	64	3.00	0.00	8.00	0.459	negative	29	3.00	0.00	5.00	0.340	negative	25	3.00	0.00	5.00	0.069
	high	39	3.00	0.00	6.00		positive	50	3.00	0.00	8.00		positive	36	3.00	0.00	8.00	
PSS-QoL/vagina	normal	59	2.00	0.00	3.00	**0.001**	negative	26	2.00	0.00	3.00	**0.033**	negative	22	1.50	0.00	3.00	0.605
	high	36	1.00	0.00	2.00		positive	47	1.00	0.00	3.00		positive	33	1.00	0.00	3.00	
Duration of disease (years)	normal	63	6.35	5.15		0.858	negative	29	7.14	5.08		0.326	negative	24	6.71	4.79		0.977
	high	39	6.15	5.68			positive	49	5.92	8.96			positive	36	6.67	6.01		
Saxon test	normal	37	1.66	0.32	4.50	**0.004**	negative	15	2.50	0.55	4.50	**<0.001**	negative	17	2.38	0.80	4.50	**<0.001**
	high	26	1.00	0.00	3.50		positive	33	1.21	0.40	2.50		positive	19	1.00	0.00	2.50	
Unstim. salivary flow rate	normal	25	0.10	0.00	1.00	0.912	negative	11	0.06	0.00	1.00	0.465	negative	13	0.10	0.00	1.00	0.131
	high	15	0.20	0.00	0.60		positive	18	0.03	0.00	0.60		positive	10	0.01	0.00	0.30	
Schirmer's test	normal	41	2.00	0.00	35.00	0.597	negative	17	6.00	0.00	35.00	**0.007**	negative	17	4.50	0.00	35.00	**0.005**
	high	26	1.75	0.00	28.00		positive	33	1.50	0.00	28.00		positive	21	1.00	0.00	28.00	
OSDI®	normal	58	46.60	20.19		**0.035**	negative	27	45.83	6.25	87.50	0.176	negative	24	39.37	19.05		0.732
	high	39	37.39	21.59			positive	48	42.71	10.42	87.50		positive	36	41.07	18.54		
Xerostomia Inventory	normal	61	26.11	8.86		0.130	negative	28	26.86	5.37		0.251	negative	24	21.21	8.74		0.095
	high	39	23.18	10.11			positive	49	24.39	9.03			positive	36	25.00	8.30		
ProFaD/somatic fatigue	normal	59	2.75	0.00	6.75	**0.026**	negative	28	3.13	0.00	6.75	0.087	negative	24	2.63	0.00	6.75	0.240
	high	36	1.88	0.00	7.00		positive	46	2.00	0.25	7.00		positive	35	2.00	0.00	7.00	
ProFaD/mental fatigue	normal	59	3.00	0.00	6.50	**0.009**	negative	28	3.75	0.00	6.50	**0.004**	negative	24	2.75	0.00	6.00	0.212
	high	38	1.50	0.00	7.00		positive	48	2.00	0.00	7.00		positive	35	2.00	0.00	7.00	
ProFaD/general discomfort	normal	58	15.37	0.20	38.00	**0.021**	negative	26	16.45	0.73	34.93	**0.037**	negative	23	8.30	0.00	34.93	0.489
	high	37	8.03	0.00	36.87		positive	47	11.93	0.00	32.97		positive	36	9.07	0.00	36.87	

N: Number of patients falling into that group; M: Mean or Median depending on whether the group is normally distributed; SD/Range: Standard deviation or range depending on whether the group is normally distributed; Significant p-values (<0.05) are highlighted in bold.

3.6.1 Correlations of immunological parameters and various scores

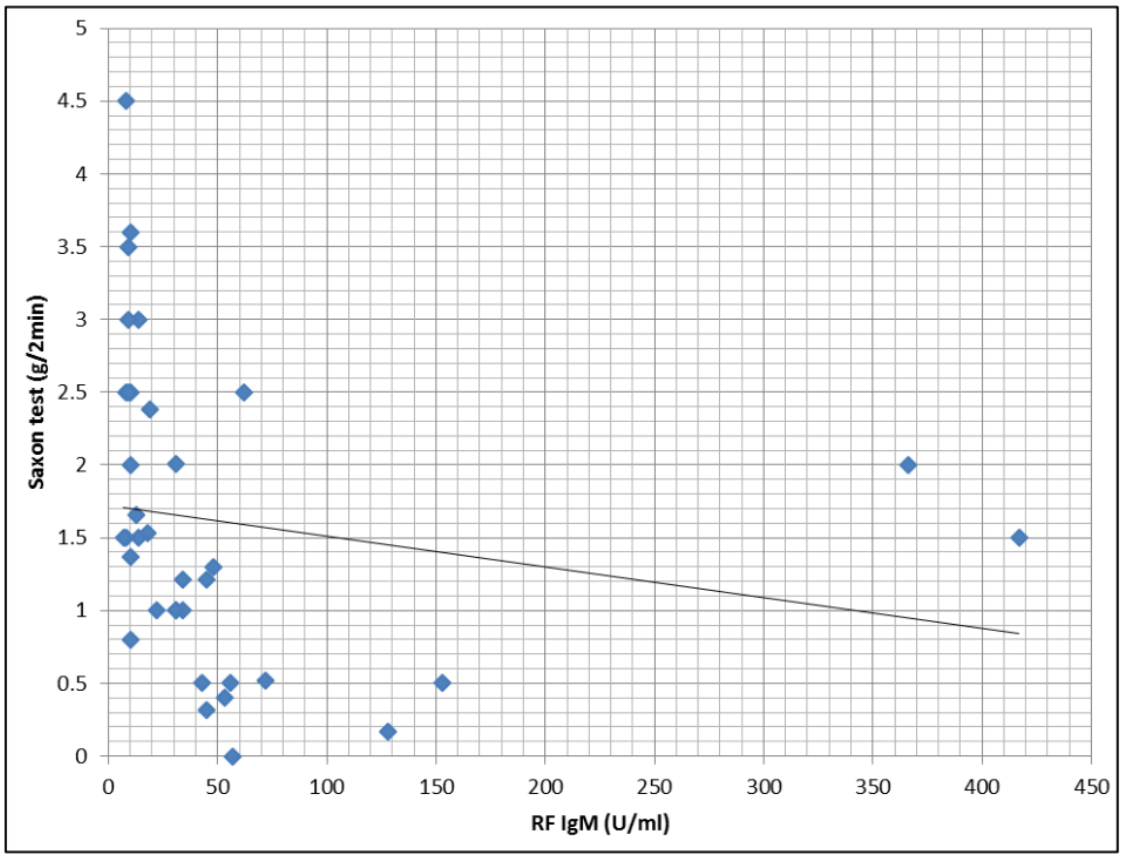
The Spearman's rho correlation coefficient (ρ) was calculated for the rheumatoid factors and IgG for various variables (table 11). IgG and ESSDAI showed a moderate correlation (ρ =0.456, p=<0.001). RF IgM and the Saxon test showed a strong negative correlation (ρ =-0.607, p=<0.001) (figure 4). RF IgA and the Schirmer's test also showed a strong negative correlation (ρ =-0.526, p=<0.001).

Table 11: Correlations of immunological parameters for various variables

	IgG	RF IgM	RF IgA
PSS-QoL/total	-0.261**	n.s.	-0.408**
PSS-QoL/physical	n.s.	n.s.	-0.307**
PSS-QoL/discomfort	n.s.	n.s.	-0.377**
PSS-QoL/dryness	n.s.	n.s.	n.s.
PSS-QoL/psychosocial	-0.245*	n.s.	n.s.
PSS-QoL/mouth	n.s.	n.s.	n.s.
PSS-QoL/vagina	-0.340**	n.s.	-0.286*
PSS-QoL/eye	n.s.	n.s.	n.s.
ESSDAI	0.456**	0.337**	0.295**
ESSPRI	n.s.	n.s.	-0.260*
ACR Score	n.s.	n.s.	0.411**
Focus Score	n.s.	n.s.	0.276*
PhGA	0.298**	n.s.	n.s.
Age	-0.321**	-0.322*	-0.457**
Duration of disease	n.s.	n.s.	n.s.
Saxon's test	-0.346**	-0.607**	-0.499**
Schirmer's test	-0.562**	n.s.	-0.526**
Xerostomia Inventory	n.s.	0.259*	n.s.
OSDI©	n.s.	n.s.	n.s.
Unstim. saliva flow rate	n.s.	n.s.	n.s.
Global sicca	n.s.	n.s.	n.s.
Eye sicca	n.s.	n.s.	n.s.
ProFaD/somatic fatigue	n.s.	n.s.	n.s.
ProFaD/mental fatigue	-0.267**	n.s.	-0.358**
ProFaD/general discomfort	n.s.	n.s.	-0.241*
Number of drugs	n.s.	n.s.	n.s.

* p < 0.05, **p < 0.01, n.s.: non-significant

Figure 4: Scatter plot of the Saxon test and RF IgM with regression line



3.7 The EULAR Sjögren's syndrome disease activity index (ESSDAI)

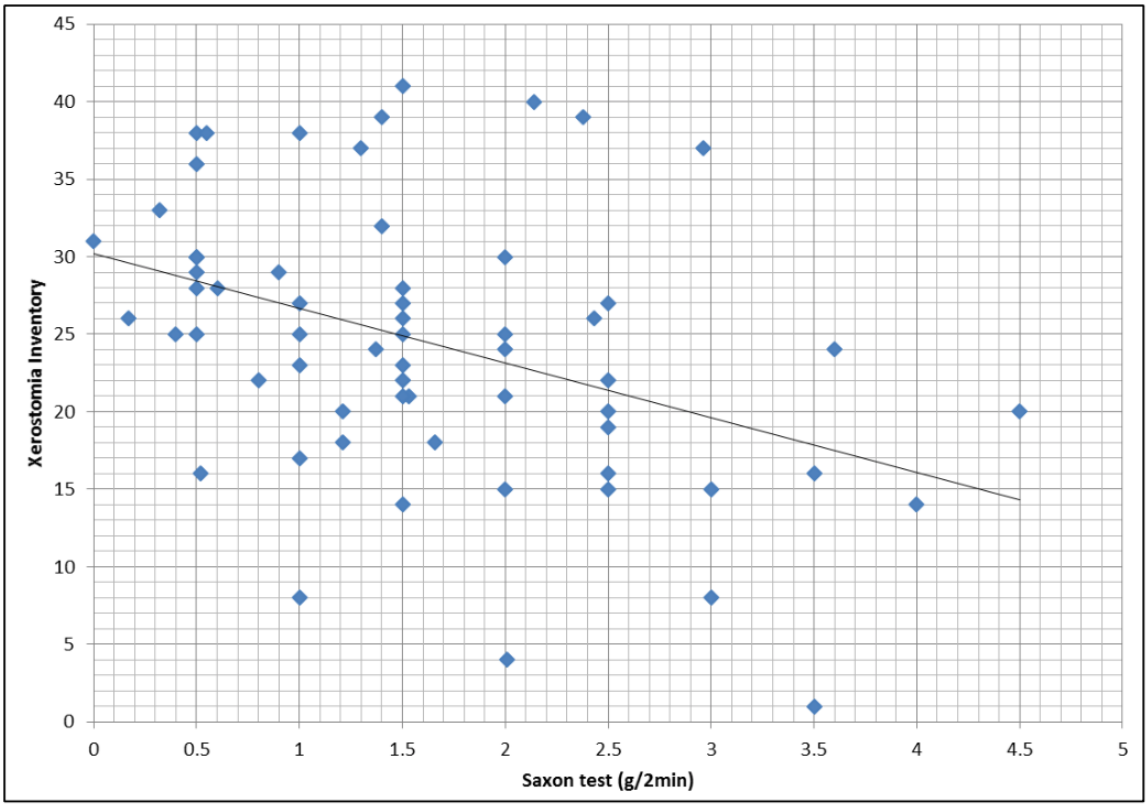
The unpaired t-test and the Mann-Whitney-U test revealed no significant differences (p=<0.05) across all variables between the ESSDAI disease activity groups.

3.8 Correlations of objective and subjective tests for mouth and eye dryness

The PSS-QoL/mouth (ρ =-0.277, p=0.028) and the Xerostomia Inventory (ρ =-0.408, p=0.001) showed a weak negative and a moderate negative correlation with the Saxon test, respectively, but not with the unstimulated salivary flow rate. Figure 5 shows a scatter plot with the regression line of the Xerostomia Inventory and the Saxon test.

The PSS-QoL/eye showed a weak negative correlation (ρ =-0.249, p=0.037) with the Schirmer's test. The Eye sicca score and the OSDI© did not show any correlations.

Figure 5: Scatter plot of the Xerostomia Inventory and the Saxon test with regression line



4 Discussion

This study shows that the change of the psychosocial burden is higher than the physical burden in PSS-patients. Furthermore, there are age-related differences in disease activity: Patients in their 50s suffer most from physical symptoms, while patients older than 70 face bigger psychosocial challenges. IgG as well as rheumatoid factors IgA and IgM showed relationships with objective and subjective dryness measurements and could be used as potential biomarkers.

4.1 Change of patient-reported outcomes and quality of life

HRQL, measured by PSS-QoL, did not significantly change during a period of two years. However, ESSPRI/fatigue significantly changed over that period (from 3.4 to 4.5).

Over two thirds of the patients (69.7%) experienced no change in their quality of life according to PSS-QoL. Patients with a worsening HRQL showed the highest value of TSH (3.23 mU/l) compared to the other groups. Nevertheless, the normal range of reference for adults is 0.45 - 4.5 mU/l, so the importance of this change may be ambiguous. On the other hand there is a study that showed that HRQL is not lower in healthy individuals with elevated TSH levels [58]. Thyroid dysfunction is almost 8 times more frequent in PSS-patients than in healthy individuals and patients suffering from both conditions have a higher risk for developing lymphoma. Further studies are needed to investigate the value of TSH levels in PSS-patients for QoL and the disease course [59,60].

No change at the physical domain of the PSS-QoL was observed in 83.3%. Unfortunately, a group comparison was not possible because of the small sample size in the other groups (better, worse). Nevertheless, a trend was observed that the protein/creatinine ratio and the level of Anti-La antibodies are higher in patients that felt physically better. Garcia-Carrasco et al. [18] found that anti-La positive patients are more likely to suffer from extra-glandular manifestations than anti-La negative patients.

The number of lymphocytes was lower in patients that felt better and higher in patients that felt worse. The mean value in the "better" group (0.83 $x10^9$/l was outside of the reference range (1 – 4 $x10^9$/l) for healthy adults. A study showed that there is increased proliferation of naïve CD4+ T cells in PSS-patients. The lack of naïve CD4+ T cells is responsible for lymphopenia which is frequently observed in PSS-patients [61]. It is known that patients who feel better over time often have higher immunological activity. The median baseline PSS-QoL score in the "better" group was 44, compared to 21 in the "worse" group and 30.5 in the "same" group (no significant differences). So patients who start out from a point of high disease activity are probably more likely to improve their HRQL. Further studies about the relationship of lymphocytes and subjective well-being in PSS-patients are needed. Missing data and the small sample size complicated my statistical evaluation.

The PSS-QoL psychosocial domain got worse in 20.6% of the patients and only 8.8% felt better. It seems that the psychosocial burden increased more in comparison to the physical domain. Patients which felt worse were significantly older (65.7 years) than patients that felt better (44.3 years). Other factors possibly influence the psychosocial well-being that are more present in older patients like reduced mobility, chronic pain, frailty or other health problems. In addition, there may be a drop in socioeconomic status with retirement. These stressors combined can result in isolation, loneliness or psychological distress [62]. So it is not certain that the psychosocial health decreases more rapidly in PSS-patients than in the general older population and a study that compares older PSS-patients to a healthy control group of older people is necessary to confirm those findings. Nonetheless depression is much more common in PSS-patients than in the general population (36.9% vs. 4.4%) [33,63]. A literature review by Bair et al. [64] from 2018 concluded that pain is associated with more depressive symptoms and worse depression outcomes such as lower HRQL and decreased work function.

It was interesting to see, that by looking at the dryness domain of the PSS-QoL, patients in the group "worse" seem to have the best saliva production, which is indicated by their unstimulated salivary flow rate, which even was within the reference range for healthy individuals (0.33ml/min) and the Saxon test, although it was lower (2.25g/2min) than the minimum amount of saliva healthy individuals produce (2.75g/2min). On the other hand, tests for eye dryness showed no significant differences between the groups. Unfortunately, data about their objective tests for dryness from two years ago were not available for

comparison with their latest results. A study by Haldorsen et al. indicates that the unstimulated salivary flow rate remains stable over a five-year period and that the Schirmer's test improves over that time. This could be explained by a typical diagnostic delay of 5 to 10 years, so most of the exocrine function may already be lost at the time the first diagnostic tests are taken [65]. Therefore, it can be stated that exocrine gland function does not worsen significantly over the evaluated time period and further studies over a period of ten years or longer may be necessary to detect significant change.

The longitudinal evaluation of question 25 from the PSS-QoL asking about the patients' general quality of life showed that half of the patients felt the same, one third felt worse and the rest (14.5%) felt better. Patients who felt better were suffering from the disease significantly shorter (3.5 years) than the other two groups. A reasonable explanation might be that, when the patients were asked about their quality of life for the first time, it was shortly after they have received their diagnose. So they might not have developed personal coping strategies yet.

The hypothesis "*There is a connection between patient-reported outcomes and a variety of objective measures that may be influencing factors",* can therefore be verified.

4.2 Age and duration of disease

By looking at the results of the PSS-QoL/total, patients between 50 and 59 and patients older than 70 years of age had a higher impaired HRQL according to PSS-QoL compared to the other age groups. The age group "50-59" showed the highest PSS-QoL/physical score and experienced the most severe sicca symptoms. Symptoms of menopause could influence the assessment of dryness. About 20% of menopausal women suffer from skin dryness, 25% suffer from vaginal dryness and more than half suffer from arthralgia [66–68]. So it is possible that the physical symptoms in that age group are aggravated by the presence of menopausal symptoms and a study including a healthy control group is therefore necessary.

Patients over the age of 70 had the highest scores in the PSS-QoL/psychosocial domain and the ProFaD/mental fatigue. It is not clear whether those findings are due to the course of the disease or other psychosocial and mental challenges that especially older people are facing and that have been already mentioned above.

Due to the cofactors mentioned above and the very similar PSS-QoL/total scores of the groups “50-59” (35.5), “60-69” (32.5) and “older than 70” (35.0), there can be no clear statement made about PSS affecting different age groups in a different way. Further research is needed, including a study design that compares different age groups of PSS-patients with healthy control groups.

Serum IgG levels were slightly higher in the age group “older than 70”, compared to the group “60-69”. A possible reason for elevated IgG levels is the prevalence of monoclonal gammopathy of undetermined significance (MGUS) in older patients (up to 7.5%) [69]. However, only 7 patients (6.5%) included in this study were diagnosed with MGUS and only one of them was over the age of 70. Furthermore, only three of the seven patients had elevated IgG levels (>16 U/ml) and the patient over the age of 70 was not among them. This could be due to MGUS being undiagnosed yet, nevertheless further research should be conducted comparing IgG levels of PSS-patients over the age of 70 with a healthy control group.

It appears that many subjective parameters peak in the “less than 3 years” group and show a second peak in the “6-10 years” group”, indicating a worsening of symptoms. A study from Theander et al. showed similar results, indicating that there was a considerable loss of function and HRQL at a median disease duration of six years, after that there was not much decline. Patients even avoided the age-expected increase in fatigue, according to their vitality item score of the short form health – 36 (SF-36). There is no clear explanation for that phenomenon, but it could be due to the development of personal coping strategies. The authors also claim that the lack of additional decline in the objective measurements may be related to the floor or ceiling properties of the tests and further decline might only be observable with more sensitive tests (Schirmer’s test, Van Bijisterfeld score and unstimulated salivary flow rate were the objective tests in that study) [70].

The hypothesis: “*There are multiple factors that affect the course of the disease differently in each age group*”, can therefore be neither verified or falsified.

4.3 Immunological parameters

The Saxon test was the only parameter that negatively correlated with all three immunological parameters.

RF IgA negatively correlated with different domains of the PSS-QoL (total, physical, discomfort, vagina) and ESSPRI. It also strongly negatively correlated with the Schirmer's test, an observation that already has been made [71]. These findings suggest that the serum IgA level, out of the three assessed immunological parameters, is the most likely to correlate with the patient's subjective well-being. RF IgA weakly negatively correlated with the ESSDAI, but the correlation between the ESSDAI and RF IgM and IgG was stronger.

RF IgM did not correlate with any domains of the PSS-QoL or any other subjective tests for sicca symptoms, except for a weak correlation with the Xerostomia Inventory, where it was the only immunological parameter that correlated. However, out of the three parameters RF IgM showed the strongest correlation with the Saxon test (ρ =-0.607), which is also a test for xerostomia.

IgG was the only immunological parameter that correlated negatively with the psychosocial-domain of the PSS-QoL. Tensing et al. reported in 2001 that serum IgG positively correlates with vitality of PSS-patients [72], so it is reasonable that IgG negatively correlates with the psychosocial burden.

No immunological parameter correlated with the somatic-fatigue domain of the ProFaD, whereas the ProFaD/general discomfort domain negatively correlated with RF IgA. This is not surprising since RF IgA also correlates with many domains of the PSS-QoL, which include similar questions. The ProFaD/mental fatigue domain negatively correlated with IgG and RF IgA. The negative correlation between fatigue and IgG levels has already been described by Segal et al. in 2008, although the authors do not claim IgG levels being predictive for fatigue [27]. Apart from that there are other potential biomarkers for fatigue that are being discussed in the *Introduction* of this work [30].

The hypothesis: "*The quantity of serum IgG and rheumatoid factors IgA and IgM correlates with subjective well-being and also objective tests for sicca symptoms*", can therefore be verified.

4.4 Objective vs. subjective tests for dryness

The results show that the Saxon test negatively correlated with the PSS-QoL/mouth, and stronger negatively correlated with the Xerostomia Inventory, whereas the unstimulated salivary flow rate correlated with neither of them. A study from Bezzina et al. from 2017 [73] correlated the ESSPRI for ocular and oral dryness with the unstimulated salivary flow rate and the Schirmer test. The study found a moderate negative correlation between the ESSPRI and the unstimulated salivary flow rate (ρ =-0.31, p<0.001), which is weaker than the correlation between the Xerostomia Inventory and the Saxon test (ρ =-0.408, p=0.001) that was found in this study. This suggests that the Saxon test in combination with the Xerostomia Inventory is a strong tool for evaluating mouth dryness on a subjective and objective basis.

The PSS-QoL/eye was the only subjective test that correlated with the Schirmer's test. This correlation (ρ =-0.249, p=0.037) was also stronger than the correlation between the ESPPRI and the Schirmer's test found in Bezzina's study (ρ =-0.13, p = 0.001). This shows that the eye domain of the PSS-QoL is a well formulated combination of questions and may be helpful for future evaluations of eye dryness.

The hypothesis that *the PSS-QoL sicca domains correlate with objective tests for sicca symptoms* can therefore be verified.

4.5 Limitations of this study

One limitation of this study was missing data. Many patients did not fill out all questionnaires or forgot to answer single questions. Since the PSS-QoL was divided into three main domains, it was possible to evaluate the different domains separately.

The comparison between groups in the longitudinal aspect of the study was of low validity, since most patients were categorized as "same". This could be, because most patients' ESSDAI scores were relatively low at both times of data collection.

Since there was a large number of statistical tests that were conducted, there is an increased risk of committing a type 1 error (false-positive), meaning that a null hypothesis is rejected

that is actually true in the population. A large sample size minimizes the risk of committing a type 1 error [74]. As mentioned above, the sample sizes sometimes were very small (3-4 patients), so that those calculations bear a greater risk of including type 1 errors. For the statistical analysis in this work the significance level was set to 0.05, meaning that there is a 5% risk of incorrectly rejecting a true null hypothesis.

5 Conclusion

In PSS fatigue and the psychosocial burden appear to increase more rapidly than siccca symptoms, which is indicated by a quite slow objective worsening of sicca test scores. This highlights the importance of a more holistic therapy approach that not only focuses on targeting sicca symptoms which might be overtreated at times but should also include psychological treatment. It also remains unclear why IgG negatively correlates with fatigue and psychosocial distress, and its role in the pathophysiological process of the disease should therefore be investigated in future studies.

There seem to be significant differences in the results of subjective and objective tests between different age groups. It remains unclear whether PSS affects different age groups differently or if certain symptoms or psychosocial problems are commonly more present in the older population. To eliminate this problem, future studies that involve the factor “age” should be conducted with a healthy control group of the same age.

The level of RF IgA appears to negatively correlate with many subjective tests, and therefore it should be subject of future studies.

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