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Associations of cigarette smoking with disease phenotype and type I interferon expression in primary Sjögren's syndrome

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Abstract

Several studies have shown a negative association between smoking and primary Sjögren's syndrome (pSS), and smoking may interfere with the immune response. The purpose of this study was to investigate if smoking affects disease activity and disease phenotype in pSS. In this cross-sectional study, consecutive pSS patients filled out the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) form and a structured questionnaire regarding smoking habits. EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) scores were calculated and blood samples were analysed for type I interferon signature using RT-PCR. Of 90 patients (93% women, median age 66.5 years), 72% were type I IFN signature positive and 6, 42 and 53% were current, former and never smokers, respectively. No significant differences by smoking status were found regarding ESSDAI total score, activity in the ESSDAI domains or type I IFN signature. Patients with a higher cumulative cigarette consumption (≥ median) had higher scores in ESSPRI total [5.0 (3.0–6.3) vs 8.0 (6.0–8.3); $p < 0.01$] and ESSPRI sicca and pain domains. Comparing type I IFN signature negative and positive patients, the latter had significantly lower activity in ESSDAI articular domain (7/25 vs 3/64; $p < 0.01$) and lower scores in ESSPRI total [7.7 (5.2–8.2) vs 6.0 (4.0–7.7); $p = 0.04$]. Smoking was not associated with disease phenotype although patients with a higher cumulative cigarette consumption had worse symptoms in some disease domains. Current smokers were few making it difficult to draw any firm conclusions about associations to current smoking.

Keywords Sjogren's syndrome · Cigarette smoking · Interferon type I · Autoimmune diseases

Abbreviations

ACR	American College of Rheumatology
AECG	American European Consensus Group
ANA	Anti-nuclear antibodies
Anti-SSA	Anti Sjögren's syndrome A antibody
Anti-SSB	Anti Sjögren's syndrome A antibody
C3	Complement component 3
C4	Complement component 4
cDNA	Complementary deoxyribonucleic acid
CNS	Central nervous system
EGM	Extra glandular manifestations
ESSDAI	EULAR Sjögren's Syndrome Disease Activity Index
ESSPRI	EULAR Sjögren's Syndrome Patient Reported Index
EULAR	European League Against Rheumatism
HLA	Human leukocyte antigen
IFN	Interferon
IQR	Interquartile range
PNS	Peripheral nervous system
pSS	Primary Sjögren's syndrome
RNA	Ribonucleic acid

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RT-PCR	Reverse transcriptase polymerase chain reaction
SD	Standard deviation

Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease that predominantly affects exocrine glands [1]. Affection of other organs, often termed extraglandular manifestations (EGM), is seen in approximately one-third of patients [2]. Apart from female sex, the predisposing factors leading to the development of pSS are not well known although genetic studies have shown associations to HLA class II genes and interferon-related genes [3]. Furthermore, viral infections and sex hormones have been suggested to contribute to disease development [4]. The pathogenic process in pSS includes dysregulation of both innate and adaptive immunity. Both cell-mediated and humoral immunity is involved, the latter mirrored by hypergammaglobulinemia and production of anti-SSA and anti-SSB antibodies [4]. It is well established that type I interferons (IFNs) are important cytokines in the pathogenesis of pSS. The presence of an activated type I IFN system is commonly evaluated by analysing the expression of a selected set of type I IFN regulated genes. The presence of an elevated expression of these genes is called an "IFN-signature". Systemic type I IFN signature is found in a large fraction of pSS patients [5]. The type I IFN signature has also been shown to be associated with presence of anti-SSA and anti-SSB antibodies, and hypergammaglobulinemia [5]. Disease activity as evaluated by the European League Against Rheumatism (EULAR) Sjögren's syndrome disease activity index (ESSDAI) has been associated to type I IFN signature previously [6, 7] although some studies fail to confirm the association [5]. On the contrary, the presence of the IFN signature has been associated with lower patient-reported symptoms as evaluated by the EULAR Sjögren's syndrome patient-reported index (ESSPRI) [5]. We recently published an epidemiological study showing that smoking was associated with a lower risk of later being diagnosed with pSS [8]. Similar findings have also been reported by others reporting a lower prevalence of smokers amongst pSS patients [9–11], and lower frequency of focal sialoadenitis [9, 11] and seropositivity for anti-SSA antibodies [9, 11] amongst smoking pSS patients. However, it is unclear whether the observed association is due to a local effect of smoking on the inflammation in the salivary glands or if smoking has an impact on systemic inflammation and consequently the phenotype of pSS.

Smoking has profound negative effects on health and influences the development of cardiovascular disease, chronic obstructive lung disease (COPD), oncogenesis as well as several chronic inflammatory diseases such as rheumatoid arthritis and Crohn's disease [12–15]. On the other hand, smoking seems to ameliorate certain diseases such as ulcerative colitis, Behcet's disease and Parkinson's disease [16–18]. Smoking has several known effects on the immune system which could be important for modulation of chronic inflammatory diseases [19]. To the best of our knowledge, no other study has previously investigated the relation between cigarette smoking, disease phenotype and type I IFN signature in patients with pSS. We hypothesised that cigarette smoking might alter the phenotype of pSS.

In the present study, we wanted to (a) investigate the relation between cigarette smoking and disease activity and burden of symptoms and (b) investigate the relation between smoking and type I IFN signature in pSS patients.

Methods

Patients

Between September 2017 and May 2018, consecutive patients with an established diagnosis of pSS, who attended the out-patient clinic at the Department of Rheumatology, Skane University Hospital, Malmö, Sweden, were asked to participate in the study. Only patients above 18 years of age and fulfilling the American-European Consensus Group (AECG) criteria were included [20]. Exclusion criteria were concomitant fulfilment of other rheumatological diseases and inability of filling out the requested questionnaires due to mental incapacity or insufficient language skills. Patients underwent a physical examination and blood samples were drawn. Based on structured clinical evaluation and laboratory analyses the ESSDAI was calculated. In addition, patients filled out the ESSPRI form and a structured questionnaire regarding smoking habits. None of the patients was using chewing tobacco. Patient characteristics are summarised in Table 1.

Questionnaire on smoking habits

The questionnaire on smoking habits included questions on whether the patient ever had been smoking regularly and if the patient declared he/she had been smoking regularly, if he/she had quit smoking. The patient was considered a current smoker if answering "Yes" to the question "Have you ever been smoking on a regular basis?" and "No" to the question "Have you quit smoking?". The patient was considered a former smoker if answering "Yes" to the question "Have you ever been smoking on a regular basis?" and "Yes" to the question "Have you quit smoking?". Never smokers were defined as answering "No" to the question "Have you ever been smoking on a regular basis?" In case a patient declared ever being smoking on a regular basis, the patient

Table 1 Primary Sjögren's syndrome patient characteristics

	n	
Females (%)		84/90 (93)
Age (years)	90	66.5 (51.8–73.0)
Current smoker (%)		5/90 (6)
Smoke duration, years	5	48 (36.5–59.5)
Pack-years, years	5	25 (11.3–40.2)
Former smokers (%)		37/90 (41)
Smoke duration, years	37	17 (11.0–30.0)
Pack-years, years	37	8.1 (3.4–17.8)
Time since smoking cessation, years	37	27 (16–39)
Time between smoke cessation and diagnosis, years	37	13 (6.5–21)
Time between smoke cessation and sicca symptoms, years	17	14 (1–21)
Never smoker (%)		48/90 (53)
Ever smokers (current + former smokers) (%)		42/90 (47)
Pack-years, years	42	8.8 (4.0–19.0)
Smoke duration, years	42	19.5 (11.8–35.0)
Years diagnosed with pSS, years	90	13 (5.0–21.3)
Years since first sicca symptom, years	41	15 (5.0–26.0)
Anti-SSA seropositives (%)		78/90 (87)
Anti-SSB seropositives (%)		53/90 (59)
ANA seropositives (%)		70/88 (80)
RF seropositives (%)		62/88 (71)
Focal sialoadenitis (%)		62/68 (91)
IgG, g/L	89	14.8 (±5.35)
C3, g/L	89	0.94 (±0.21)
C4, g/l	89	0.18 (±0.06)
ESSDAI total	89	3 (1.0–7.5)
ESSDAI high activity		7/89 (8)
ESSDAI moderate activity		30/89 (34)
ESSDAI low activity		52/89 (58)
ESSPRI total	89	6.7 (4.7–8.0)
Type I IFN positive		64/89 (72)

Data are presented as *n* cases/*n* total (%). For continuous data: mean (±SD)/median (q1–q3)

ANA anti-nuclear antibodies, *C* complement component, *ESSDAI* EULAR Sjögren's Syndrome Disease Activity Index, *ESSPRI* EULAR Sjögren's Syndrome Patient Reported Index, *IFN* interferon, *IgG* immunoglobulin G, *RF* rheumatoid factor

was asked what year he/she started and stopped smoking as well a question on current tobacco consumption and mean tobacco consumption during years of smoking. A translated version of the form is found in the supplementary materials (Supplementary material).

Patients were divided into the following groups according to smoking status: (1) current smokers, (2) former smokers and (3) never smokers. Cigarette consumption, defined as pack-years was also calculated from the questionnaire. A pack-year is defined as twenty cigarettes smoked every day for one year, i.e. a cumulative consumption of 7300 cigarettes. The average number of pack-years amongst ever smokers (current + former smokers) was calculated and patients were divided into those having a cigarette consumption above or equal to the median and those with a consumption below the median.

ESSDAI and ESSPRI

The ESSDAI index and the ESSPRI index are validated indices, widely used in pSS studies, and are described in detail elsewhere. In short, ESSDAI measures disease activity in twelve different domains where different organ systems are assessed as well as laboratory analyses mirroring disease activity. The total score is calculated by adding the individual domains scores which gives a total score ranging between 0 and 123. Low, moderate and high disease activity is defined as a score of 0–4, 5–13 and ≥ 14 respectively. In

the ESSPRI, patients score their symptoms in three different domains: dryness, pain, and fatigue on a Likert scale between 0 and 10. The total ESSPRI score is calculated as the mean of the different domains and thus has a range of 0–10 [21–23].

Laboratory analyses

Blood samples were collected during routine follow-up appointments at office hours using a standardised (non-fasting) procedure. Immunoglobulin (Ig)G, C3 and C4 were measured by nephelometry using an Immage800 (Beckham Coulter Inc., Brea, CA, USA). Data on the presence of rheumatoid factor (RF), antinuclear antibodies (ANA), anti-SSA and anti-SSB antibodies, all measured by validated methods in clinical care, is registered continuously in all pSS patients in our registry (Malmö Sjögren's Syndrome Registry) and were not re-analysed for the current study. Currently, RF is measured by Phadia ImmunoCap250, anti-SSA/SSB by EuroblotOne, and ANA is by indirect immunoflourescence using HEp 2010 cells as substrate.

RT-PCR analysis of type I IFN signature

Blood was collected in clotting tubes for serum preparation and in PAXgene RNA tubes (PreAnalytix, Hombrechtikon, Switzerland) for whole blood RNA analysis. Samples were stored in − 80 °C until analysis. RNA isolation, cDNA preparation and RT-PCR were performed according to the manufacturer's protocol. The protocol and selection of expressed genes is previously described in detail [5]. The type I IFN-induced genes analysed include IFI44, IFI44L, IFIT1, IFIT3 and MxA. Patients were divided into groups that were positive or negative for type I IFN using a threshold of mean healthy controls (HC) + 2 $S.D_{HC}$ based on the previous analysis [5].

Statistics

Due to small sample sizes, continuous data were generally considered non-normally distributed and thus data is presented as median (q1–q3) and the Kruskal–Wallis test was used for multiple group comparisons and the Mann–Whitney *U* test for comparison between groups. For continuous data including more than 80 cases, data were analysed for normality by visual inspection of histograms and Q–Q-plots and Shapiro–Wilk test. If normally distributed, data are presented as mean [± standard deviation (SD)] and the one-way analysis of variation (ANOVA) was used for multiple group comparisons and the independent samples *T* test for comparison between groups. The χ^2-test or Fisher's exact test were used for discrete variables. Due to co-linearity of different variables, Bonferroni correction was not used. A p value < 0.05 was considered significant. The statistical analyses were performed using SPSS version 22 for Mac.

Results

Of 109 consecutive patients, 90 agreed to participate and were included in the study. Median age was 66.5 (51.8–73.0) years, 93% were females. In addition to fulfilling the AECG criteria, all patients also fulfilled the American College of Rheumatology (ACR)/EULAR criteria for pSS [24].

Smoking habits and IFN signatures of the study population

No patient declared any tobacco smoking apart from cigarette smoking. Six percent of pSS patients were current smokers, whilst 41% and 53% were former and never smokers, respectively. The median time since smoke cessation amongst former smokers was 27 (16-39) years. The median cigarette consumption amongst ever smokers (former + current smokers) was 8.8 (4.0–19.0) pack-years. 72% of pSS patients showed a type I IFN signature. Further characteristics are found in Table 1.

Smoking habits, ESSDAI/ESSPRI and patient characteristics

No significant differences in ESSDAI total score or activity in the ESSDAI domains were found between never, former or current smokers, nor between patients with a cigarette consumption as evaluated by pack-years below vs. ≥ 8.8 pack-years (Tables 2, 3). Comparing patients with a low (< 8.8 pack-years) and high (≥ 8.8 pack-years) cigarette consumption, a significantly higher ESSPRI total score [5.0 (3.0–6.3) vs 8.0 (6.0–8.3); $p = 0.01$], ESSPRI sicca score [6.0 (5.0–9.0) vs 8.0 (8.0–9.0); $p = 0.05$] and ESSPRI pain score [4.0 (2.0–5.0) vs 7.0 (5.0–9.0); $p = 0.01$] were found in the high consumption group (Table 3).

Focal sialoadenitis was present in 3/5 of current smokers, and in over 90% of former smokers and never smokers. Amongst former smokers, there were no significant differences in total ESSDAI score, presence of activity in the ESSDAI domains or total ESSPRI score comparing patients below median (27 years) and ≥ median time since smoke cessation (data not shown). No statistically significant differences in type I IFN signature positivity by smoking status were found (Table 2). Additionally, no statistically significant differences were found in levels of IgG, C3, C4, presence of anti-SSA, anti-SSB, ANA, or RF by smoking status or cigarette consumption (Tables 2, 3).

Table 2 Associations between smoking status and clinical characteristics in primary Sjögren's syndrome patients

	Never smokers, $n=48$	Former smokers, $n=37$	Current smokers, $n=5$	p value
ESSDAI total	3 (1.0–8.0)	4 (1.0–7.5)	1.0 (0.0–8.5)	0.55
ESSDAI activity in each domain, *n* (%)				
Constitutional	6/47 (13)	7/37 (19)	0/5 (0)	0.61
Lymphadenopathy	3/47 (6)	1/37 (3)	0/5 (0)	0.71
Glandular	5/47 (11)	5/37 (14)	0/5 (0)	0.86
Articular	4/47 (9)	4/37 (11)	2/5 (40)	0.12
Cutaneous	3/47 (6)	3/37 (8)	0/5 (0)	1.0
Pulmonary	5/47 (11)	6/37 (16)	1/5 (20)	0.51
Renal	4/47 (9)	4/37 (11)	0/5 (0)	0.83
Muscular	0/47 (0)	1/37 (3)	0/5 (0)	0.47
PNS	2/47 (4)	2/37 (5)	0/5 (0)	1.0
CNS	2/47 (4)	0/37 (0)	0/5 (0)	0.56
Haematological	11/47 (23)	8/37 (22)	0/5 (0)	0.76
Biological	25/47 (53)	20/37 (54)	2/5 (40)	0.89
ESSPRI total score	6.7 (4.7–7.7)	6.0 (4.5–8.0)	8.0 (6.2–8.7)	0.24
ESSPRI domain scores				
Sicca	7.0 (5.0–8.0)	8.0 (5.0–9.0)	9.0 (8.5–9.5)	0.06
Fatigue	7.0 (5.0–8.0)	7.0 (3.0–8.0)	8.0 (4.0–9.0)	0.64
Pain	5.0 (1.0–7.0)	5.0 (3.0–8.0)	8.0 (3.5–9.5)	0.18
Anti-SSA seropositive (%)	40/47 (85)	32/37 (87)	5/5 (100)	1.00
Anti-SSB seropositives (%)	28/47 (60)	22/37 (60)	2/5 (40)	0.78
ANA seropositives (%)	38/46 (83)	28/36 (78)	4/5 (80)	0.92
RF seropositives (%)	33/46 (72)	25/36 (69)	3/5 (60)	0.89
Focal sialoadenitis (%)	36/38 (95)	22/24 (92)	3/5 (60)	0.06
IgG (g/L)	14.4 (±5.09)	15.7 (±5.7)	12.8 (±4.99)	0.40
C3 (g/L)	0.92 (±0.19)	0.94 (±0.23)	1.04 (±0.29)	0.43
C4 (g/L)	0.18 (±0.06)	0.18 (±0.06)	0.20 (±0.07)	0.67
Type I IFN positive (%)	33/47 (70)	29/37 (78)	2/5 (40)	0.16

ANA anti-nuclear antibodies, *C* complement component, *CNS* central nervous system, *ESSDAI* EULAR Sjögren's Syndrome Disease Activity Index, *ESSPRI* EULAR Sjögren's Syndrome Patient Reported Index, *IFN* interferon, *IgG* immunoglobulin G, *PNS* peripheral nervous system, *RF* rheumatoid factor

*$p<0.05$; data are presented as *n*/*n* total (%). For continuous data: mean (±SD)/median (q1–q3). Comparisons between the means or medians were performed using One-way ANOVA or Kruskal–Wallis test. Discrete variables were compared using χ^2 test or Fisher's exact test. ESSDAI is presented as ESSDAI total score as well as presence of activity in each ESSDAI domain. ESSPRI is presented as ESSPRI total as well as domain scores

IFN signature, ESSDAI/ESSPRI and patient characteristics

When studying associations between type I IFN signature and ESSDAI scores, only presence of activity in the ESSDAI articular domain was significantly lower in the type I IFN signature positive group in comparison with the type I IFN signature negative group (7/25 vs 3/64; $p<0.01$). In addition, the ESSPRI total score [6.0 (4.0–7.7) vs 7.7 (5.2–8.2); $p=0.04$], ESSPRI sicca score [7.0 (5.0–8.0) vs 8.0 (6.0–9.0); $p=0.03$] and ESSPRI pain score [4.5 (1.3–7.0) vs 6.0 (5.0–8.0); $p=0.02$] were also significantly lower in the type I IFN positive group (Table 4). Type I IFN signature positive patients were significantly more often seropositive for anti-SSA (97% vs 60%; $p<0.01$), anti-SSB (69% vs 32%; $p<0.01$), ANA (89% vs 58%; $p<0.01$), RF (81% vs 42%; $p<0.01$), and had higher titers of IgG (mean 15.7 g/L; SD 5.26 vs 12.5 g/L; SD 4.95, $p=0.01$) compared to type I IFN signature negative patients. However, no differences in the presence of focal sialoadenitis, or levels of complement 3 or 4 were found between these groups (Table 4).

Discussion

In this study, 6, 41 and 53% of the patients with pSS were current smokers, former smokers and never smokers, respectively. Seventy-two percent were type I IFN signature

Table 3 Associations between smoking consumption and clinical characteristics in primary Sjögren's syndrome patients

	Pack-years < 8.8 years	Pack-years ≥ 8.8 years	*p* value
ESSDAI total	4 (1.0–7.0)	4.0 (0.0–9.0)	0.87
ESSDAI activity in each domain, *n* (%)			
Constitutional	3/19 (16)	4/23 (17)	1.0
Lymphadenopathy	1/19 (5)	0/23 (0)	0.45
Glandular	1/19 (5)	4/23 (17)	0.36
Articular	4/19 (21)	2/23 (9)	0.38
Cutaneous	1/19 (5)	2/23 (9)	1.0
Pulmonary	4/19 (21)	3/23 (13)	0.68
Renal	0/19 (0)	4/23 (17)	0.11
Muscular	0/19 (0)	1/23 (4)	1.0
PNS	1/19 (5)	1/23 (4)	1.0
CNS	0/19 (0)	0/23 (0)	–
Haematological	5/19 (26)	3/23 (13)	0.43
Biological	10/19 (53)	12/23 (52)	1.0
ESSPRI total score	5.0 (3.0–6.3)	8.0 (6.0–8.3)	0.01*
ESSPRI domain scores			
Sicca	6.0 (5.0–9.0)	8.0 (8.0–9.0)	0.05*
Fatigue	5.0 (2.0–8.0)	8.0 (5.0–9.0)	0.09
Pain	4.0 (2.0–5.0)	7.0 (5.0–9.0)	0.01*
Anti-SSA seropositive (%)	18/19 (95)	19/23 (83)	0.36
Anti-SSB seropositives (%)	13/19 (68)	11/23 (48)	0.22
ANA seropositives (%)	13/19 (68)	19/22 (86)	0.26
RF seropositives (%)	12/19 (63)	16/22 (73)	0.51
Focal sialoadenitis (%)	10/12 (83)	15/17 (88)	1.0
IgG (g/L)	15.9 (±6.98)	14.9 (±4.50)	0.68
C3 (g/L)	0.94 (±0.25)	0.97 (±0.24)	0.73
C4 (g/L)	0.19 (±0.08)	0.19 (±0.05)	0.98
Type I IFN positive (%)	15/19 (79)	16/23 (70)	0.73

ANA anti-nuclear antibodies, *C* complement component, *CNS* central nervous system, *ESSDAI* EULAR Sjögren's Syndrome Disease Activity Index, *ESSPRI* EULAR Sjögren's Syndrome Patient Reported Index, *IFN* interferon, *IgG* immunoglobulin G, *PNS* peripheral nervous system, *RF* rheumatoid factor

*$p < 0.05$; Data are presented as *n*/*n* total (%). For continuous data: mean (±SD)/median (q1–q3). Means or medians were compared using independent *T* test or Mann–Whitney *U* test. Discrete variables were compared using χ^2 test or Fisher's exact test. ESSDAI is presented as ESSDAI total score as well as the presence of activity in each ESSDAI domain. ESSPRI is presented as ESSPRI total as well as domain scores

positive. A higher cumulative cigarette consumption was associated with higher scores in ESSPRI total and ESSPRI sicca and pain domains. On the contrary, type I IFN signature was associated to lower scores in ESSPRI total, and ESSPRI sicca and pain domains although no association was found between a higher cumulative cigarette consumption and type I IFN signature. Concerning ESSDAI scores, there were no associations with higher cigarette consumption, nor was type I IFN positivity significantly associated with ESSDAI-scores besides a significant negative association with the presence of activity in the ESSDAI articular domain.

When dividing patients into never, former and current smokers, no significant associations were found with ESSDAI-scores, ESSPRI scores or type I IFN positivity. However, due to the low numbers of current smokers in the current study, caution should be taken regarding conclusions about the effect of current smoking.

Several previous studies [9–11, 25] have shown a negative association between smoking and pSS diagnosis. However, it is unclear whether the reported negative association is due to a local effect of smoking in the exocrine glands or to a more systemic effect. A possible explanation of the lower frequency of current smokers amongst pSS patients could be that the dryness of the oral cavity and airways increases the irritation of the smoke thereby making the patients more prone to quit smoking. Although the symptoms of dryness may have impact, one study comparing pSS patients with non-pSS sicca patients [9] and another study using data on

Table 4 Clinical characteristics stratified on type I interferon activation

	IFN I neg, $n=25$	IFN I pos, $n=64$	p value
ESSDAI total score	3.0 (0.0–8.0)	3.0 (1.0–7.8)	0.85
ESSDAI activity in each domain			
Constitutional	5/25 (20)	8/64 (13)	0.50
Lymphadenopaty	1/25 (4)	3/64 (5)	1.0
Glandular	2/25 (8)	8/64 (13)	0.72
Articular	7/25 (28)	3/64 (5)	< 0.01*
Cutaneous	2/25 (8)	4/64 (6)	1.0
Pulmonary	1/25 (4)	11/64 (17)	0.17
Renal	2/25 (8)	6/64 (9)	1.0
Muscular	0/25 (0)	1/64 (2)	1.0
PNS	1/25 (4)	3/64 (5)	1.0
CNS	1/25 (4)	1/64 (2)	0.49
Haematological	4/25 (16)	15/64 (23)	0.44
Biological	13/25 (52)	34/64 (53)	0.92
ESSPRI total score	7.7 (5.2–8.2)	6.0 (4.0–7.7)	0.04*
ESSPRI domain scores			
Sicca	8.0 (6.0–9.0)	7.0 (5.0–8.0)	0.03*
Fatigue	8.0 (5.0–9.0)	7.0 (5.0–8.0)	0.26
Pain	6.0 (5.0–8.0)	4.5 (1.3–7.0)	0.02*
Anti-SSA seropositives	15/25 (60)	62/64 (97)	< 0.01*
Anti-SSB seropositives	8/25 (32)	44/64 (69)	< 0.01*
ANA seropositives	14/24 (58)	56/63 (89)	< 0.01*
RF seropositives	10/24 (42)	51/63 (81)	< 0.01*
Focal sialoadenitis	16/18 (89)	45/49 (92)	0.66
IgG (g/L)	12.5 (±4.95)	15.7 (±5.26)	0.01*
C3 (g/L)	0.94 (±0.21)	0.93 (±0.22)	0.83
C4 (g/L)	0.19 (±0.07)	0.18 (±0.06)	0.42

ANA anti-nuclear antibodies, *C* complement component, *CNS* central nervous system, *ESSDAI* EULAR Sjögren's Syndrome Disease Activity Index, *ESSPRI* EULAR Sjögren's Syndrome Patient Reported Index, *IFN* interferon, *IgG* immunoglobulin G, *PNS* peripheral nervous system, *RF* rheumatoid factor

*$p<0.05$; data are presented as *n*/*n* total (%). For continuous data: mean (±SD)/median (q1–q3). Independent *T* test for continuous, normally distributed variables, Means or medians were compared using independent *T* test or Mann–Whitney *U* test. Discrete variables were compared using χ^2 test or Fisher's exact test. ESSDAI is presented as an ESSDAI total scores as well as the presence of activity in each ESSDAI domain. ESSPRI is presented as ESSPRI total as well as domain scores

smoking acquired years before the individuals were diagnosed with pSS [8] still found a lower frequency of current smokers amongst pSS patients which may indicate that dryness might not be the only explanation. In the current study, we investigated whether smoking habits is associated with the phenotypic expression of pSS. We did not find any evidence that previous smoking alters the phenotype of pSS, neither as evaluated by ESSDAI-scores nor by type I IFN signature. The higher ESSPRI sicca and pain domain scores amongst patients with a higher cumulative cigarette consumption (≥ 8.8 packyears) do signal a long term effect by smoking. E.g. long term smoking has previously been shown to be associated to reduced amount and quality of saliva [26] why harmful effects on the salivary glands, not affecting the development of pSS, might be an explanation for the increased sicca symptoms. Furthermore, there are previous reports of smoking being a risk factor for chronic pain [27], and thus the higher scores in ESSPRI pain amongst patients with higher cumulative cigarette consumption might not be associated with pSS disease activity.

Current smokers in this cohort of consecutive patients were too few to make any conclusions regarding this group. It would be of interest to study a larger group of current smoking pSS patients to investigate if the numerical differences seen in some of the ESSDAI scores and ESSPRI scores in this study (Table 2) can be reproduced, especially since cigarette smoking has been shown to suppress IFN I signalling [28].

We did not find any association between type I IFN signature and ESSDAI total score in the current cohort although there was a significantly lower prevalence of activity in the ESSDAI articular domain amongst type I IFN signature positive patients. Previous studies have shown that the association between the type I IFN signature and the ESSDAI varies between studied cohorts [5–7]. This could be due to the insufficient sensitivity of the ESSDAI to tap systemic inflammation or that the systemic inflammation measured by ESSDAI is not solely driven by type I IFN [29, 30]. However, previously demonstrated associations between type I IFN signature, autoantibodies and hypergammaglobulinemia in two previous pSS cohorts [5] were reproduced in the current pSS cohort.

Concerning symptoms, we found an inverse association between type I IFN signature and ESSPRI total, sicca and pain scores. A negative association between ESSPRI and IFN signature and other proinflammatory cytokines has previously been described [5, 31]. A possible explanation for this finding could be that the mechanisms regulating inflammation also affect symptoms such as fatigue and pain as proposed by Howard Tripp et al. [31]. There are also reports that IFNα inhibits nociceptive transmission in the spinal cord which could provide an explanation to the negative association between type I IFN signature and the ESSDAI pain domain [32]. Furthermore, there are also reports that treatment with low dose IFNα improves salivary flow in pSS patients [33], which can be due to an increased expression of the aquaporine-5 gene [34].

Strengths of the current study include the use of consecutive, well-characterised patients in routine clinical care. However, there were several limitations of the study: Smoking exposure in this cohort of consecutive patients

was mostly long before the study started and the amount of consumed cigarettes varied a lot. Therefore, the population of ever smokers and former smokers was heterogenous. As expected, the number of current smokers was low, making it difficult to draw conclusions about the association to current smoking. However, the prevalence of daily smokers in the general population has reduced [35]. Therefore, it might be difficult to include a larger number of current daily smokers in future studies. Retrospectively acquired data on smoking also has limitations due to recall bias. It is also possible that patients might interpret certain questions in the questionnaire differently such as whether they have been smoking regularly. Potential confounders of sicca symptoms, e.g. use of antidepressants or other drugs with anti-cholinergic side effects, were not available. Most patients had long-standing pSS, and we cannot exclude that different patterns may be observed in patients with recent onset of clinical disease.

Conclusion

Patients with a higher cumulative smoking exposure scored significantly higher in ESSPRI total, and ESSPRI sicca and pain domains scores whilst type I IFN signature positive patients scored significantly lower in ESSPRI total, sicca and pain domains. No difference in type I IFN positivity was found between the group of high and low cumulative cigarette consumption nor were there any differences in ESSDAI total score or activity in the ESSDAI domains.

No significant differences were found between never, former or current smokers in ESSDAI scores, ESSPRI scores or type I IFN positivity. Of note, current smokers were few in this study making it difficult to draw any firm conclusions regarding this group.

In conclusion, there does not seem to be a strong and consistent association between former smoking and disease activity, patient-reported symptoms or laboratory signs of systemic inflammation in pSS, at least not in established disease. A higher cumulative consumption of cigarettes is associated with more symptoms although this might have other explanations than pSS disease activity.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical statement The current study was approved by the regional ethical review board for southern Sweden (Lund, Sweden: 2015/311 and 2017/94). All patients gave written informed consent in accordance with the declaration of Helsinki.

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