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ORIGINAL ARTICLE

Serological lymphocytic activity and patient-reported outcomes in Sjögren's syndrome

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Abstract

This study was set to investigate whether serum markers of lymphocytic activity are associated with patient-reported outcomes in Sjögren's syndrome (SS). Forty-six patients with SS were included in this cross-sectional study. Patients with monoclonal gammopathy, history of malignant lymphoma, or with secondary SS were excluded. Serum levels of IgG, β2-microglobulin (β2M), soluble interleukin-2 receptor (sIL2-R), and free light chains (FLC) were assessed. Systemic disease activity was measured by the EULAR SS disease activity index (ESSDAI). Patient-reported symptoms were recorded by visual analogue scales (VAS) of pain, fatigue, and dryness, as compiled in the EULAR SS patient-reported index (ESSPRI). Depressive symptoms were determined by the Patient Health Questionnaire 9 (PHQ-9). Serum concentrations of κFLC ($r = 0.491$, $p = 0.001$), λFLC ($r = 0.326$, $p = 0.027$), and β2M ($r = 0.421$, $p = 0.004$) correlated with the ESSDAI, whereas sIL-2R and IgG did not. No correlations between serum markers of lymphocytic activity and the ESSPRI, or single VAS measures of pain, dryness, or fatigue, were found. In patients with VAS fatigue scores in the upper quartile, sIL-2R serum levels were even decreased ($p = 0.019$). Only depressive symptoms as determined by PHQ-9 were positively correlated with fatigue ($r = 0.536$, $p < 0.001$). In this well-defined cohort of patients with SS, serological lymphocytic activity was not correlated with patient-reported outcomes and sIL-2R levels were even decreased in patients with high fatigue scores. Only depressive symptoms were correlated with fatigue. This highlights the need to further understand the link between inflammation and disease characteristics in SS.

Keywords ESSDAI · ESSPRI · FLC · Free light chains · IgG · Immunoglobulin G · PHQ-9 · sIL-2R · Sjögren's syndrome · Soluble interleukin-2 receptor · β2-microglobulin

Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease, which is usually slowly progressive. It is characterized by lymphocytic infiltrations of the exocrine glands leading to sicca symptoms, but may also cause extra-glandular manifestations like interstitial lung disease, arthritis, cutaneous vasculitis, and central or peripheral nervous system (PNS) involvement [1]. Serological markers of lymphocytic activity like β2-microglobulin (β2M), soluble interleukin-2 receptor (sIL-2R), and free light chains (FLCs) have been shown to correlate with systemic disease activity and might be useful for disease monitoring [2–4].

Beside specific symptoms related to systemic disease manifestations, patients with SS are suffering particularly from pain, sicca symptoms, and fatigue. These patient-reported symptoms seem to be much more important for health-related quality of life (HRQol) than systemic disease manifestations as captured by the ESSDAI [5]. Whereas sicca symptoms can be clearly attributed to inflammation of the salivary and lacrimal glands, pain is only partially explained by arthritis or peripheral nervous system involvement and is often rather unspecific and diffuse. Impressively, approximately 70% of patients are suffering from fatigue (reviewed in [6]).

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Fatigue has been hypothesized to result from *sickness behavior*, which is an evolutionary adaptive response to infection or to a pro-inflammatory state: decreased activity or sleepiness is conserving energy and beneficial for recovery from infection [7]. However, in SS, it has been shown that several pro-inflammatory cytokines, e.g., tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), are inversely correlated to fatigue [8], and neither rheumatoid factor (RF), antinuclear antibodies (ANAs), anti-SS-A/Ro or anti-SS-B/La, concentrations of immunoglobulins (IgG, IgM, IgA), nor CRP are associated with fatigue [9].

Patient-reported outcomes are important measures in clinical trials and visual analogue scales (VAS) of pain, sicca symptoms, and fatigue are compiled in the EULAR SS patient-reported index (ESSPRI) [10]. Conflicting results have been published on the correlation between markers of lymphocytic activity and these patient-reported outcomes. Whereas one study showed a slight correlation between β2M and the ESSPRI ($r = 0.214$, $p = 0.043$) [2], another study revealed no associations between the ESSPRI and β2M or FLCs [3]. Whether serum concentrations of sIL-2R are correlated to the ESSPRI or single VAS measures of sicca, pain, or fatigue has not been published so far.

Patient-reported symptoms are strongly linked to HRQoL in SS [5]. Accordingly, clinical trials are increasingly focused on the ESSPRI. To treat these patient-reported measures, it is fundamental to understand the link to inflammation and therewith the potential of targeting the immune system. Hence, the aim of this study was to investigate whether serum markers of lymphocytic activity are associated with patient-reported outcomes in SS.

Methods

Patients

Patients with SS were screened at the outpatient clinic of the Department of Rheumatology and Clinical Immunology, University Medical Centre Freiburg, Germany. The American College of Rheumatology (ACR) criteria were used for classification [11]. Patients with monoclonal gammopathy, history of malignant lymphoma, or with secondary SS were excluded. Systemic disease activity was measured by the ESSDAI. This index is compiled of 12 specific domains (constitutional, lymphadenopathy, glandular, articular, cutaneous, pulmonary, renal, muscular, peripheral nervous system, central nervous system, hematological, biological) [12]. Patient-reported symptoms were assessed by the ESSPRI measuring dryness, pain, and fatigue on three VASs (0–10). The ESSPRI is calculated as the average value of these scales and ranges between 0 and 10 [5]. Depressive symptoms were measured using the Patient Health Questionnaire 9 (PHQ-9), ranging from 0 to 27: 1–4 no depression, 5–9 mild depression, 10–14 moderate depression, 15–19 moderately severe depression, and 20–27 severe depression [13]. This study was conducted in compliance with the Declaration of Helsinki and approved by the local ethics committee of the University of Freiburg, Germany.

Laboratory analysis

The analyses of FLC serum levels, creatinine, immunoglobulins, RF, ANAs, complement, β2M, sIL-2R, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were conducted in the routine diagnostic laboratories of the University Medical Centre Freiburg. The estimated glomerular filtration rate (eGFR) was calculated using the *Modification of Diet in Renal Disease formula* (MDRD).

Statistical analysis

SPSS (version 20.0, IBM Corp., New York, United States) and GraphPad Prism (version 6.01, La Jolla, USA) were used for statistical analysis. Continuous values are shown as medians and interquartile range (IQR). Spearman's rank correlation coefficients were computed to evaluate correlations. For the comparison of two groups, the Mann-Whitney *U* test was used. With regard to the number of cases ($n = 46$), we have not adjusted for multiple testing and focused data interpretation on the correlation coefficient (r). p values < 0.05 were considered significant.

Results

Study population

In this prospective study, 51 patients with SS were screened. One patient was excluded because of secondary SS, three patients due to monoclonal gammopathy or lymphoma, and one patient because of dementia precluding informed consent. Finally, 46 patients with SS could be included in this study. Nine patients were on corticosteroid treatment (median dose: 5.0 (13.8–5.0) mg/day), only 2 patients were on other immunosuppressive therapies (both on mycophenolate mofetil), and 11 patients received mild immunomodulatory anti-malaria drugs. For patients' characteristics, see Table 1.

Table 1 Patients' characteristics

	Subjects (*n*)	Value
Age (years)	46	55 (62–46)
Sex (f/m)	46	42/4
Disease duration (years)	46	3.8 (8.2–1.5)
ANA positive (*n*/%)	46	46/100
Anti-SS-A/Ro (*n*/%)	46	36/78.3
Anti-SS-B/La (*n*/%)	46	25/54.4
β2-microglobulin (mg/l)	46	2.76 (3.45–2.29)
sIL-2R (U/ml)	41	496 (861–370)
κ FLC (mg/l)	46	23.2 (33.0–19.7)
λ FLC (mg/l)	46	22.2 (31.1–15.6)
FLC ratio	46	1.16 (1.39–0.93)
IgG (g/l)	46	15.0 (17.0–12.1)
IgM (g/l)	46	1.3 (2.0–0.9)
IgA (g/l)	46	2.8 (3.5–2.3)
RF positive (*n*/%)	45	28/62.2
C3 (g/l)	41	1.02 (1.10–0.90)
C4 (g/l)	41	0.16 (0.22–0.12)
eGFR (ml/min)	46	86 (95–73)

All values are depicted as median (IQR) if not stated otherwise. Not all parameters could be obtained from all patients. *ANA* antinuclear antibodies, *sIL-2R* soluble interleukin-2 receptor, *FLC* free light chains, *Ig* immunoglobulin, *RF* rheumatoid factor, *C* complement component, *eGFR* glomerular filtration rate estimated with MDRD formula

Serological makers of lymphocytic activity

Median serum levels of IgG, β2M, sIL-2R, κFLCs, and λFLC are depicted in Table 1. One patient revealed a very high κFLC serum concentration (676 mg/l), with an elevated κ/λ ratio, normal immunofixation, and without history of malignancy. All statistical analyses were undertaken with and without this outlier. As there were no differences in statistical significance, we decided to exclude this single value to ensure good data visualization and concise data presentation. The serum concentrations of β2M correlated with eGFR ($r = -0.302$, $p = 0.041$), whereas sIL-2R ($r = -0.034$, $p = 0.835$) and FLCs (κFLC: $r = -0.279$, $p = 0.06$, λFLC: $r = -0.245$, $p = 0.101$) did not correlate. No correlations to corticosteroid treatment were observed (data not shown).

Systemic disease activity and serological markers of lymphocytic activity

The median ESSDAI in our cohort was 4 (11–3). Serum concentrations of β2M ($r = 0.421$, $p = 0.004$), κFLC ($r = 0.491$, $p = 0.001$), and λFLC ($r = 0.326$, $p = 0.027$) correlated with the ESSDAI, whereas IgG ($r = 0.231$, $p = 0.122$) and sIL-2R ($r = 0.247$, $p = 0.119$) did not (see Fig. 1).

Patient-reported outcomes and serological markers of lymphocytic activity

Thirty-seven patients completed the ESSPRI. The median ESSPRI was 6.0 (6.9–4.3); no correlation between the ESSDAI and the ESSPRI was observed ($r = 0.198$, $p = 0.240$). Neither serum concentrations of IgG ($r = -0.263$, $p = 0.115$), β2M ($r = 0.002$, $p = 0.993$), sIL-2R ($r = -0.068$, $p = 0.704$), κFLCs ($r = 0.100$, $p = 0.557$), nor λFLCs ($r = -0.093$, $p = 0.584$) correlated with the ESSPRI (see Fig. 1). When analyzing single VAS measures of dryness, pain, and fatigue, no correlations with IgG, β2M, sIL2-R, κFLCs, or λFLCs were found (data not shown). Neither age, disease duration, body mass index, ESR, CRP nor C3, C4, RF positivity, anti-Ro/SS-A positivity, or anti-La/SS-B positivity were associated with fatigue (data not shown). When analyzing patients with VAS measures in the upper quartile (fatigue ≥ 8, pain ≥ 7, dryness ≥ 8), no differences were found for β2M and FLCs, whereas sIL2-R serum concentrations were even lower in patients with high fatigue scores ($p = 0.019$) (see Fig. 2). Depressive symptoms as determined by PHQ-9 strongly correlated with fatigue ($r = 0.563$, $p < 0.001$) (see Fig. 3), but not with pain ($r = 0.247$, $p = 0.140$) or dryness ($r = 0.063$, $p = 0.712$). No correlations between TSH levels ($n = 44$) and the ESSPRI, or single VAS measures of pain, dryness, and fatigue were found (data not shown).

Discussion

In this study, we were able to show that serological lymphocytic activity is not associated with patient-reported outcomes in SS. When analyzing patients with VAS scores in the upper quartile, sIL2-R concentrations were even decreased in patients with high levels of fatigue. The only measure which was positively associated with fatigue was depression. In contrast, systemic disease activity, as determined by the ESSDAI, was strongly correlated with β2M and FLC serum concentrations.

Our study confirms recently published data showing correlations between FLCs, β2M, and the ESSDAI [2, 3]. Moreover, it is the first study to show that sIL-2R serum concentrations are not correlated with the ESSDAI. The release of sIL-2R is a hallmark of unspecific T lymphocyte activation [14], whereas FLCs and β2M are markers of B, and B and T cell activation [15, 16]. This might indicate that markers of B, or B and T cell activation, are stronger surrogates of systemic disease activity in SS than markers of T cell activity. This is consistent with the pathogenesis involving both an abnormal T and B cell response to autoantigens, but particularly a phenotype of B cell hyperactivity [17]. In accordance with published data, we found that IgG is not correlating with the ESSDAI, although it is even included in

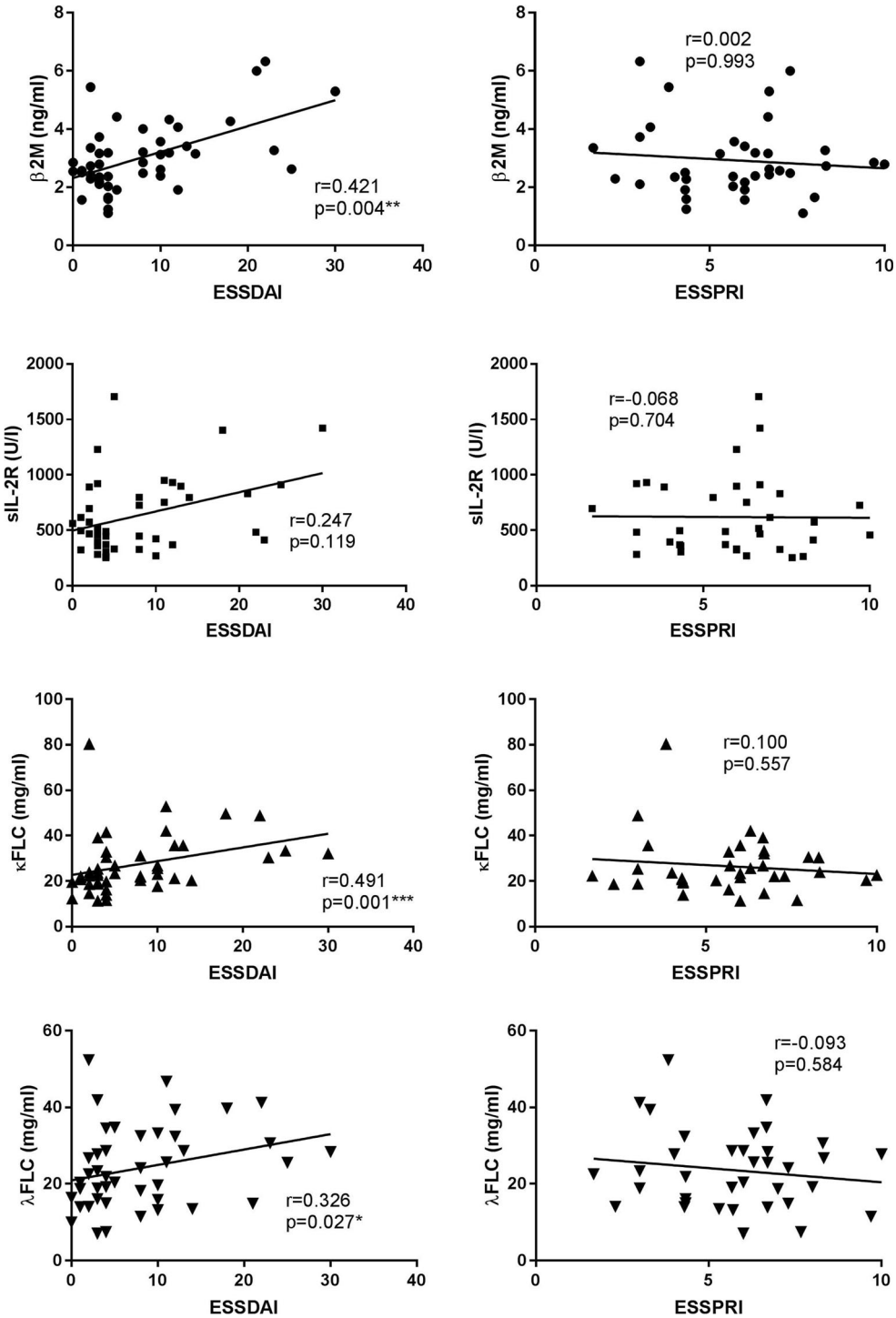

Fig. 1 Correlations between the ESSDAI, the ESSPRI, and serum markers of lymphocytic activity. β2M β2-microglobulin, sIL-2R soluble interleukin-2 receptor, FLC free light chain, ESSDAI EULAR Sjögren's syndrome disease activity index, ESSPRI EULAR Sjögren's syndrome patient-reported index

the index [2]. This might be explained by the rather low weight of IgG in the calculation of the ESSDAI, and disease dynamics, as IgG has a longer half-life (20–25 days) in comparison to FLCs (2–6 h) [15].

In contrast to systemic disease activity, neither the ESSPRI nor single measures of pain and dryness were correlating with serum levels of IgG, β2M, sIL-2R, or FLCs. This is consistent with the concept that dryness is the result of long-lasting local inflammation of the exocrine glands, which might be irrespective of current systemic activity of lymphocytes. Pain is often unspecific and diffuse in SS and only occasionally linked to arthritis or PNS involvement. Accordingly, we found no link between markers of lymphocytic activity and pain. Fatigue in pSS has been hypothesized to result from *sickness behavior* as an evolutionary response to infection or a pro-inflammatory state [18]. However, there is recent data showing that pro-

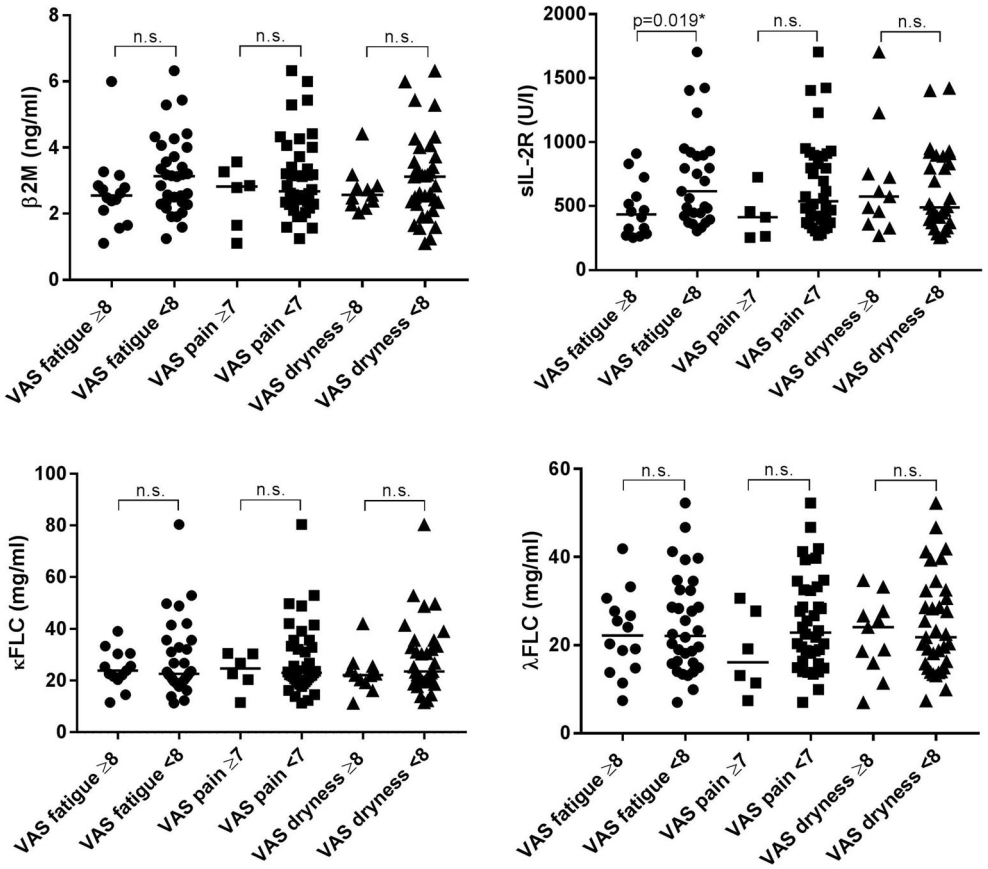

Fig. 2 Patient-reported outcomes and serum markers of lymphocytic activity. β2M β2-microglobulin, sIL-2R soluble interleukin-2 receptor, FLC free light chain, VAS visual analogue scale

inflammatory cytokines are even inversely correlated to fatigue [8]. Our study supports this evidence showing no correlation between IgG, β2M, sIL-2R, or FLCs and fatigue, and even decreased levels of sIL-2R in patients with high fatigue scores. In a large placebo-controlled trial, it has been shown that B cell depleting therapy (rituximab) has some early effects on VAS measures of fatigue; however, the primary endpoint at week 24 failed [19]. Although anti-B cell activating factor (BAFF) therapy (belimumab) showed encouraging effects on systemic disease activity (ESSDAI), fatigue scores did not improve [20]. Consistently, a placebo-controlled trial investigating hydroxychloroquine in SS revealed no effects on fatigue at week 24 [21]. In our study, only depression was positively correlated to fatigue.

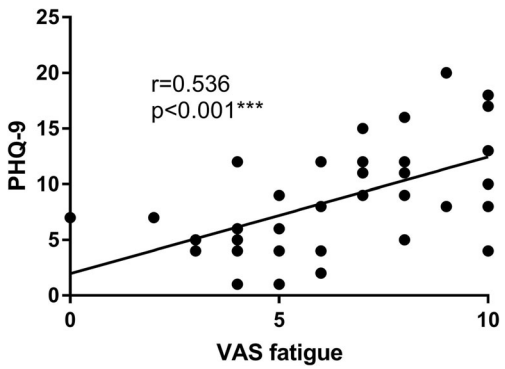

Fig. 3 Correlation between depressive symptoms as determined by PHQ-9 and fatigue. *PHQ-9* Patient Health Questionnaire 9, *VAS* visual analogue scale

This study is unique as it analyzes different serological markers of B and T lymphocyte activation, systemic disease activity, patient-reported outcomes, and depression in a homogenous population of SS patients, without history of lymphatic malignancies, monoclonal gammopathy, or secondary SS. Nevertheless, this study has limitations. Firstly, it is limited by its cohort size and cross-sectional design. Secondly, the VAS fatigue score mainly measures physical fatigue and no other dimensions of the symptom (e.g., mental fatigue). However, physical fatigue is highly important in SS, and the VAS fatigue score is used in most clinical trials as part of the ESSPRI (reviewed in [6]).

To conclude, in this well-defined cohort of patients with SS, serological lymphocytic activity was not correlated with patient-reported outcomes. Patients with high fatigue scores had even decreased serum levels of sIL-2R. Only depressive symptoms were positively correlated with fatigue. Patient-reported outcomes are more relevant for HRQoL in SS than systemic disease manifestations [5], and clinical trials focusing on these endpoints are emerging. Our study highlights the need to further understand the link between inflammation and

diseases characteristics, and to thoroughly define study cohorts in clinical trials testing new immunosuppressive treatment regimes in SS.

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Compliance with ethical standards

Ethical standards This study has been approved by the ethics committee of the University of Freiburg and was conducted in compliance with the Declaration of Helsinki and its later amendments. All patients gave their informed consent prior to inclusion in the study.

Disclosures None.

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