Evaluation of soluble CD25 as a clinical and autoimmune biomarker in primary Sjögren's syndrome

J. Chen, Y. Jin, C. Li, Y. Gan, J. Li, S. Chen, X. Sun, J. He, Z. Li

Department of Rheumatology and Immunology, Peking University People's Hospital, Beijing, China.

Jiali Chen, MD
Yuebo Jin, MD
Chun Li, MD
Yuzhou Gan, MD
Jing Li, PhD
Shi Chen, MD
Xiaolin Sun, PhD
Jing He, MD
Zhanguo Li, MD, PhD

Please address correspondence to:
Jing He,
Department of Rheumatology and Immunology,
Peking University People's Hospital,
11 Xizhimen South Street,
Beijing 100044, China.
E-mail: hejing1105@126.com

and
Zhanguo Li,
E-mail: zgli99@aliyun.com

Received on May 4, 2020; accepted in revised form on June 15, 2020.

Clin Exp Rheumatol 2020; 38 (Suppl. 126): S142-S149.

Key words: soluble CD25, disease activity, anaemia, primary Sjögren's syndrome

Funding: this work was supported by the National Natural Science Foundation of China (81701607, 81801615 and 81671602).
Competing interests: none declared.

ABSTRACT

Objective. *Serum soluble CD25 (sCD25) is associated with T cell activation and regarded as a marker of disease activity in autoimmune disorders. The aim of our study was to investigate the clinical relevance of sCD25 in patients with primary Sjögren's syndrome (pSS).*

Methods. *Sixty-five pSS patients and 60 healthy controls (HCs) with comparable age and gender were recruited. Serum samples were collected and sCD25 concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Clinical and laboratory changes were examined after 12 weeks of treatment, and data were recorded in detail. The European League Against Rheumatism Sjögren's syndrome disease activity index (ESSDAI) was used to evaluate disease activity.*

Results. *Serum sCD25 levels were significantly increased in pSS patients, compared to HCs ($p<0.001$). The increased sCD25 were positively associated with ESSDAI scores ($p=0.006$), especially the haematological domain ($p=0.002$), and erythrocyte sedimentation rate, and levels of C-reactive protein, immunoglobulin G and γ-globulin ($p<0.001$, <0.001, 0.045 and 0.011, respectively). High sCD25 was strongly associated with anaemia ($p=0.014$) and was inversely correlated with haemoglobin levels ($p=0.002$). In further analysis, we found that patients with autoimmune haemolytic anaemia (AIHA) had the highest levels of sCD25, followed by patients with chronic disease of anaemia (ACD) and iron-deficiency anaemia (IDA). With the improvement after treatment, serum sCD25 levels were significantly decreased, accompanied by resolved anaemia compared to baseline ($p=0.001$).*

Conclusion. *sCD25 was associated with disease severity, especially anaemia in patients with pSS and may serve as an indicator of disease activity.*

Introduction

Primary Sjögren's syndrome (pSS) is a common systemic autoimmune disease characterised by lymphocytic infiltration in lachrymal and salivary glands, leading to xerostomia and xerophthalmia (1). Moreover, approximately 30-40% of patients with pSS develop extraglandular complications, including haematological, pulmonary or renal manifestations (1). The disease displays an array of haematological manifestations, from asymptomatic cytopenia to life-threatening complications such as severe haemolysis anaemia and B-cell lymphoma (2). Anaemia is frequently observed in patients with pSS, and the causes include chronic disease (ACD), iron deficiency (IDA) and autoimmune haemolysis (AIHA) (3). Serious and refractory anaemia complications in pSS have been previously reported (4). However, the specific pathogenesis and prognostic markers associated with anaemia in pSS require further characterisation.

Interleukin (IL) 2 receptor has three polypeptide subunits, including CD25 (IL-2Rα), CD122 (IL-2Rβ) and CD132 (the common γ chain) (5). It is well known that soluble sCD25 is generated as a consequence of proteolytic cleavage, mainly from the membrane of activated T cells, and the serum concentrations of sCD25 are associated with the proliferation of activated T cells (5). High serum concentrations have been reported in patients with Sjögren's syndrome (SS) and other autoimmune diseases, such as systemic lupus erythematosus (SLE) (6), inflammatory myositis (IM) (7) and rheumatoid arthritis (RA) (8). The measurement of sCD25 levels during the progression of diseases may be useful for monitoring autoimmune disease. A previous study showed that salivary and serum sCD25 concentrations were higher in patients with glandular involvement (9). Another study showed that elevated

sCD25 concentrations may indicate the progression of extraglandular manifestations, including renal damage, vasculitis, lung involvement, lymphadenopathy, and Raynaud's phenomenon (10). However, the correlation between serum sCD25 levels and haematological involvements is still unclear. In this study, we analysed the concentrations of serum sCD25 to explore the role of sCD25 as a biomarker for haematological involvements.

Methods

Patients

Patients diagnosed with pSS, according to the 2002 American-European Consensus Group (AECG) classification criteria (11), were recruited from Peking University People's Hospital's Department of Rheumatology and Immunology between March 2019 and September 2019. Sixty-five patients with pSS were enrolled in this study. Sixty healthy controls (HCs) with no evidence of autoimmune disease or other diseases were gender- and age-matched with these patients. All participants were in the age range of 18–70 years. The study was approved by the Peking University People's Hospital Ethics Committee on December 2017 (no. 2017PHB167-01) and conducted according to the provisions of the Declaration of Helsinki and other relevant regulations. All participants provided written consent to the study.

Clinical and laboratory data collection and evaluation

Clinical and laboratory data were recorded at the beginning of the study and at a 12-week follow-up examination. The following characteristics were tracked for each patient in the study: age, gender, disease duration, glandular and extraglandular manifestations, blood cell counts (including leukocyte, lymphocyte, haemoglobin and platelet), erythrocyte sedimentation rate, C-reactive protein, γ-globulin, immunoglobulins G, anti-nuclear antibody (ANA), anti-SSA antibody, anti-SSB antibody, and rheumatoid factor (RF). Systemic features of patients with pSS were described and assessed according to the European League Against Rheumatism (EULAR) Sjögren's syndrome disease activity index (ESSDAI), which includes 12 domains and defines active disease as ESSDAI ≥5 points (12). Moreover, in evaluating the haematological domain of ESSDAI, patients with iron deficiency anaemia were scored as 0 (13). Any therapeutic medications patients were being treated with also were recorded.

For patients with haematological involvement, leukopenia was defined as white blood cell count <4000/mm^3, thrombocytopenia was defined as platelet count <100,000/mm^3 and anaemia was defined as haemoglobin levels <110 g/L for women and <120 g/L for men. Classic criteria were used for diagnosis of autoimmune haemolytic anaemia (AIHA), iron deficiency anaemia (IDA), and anaemia of chronic disease (ACD), as follows. Patients with a positive result for the direct anti-globulin test with laboratory evidence of haemolysis were diagnosed with AIHA (14). Patients with laboratory evidence of microcytic hypochromic anaemia accompanied with low ferritin, low iron, low transferrin saturation, and raised total iron-binding capacity were diagnosed with IDA (15). Patients with an increased concentration of inflammatory markers, altered iron homeostasis including low serum levels of iron, and reduced saturation of iron transferrin along with normal or increased serum concentrations of ferritin, and ruling out other diseases, were diagnosed with ACD (16).

Sample collection and measurement of sCD25

Peripheral blood samples were collected from all participants and processed within 2 hours. Samples were centrifuged at 3500 rpm×10 min and divided into aliquots of 100 μl. Processed samples were either analysed or stored at -80°C. All samples were measured immediately to prevent unnecessary freeze-thaw cycles. An enzyme-linked immunosorbent assay (ELISA) was used to measure the serum levels of sCD25 with an R&D human sCD25 kit (Quantikine ELISA, R&D Systems Inc, USA & Canada). The normal reference range of sCD25 was decided by the 95% confidence interval of healthy controls, and patients with pSS were divided into low sCD25 and high sCD25 groups according to the upper limits of normal (ULN). All processes were conducted according to manufacturer's instructions.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) for normal distribution or median (interquartile range, IQR) for abnormal distribution. Categorical variables are shown as numbers (percentages) of the total sample. Spearman's correlation test was used to determine the relationships between sCD25 and clinical parameters. The statistical significance between groups was assessed using the Mann-Whitney U-test, Student *t-test*, Chi-square (χ^2) test and paired *t*-test. Data analyses were calculated using SPSS (v. 20.0, IBM) or Graph Pad Prism (v. 5.0, Graph Pad Software). A significant difference was defined as $p<0.05$.

Results

Characteristics of patients with pSS

A total of 65 pSS patients and 60 healthy controls were enrolled in our study. Sixty-three out of 65 (96.9%) pSS patients were female, and the median age was 56 (50 to 65) years old. The median disease duration was 7 (3 to 12) years and the median ESSDAI score was 7 points (4 to 9 points). Among the pSS patients, the most common systemic manifestation was haematological involvement (51, 78.5%), including leukopenia (27, 41.5%), anaemia (23, 35.4%) and thrombocytopenia (18, 27.7%). Pulmonary involvement (20, 30.8%) and lymphadenopathy (15, 23.1%) were also prevalent manifestations. Thirty-one patients (47.7%) were treated with prednisolone, and 16 (24.6%) were treated with hydroxychloroquine (HCQ). Some patients were being treated with immunosuppressive agents including cyclosporin A (5, 7.7%, CsA), mycophenolate mofetil (2, 3.1%, MMF), leflunomide (7, 10.8%, LEF), and cyclophosphamide (3, 4.6%, CYC).

Serum sCD25 were increased and correlated with disease activity

In pSS patients, the serum levels of

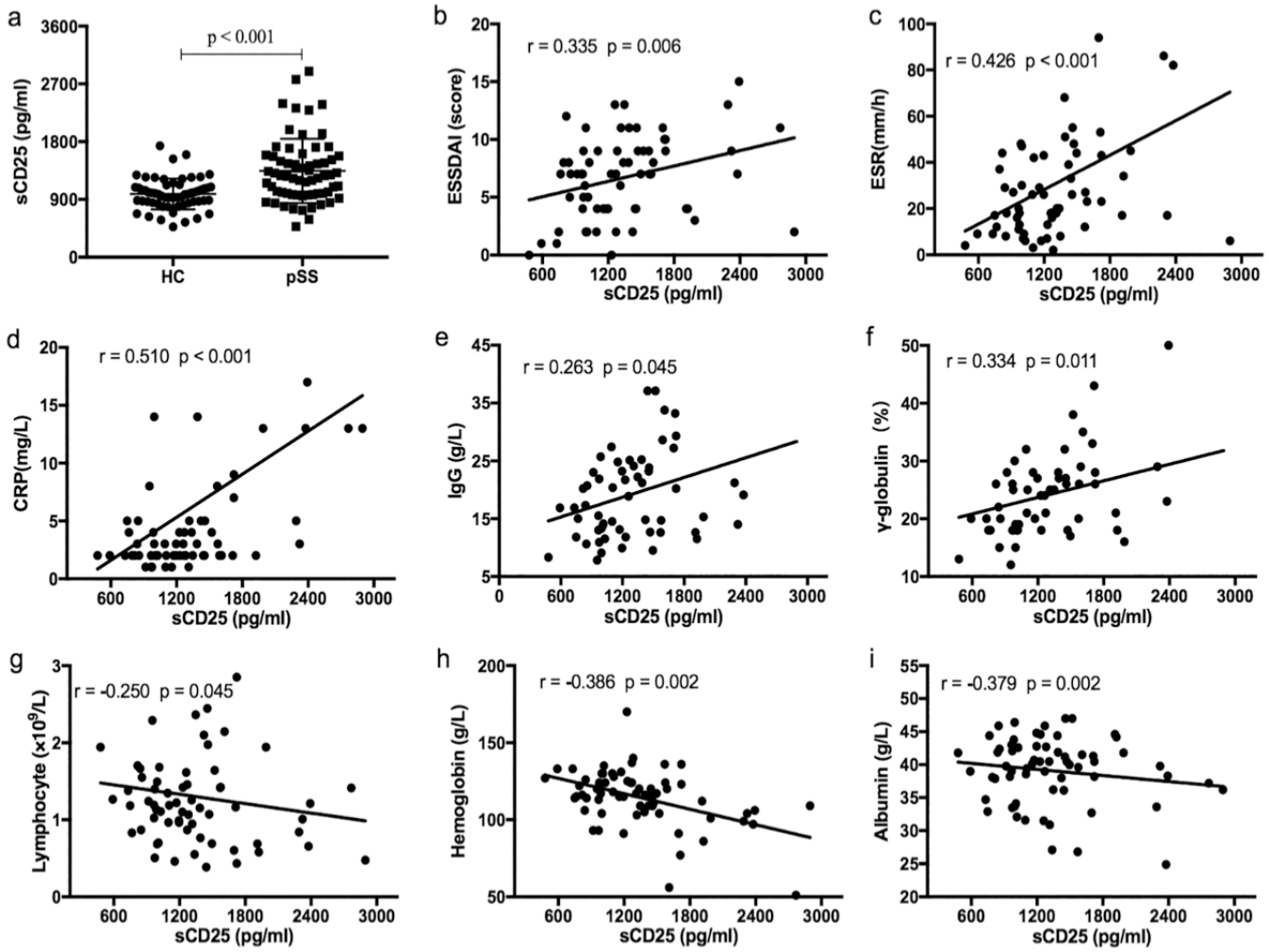

Fig. 1. Elevated serum sCD25 and correlations with clinical parameters in patients with pSS. (**a**) Serum sCD25 levels were higher in patients with pSS (n=65) compared to those in HCs (n=60). Levels of serum sCD25 were positively correlated with ESSDAI scores (**b**), ESR (**c**), CRP (**d**), IgG (**e**) and γ-globulin (**f**), while there were negative correlations between sCD25 levels and lymphocytes (**g**), haemoglobin (**h**), and albumin (**i**). Results are the mean ± SD. Student t-test; Spearman Correlation.
sCD25: soluble CD25; pSS: primary Sjögren's syndrome disease; HC: healthy controls. ESSDAI: European league against rheumatism (EULAR) Sjögren's syndrome disease activity index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; IgG: immunoglobulin G.

sCD25 were significantly higher than those of HCs (1342.1±500.8 pg/ml *vs*. 983.6±240.3 pg/ml, $p<0.001$, Fig. 1a). The ULN of serum sCD25 was 1018.5 pg/ml in our study, so 44 out of 65 (67.7%) pSS patients fell into the high sCD25 group. Patients in the high sCD25 group had higher disease activity, and their median ESSDAI score was 8 points versus 6 points for the low sCD25 group ($p=0.040$). In addition, more patients in the high sCD25 group had haematological involvement ($p=0.025$), including anaemia and thrombocytopenia ($p=0.014$ and 0.024). Moreover, patients in the high sCD25 group had high levels of γ-globulin, IgG, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) ($p=0.003$, 0.016, 0.027 and 0.047, respectively), while there were no significant differences in other demographic and laboratory parameters between the high and low sCD25 groups (Table I).

The serum concentrations of sCD25 were positively correlated with ESSDAI scores (r=0.335, $p=0.006$, Fig. 1b). Also, there were several significant correlations between sCD25 and other parameters associated with disease activity and systemic involvements. Serum levels of sCD25 were positively correlated with ESR (r=0.426, $p<0.001$, Fig. 1c), CRP (r=0.51, $p<0.001$, Fig. 1d), IgG (r=0.263, $p=0.045$, Fig. 1e) and γ -globulin (r=0.334, $p=0.011$, Fig. 1f). Results also indicated that the serum levels of sCD25 were inversely correlated with levels of lymphocytes (r=-0.250, $p=0.045$, Fig. 1g), haemoglobin (r=-0.386, $p=0.002$, Fig. 1h) and albumin (r=-0.379, $p=0.002$, Fig. 1i).

Increased sCD25 was correlated with anaemia

A significant positive correlation between sCD25 and haematological involvement was observed (r=0.371, $p=0.002$, Table II). Serum sCD25 concentrations were higher in patients with haematological involvement than in those without (1432.2±70.8 pg/ml *vs*. 1014.0±86.4 pg/ml, $p=0.002$, Fig. 2a). Haematological involvement includes leukopenia, anaemia and thrombocytopenia, and patients with anaemia had higher levels of sCD25 than those with-

Table I. Clinical characteristics of patients in the high and low sCD25 group.

Variables	Low sCD25 (n=21)	High sCD25 (n=44)	*p*-value
Demographic characteristics			
Gender, female	20 (95.2)	44 (100.0)	0.323
Age, years	56 (30, 60)	60 (50, 69)	0.095
Disease duration, years	6 (2, 10)	7.5 (4, 14)	0.411
Systemic involvements			
Lymphadenopathy	3 (14.3)	12 (27.3)	0.350
Articular	2 (9.5)	5 (11.4)	1.000
Pulmonary	6 (28.6)	18 (40.9)	0.335
Haematological	13 (61.9)	38 (86.4)	0.025*
Leukopenia	10 (15.4)	18 (40.9)	0.609
Anaemia	3 (14.3)	20 (45.5)	0.014*
Thrombocytopenia	2 (10.5)	16 (36.4)	0.024*
Laboratory parameters			
γ-globulin, %	18.5 (17.7, 24.2)	26.0 (20.6, 28.9)	0.003**
Immunoglobulin G, g/L	14.6 (11.1, 19.5)	21.5 (14.6, 26.7)	0.016*
C-reactive protein, mg/L	1.9 (1.4, 3.7)	2.8 (2.0, 5.2)	0.027*
Erythrocyte sedimentation rate, mm/h	26 (18, 48)	29 (13, 44)	0.047*
Rheumatoid factor, IU/mL	364 (88, 514)	546 (154, 618)	0.223
Anti-nuclear antibody, positive	14 (66.7)	37 (84.1)	0.110
Anti-SSA positive	16 (76.2)	35 (79.5)	0.758
Anti-SSB, positive	7 (33.3)	20 (45.5)	0.354
Disease activity index			
ESSDAI score	6 (2, 8)	8 (4, 10)	0.040*

Data are presented as n (%) and median (IQR).
*p<0.05, **p<0.01; Mann-Whitney U-test and Chi-square (χ^2) test.
IQR: interquartile range; sCD25: soluble CD25; ESSDAI: European league against rheumatism (EULAR) Sjögren's syndrome disease activity index.

Table II. Spearman correlations between sCD25 and each domain of ESSDAI.

Domains of ESSDAI	*r*	*p*-value
Haematological	0.371	0.002**
Constitutional	0.109	0.386
Lymphadenopathy	0.305	0.014*
Glandular	-0.124	0.326
Articular	0.089	0.483
Cutaneous	-0.097	0.444
Pulmonary	0.178	0.157
Renal	0.000	1.000
Muscular	0.000	1.000
Peripheral nervous system	0.073	0.562
Central nervous system	0.073	0.562
Biological	0.065	0.607

ESSDAI: European league against rheumatism (EULAR) Sjögren's syndrome disease activity index.
**p<0.01; *p<0.05.

out anaemia (1688.9±122.6 pg/ml *vs.* 1221.0±56.4 pg/ml, p<0.001, Fig. 2b). However, there was no significant difference in other clinical parameters between patients with anaemia and without (Supplementary Table S1). Furthermore, there was no independently significant risk factor for anaemia in multivariate binary logistic regression analyses (Suppl. Table S2).

For the 23 patients diagnosed with anaemia, the mean concentration of haemoglobin was 96.5 g/L (range from 51 g/L to 109 g/L). Among these patients, 15 (65.2%) were diagnosed with ACD, four (17.4%) with IDA and four (17.4%) with AIHA. The mean concentration of sCD25 was 2290.9±218.5 pg/ml for patients with AIHA, 1587.3±135.0 pg/ml for ACD and 1218.2±166.7 pg/ml for IDA. Patients with AIHA and ACD had much higher levels of sCD25 compared to HCs (p<0.001) while there was no difference between patients with IDA and HCs. Furthermore, sCD25 levels of AIHA patient samples were higher than those with ACD and IDA (p=0.025, 0.008, respectively). There was no statistical difference between the sCD25 levels of ACD and IDA patients (Fig. 2c).

Decreased sCD25 was associated with remission of anaemia

All 23 patients with anaemia in our study enrolled in a 12-week follow-up examination. The mean ESSDAI score for these patients decreased from 9 to 6 points (p<0.001) after treatment for 12 weeks. There was also a significant reduction in the mean haematological domain score, changing from 3.1 to 1.4 points (p<0.001). Among these patients, 20 (87.0%) were treated with prednisolone, and the dose was changed from 28.6 mg/day to 16.9 mg/day over the course of 12 weeks. Other medications included HCQ (8, 34.8%), CsA (6, 26.1%), MMF (2, 8.7%), LEF (3, 13.0%), AZA (3, 13.0%) and iron supplement (4, 17.4%). The detailed characteristics of these patients before and after treatment are presented in Table III.

The mean haemoglobin levels of samples taken during the follow-up examinations increased from 96.5 g/L to 108.7 g/L (p=0.015), with a corresponding significant decrease in mean sCD25 levels (1688.9±122.6 pg/ml *vs.* 1190.4±587.8 pg/ml, p<0.001, Fig. 3a). These patients were considered to be in remission if their haemoglobin levels recovered to normal after treatment. According to observed changes in their haemoglobin levels, patients were divided into remission (8 patients) and non-remission (15 patients) groups. Serum sCD25 levels were significantly reduced in patients with remission (906.9±115.8 pg/ml) compared to their baseline (1688.9±122.6 pg/ml, p=0.001) and those without remission (1539.7±154.3 pg/ml) (p=0.013). There was no significant decrease in patients without remission (Fig. 3b).

In these eight responsive patients, four patients (50.0%) had ACD, two (25.0%) had IDA, and two (25.0%) had AIHA. Compared to the baseline level, sCD25

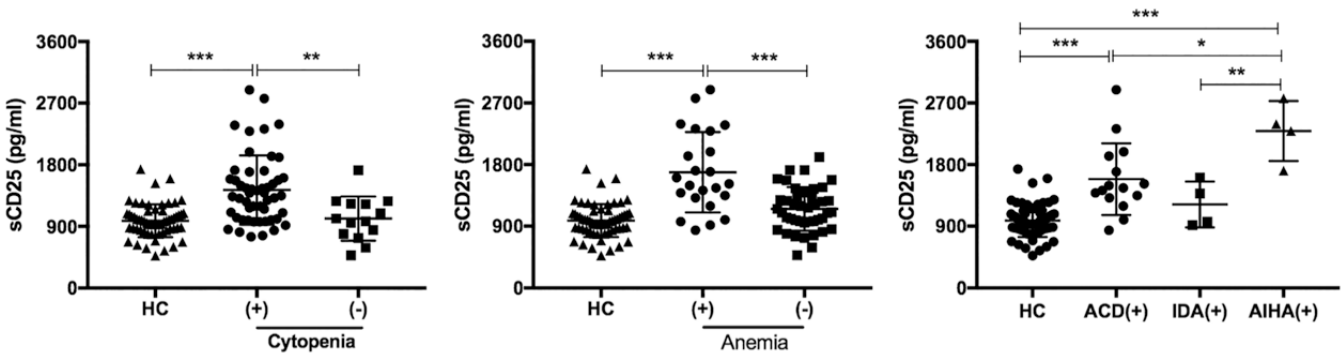

Fig. 2. Elevated levels of sCD25 in patients with anaemia. (**a**) Serum sCD25 levels were higher in patients with cytopenia (n=51) compared to those in HCs (n=60) and pSS patients without cytopenia (n=14). (**b**) Patients with anaemia (n=23) had higher sCD25 levels than those without anaemia (n=28). (**c**) The serum levels of sCD25 in AIHA patients (n=4) were higher than those in patients with ACD (n=15) and IDA (n=4). Results are the mean ± SD.
*p<0.05, **p<0.01; ***p<0.001, Student t-test.
sCD25: soluble CD25; HC: healthy controls; ACD: anaemia of chronic disease; IDA: iron-deficiency anaemia; AIHA: autoimmune haemolysis anaemia.

Table III. Characteristics of patients with anaemia before and after treatment.

Variables	Baseline	3 months later	p-value
Total ESSDAI	9 (4)	6 (3)	<0.001***
Haematological domain of ESSDAI	3.1 (1.5)	1.4 (1.1)	<0.001***
Prednisolone, mg/day	28.6 (18.6)	16.9 (9.8)	<0.001***
Leukocyte, 10^9/L	4.5 (1.6)	4.6 (1.5)	0.841
Lymphocyte, 10^9/L	1.4 (0.7)	1.5 (0.6)	0.339
Haemoglobin, g/L	96.5 (15.8)	108.7 (8.4)	0.015*
Platelet, 10^9/L	196 (53)	214 (56)	0.001**
C-reactive protein, mg/L	1.06 (0.65)	1.23 (1.3)	0.518
Erythrocyte sedimentation rate, mm/h	28.8 (13.0)	24.2 (10.2)	0.077
Immunoglobulin G, g/L	25.7 (6.7)	24.2 (6.0)	0.013*
γ-globulin, %	28.1 (5.5)	26.9 (3.7)	0.131
sCD25, pg/ml	1688.9 (587.9)	1190.4 (636.4)	0.002**

Data are presented as n (%) and mean (SD).
***p<0.001; **p<0.01; *p<0.05. Paired t-test.
ESSDAI: European league against rheumatism (EULAR) Sjögren's syndrome disease activity index.

were significantly reduced in patients with resolved ACD (1587.3±135.0 pg/ml *vs*. 1039.7±134.0 pg/ml, p=0.016, Fig. 3c). Also, the levels of sCD25 in patients with resolved AIHA and IDA were sharply decreased, while there was a slight reduction or even increase in other patients without remission. However, statistical significance could not be established due to the small sample size (Fig. 3d and 3e).

These patients were further categorised into two groups based on changes in their sCD25 levels after treatments: patients with decreased sCD25 levels (n=17) and patients with increased sCD25 levels (n=6). The proportion of remission was 41.2% (7 out of 17 patients) in the group with decreased sCD25 and 16.7% (1 out of 6 patients) in the group with increased sCD25, but the difference was not significant (p=0.369).

Discussion

As has been shown in previous studies, our study showed that the serum concentrations of sCD25 were higher in patients with pSS compared with healthy controls. As there was no significant difference in sCD25 levels of resolved patients compared to the healthy controls, we conclude that sCD25 levels are mainly influenced by the inflammatory disease course. Indeed, the presence of activated $CD4^+$ T cells in exocrine glands, especially T helper 17 (Th17) cells, and elevated cytokines in serum, such as IL-17A, IL-6, interferon (IFN), and tumour necrosis factor (TNF), strongly indicated an ongoing inflammatory process within impaired tissues of patients with pSS (17). Therefore, the serum levels of sCD25 released from activated T cells during the active course (5), may serve as a biomarker to evaluate the disease activity of pSS. For the pSS patients in our study, sCD25 levels were positively correlated with disease activity and laboratory features, such as ESSDAI, ESR, CRP, IgG and γ-globulin, which agrees well with previous studies showing that sCD25 is positively correlated with IgG, CRP (10, 18). Notably, significantly elevated levels of sCD25 were found in patients with haematological involvement, especially those with anaemia. It has been shown that sCD25 could denote the progression of systemic involvements including renal damage, vasculitis, lung involvement, lymphadenopathy and Raynaud's phenomenon (10). Although several studies have reported that sCD25 was a reliable biomarker for assessing the disease activity of lymphoma (19), to our knowledge, this is the first study to report the relationship between sCD25 and haematological disorders of anaemia.

Although sCD25 has been regarded as a biomarker for autoimmune diseases (5), the roles of sCD25 in the pathogenesis of these diseases have not been fully characterised. Several studies have reported that sCD25 can act as an early inhibitor of T-cell response related to IL-2 signalling (20). In the autoimmune encephalomyelitis (EAE) model, sCD25 can enhance the Th17 response and exacerbate EAE by prohibiting signalling of the IL-2 receptor by sequestering the local IL-2 (21). Although CD25 has a lower affinity for IL-2 than the trimeric IL-2 receptor, sCD25 can efficiently bind to secreted IL-2, suggesting its ability to serve as a decoy receptor for IL-2 to play a patho-

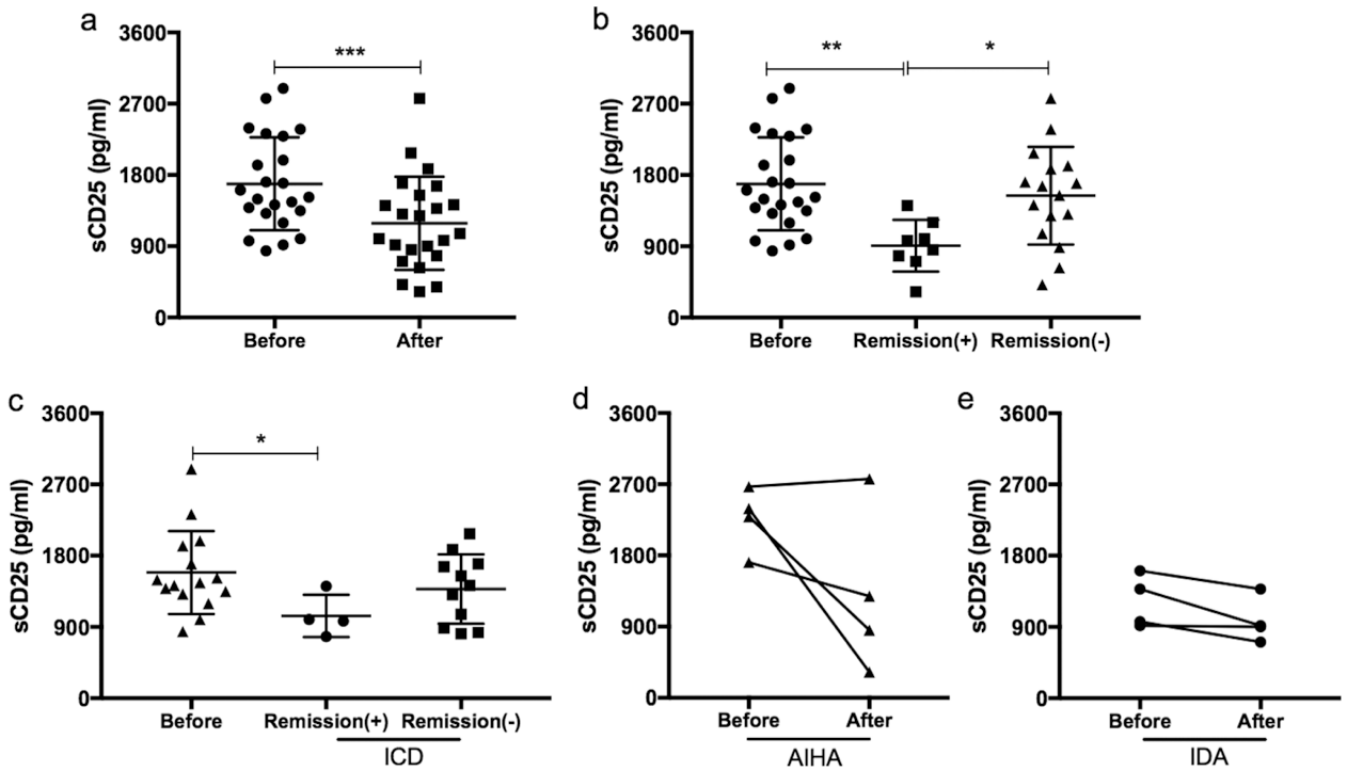

Fig. 3. Decreased serum sCD25 levels were associated with remission of anaemia after treatment. (**a**) The levels of sCD25 were significantly decreased after treatment in patients with anaemia (n=23). (**b**) Serum sCD25 levels were significantly decreased in patients with remission (n=8) compared to the baseline and anaemia patients without remission (n=15). (**c**) Serum sCD25 levels in ACD patients with remission (n=4) were lower than the baseline (n=15) and ACD patients without remission (n=11). (**d** and **e**) The changes in serum sCD25 levels for AIHA (n=4) and IDA (n=4) patients, respectively, before and after treatment.

Results are the mean ± SD. *p<0.05, **p<0.01, ***p<0.001, Student t-test.

sCD25: soluble CD25; ACD: anaemia of chronic disease; ICD: iron deficiency anaemia; AIHA: autoimmune haemolysis anaemia.

genic role in the autoimmune disease (21). It is well-known that IL-2 signalling deficiency in IL-2 knockout mice can cause severe autoimmune disease (22). Previous studies have shown that defective IL-2 and Treg cells, along with activated Th17 cells, are involved in the pathogenesis of pSS (23, 24), which may suggest a direct or indirect relationship between sCD25 and this disease.

Anaemia has been reported in mice with deficiencies of IL-2, IL-2Rα, IL-2Rβ, IL-2Rγ, JAK3 or STAT5 (25). However, it is still unknown why a deficiency in IL-2 signalling could cause an erythroid defect. A recent study has reported that IL-2 signalling is essential for normal bone marrow erythropoiesis via maintenance of Treg cells, which might facilitate IL-2 as a clinical therapy against anaemia in people with autoimmune diseases associated with IL-2 deficiency (25). Accordingly, one study reported that low-dose IL-2 was an effective therapy for SLE, which is one of the most important aetiologies for secondary AIHA (26). Moreover, another study on pSS reported that low-dose IL-2 can increase Treg cells and restore the ratio of Th17/Treg, which helps to resolve disease (27). In our study, patients with anaemia had higher sCD25 levels, which were negatively correlated with levels of haemoglobin. Samples taken during patients' follow-up examinations had significantly decreased levels of sCD25 for patients with remission while no significant difference for patients without remission. Moreover, the rate of remission in the decreased sCD25 group was higher than that for the increased sCD25 group (41.2% *vs.* 16.7%), though there was no significant difference due to the small sample size. In summary, sCD25 may serve as an index to predict the development of anaemia and therapeutic response in patients with pSS. More study is need to determine whether elevated sCD25 levels are related to the pathogenesis of anaemia.

ACD, also referred to as "anaemia of inflammation," is the type of anaemia most frequently associated with chronic diseases, especially autoimmune diseases (28). Immunity-associated mechanisms leading to ACD are the anti-proliferative effects of cytokines, such as TNF-α, IL-6, toward the differentiation and promotion of erythroid progenitor cells (28). Patients with pSS had significant differences in levels of TNF-α and IL-6 compared to healthy controls, but there was no significant correlation found between these increased levels and disease activity (data not shown). Serum sCD25 levels in patients with ACD were elevated compared to healthy controls, and significantly decreased in completely resolved patients compared to patients without remission. These results are useful for evaluating the progression of ACD. This tendency also was observed in patients with AIHA.

AIHA, which rarely occurs in patients with SS, is associated with a higher

frequency of autoantibodies. This is a result of B cell hyperactivity, which is activated or reinforced by autoreactive effector T cells and impaired regulatory T cells (Treg) (29). Some previous studies have shown that pSS patients with AIHA had more positivity of autoimmune antibodies, including ANA, RF, anti-SSA, and anti-SSB (3, 14). In our study, no differences were found between the types and titres of antibodies in patients with AIHA and those with other types of anaemia (data not presented here). Patients with AIHA had the highest serum sCD25 levels, which indicates that autoreactive T cells had an important effect on the pathogenesis of AIHA in patients with pSS.

Although anaemia patients had similar disease activity indexes and their mean ESSDAI score was 7 points, the levels of sCD25 in patients with IDA were notably low. Iron is a critical substance for metabolic processes and energy production in cells. A shortage of iron will impair the function of cellular immunity and secretion of IL-2 (30), and this condition may lead to low serum sCD25 levels in patients with IDA. Similarly, we also observed that patients with leukopenia had lower levels of sCD25 than patients with anaemia or thrombocytopenia (1255.0±78.8 *vs*. 1688.9±122.6 and 1459.0±103.3 pg/ml). Thus, serum sCD25 levels may be used to help clinicians distinguish different types of anaemia and understand the pathogenesis of anaemia in patients with pSS.

There are some limitations to this study. One limitation was the small sample size of our study, which may explain why serum sCD25 levels of AIHA patients were not significantly different after treatment, and why the remission rates of the decreased and increased sCD25 groups were not significantly different. Moreover, the patients were from one single medical centre, with only short-term follow-up, which also may have led to biases. Another limitation of this study was that the different immunosuppressive drugs could influence the immune cells. During the follow-up exams, the mean prednisolone doses were 30 mg/day and 27 mg/day for patients with remission and non-remission, respectively. There also were slight differences in the types and doses of immunosuppressive agents (p>0.05). Our results showed that AIHA patients with remission had strikingly reduced sCD25 levels, which may be a benefit from the high doses of prednisolone (55 mg/day) and CsA (100 mg/day) they received. Future studies should focus on a larger controlled cohort that includes long-term follow-up.

These data demonstrate that sCD25 levels can serve as a useful index to evaluate disease activity and systemic involvement of anaemia in patients with pSS. Serum concentrations of sCD25 were varied based on patients' specific diagnosis (AIHA, ACD and IDA), which may imply in the mechanisms of anaemia associated with pSS and other chronic autoimmune diseases.

Acknowledgements

The authors are grateful to all those who participated in this study and to the staff from the inpatient and outpatient clinics.

References

1. MARIETTE X, CRISWELL LA: Primary Sjögren's syndrome. *N Engl J Med* 2018; 379: 97.
2. BAIMPA E, DAHABREH IJ, VOULGARELIS M, MOUTSOPOULOS HM: Hematologic manifestations and predictors of lymphoma development in primary Sjögren syndrome: clinical and pathophysiologic aspects. *Medicine* (Baltimore) 2009; 88: 284-93.
3. ZHOU JG, QING YF, JIANG L, YANG QB, LUO WF: Clinical analysis of primary Sjögren's syndrome complicating anaemia. *Clin Rheumatol* 2010; 29: 525-9.
4. ASSIMAKOPOULOS SF, MICHALOPOULOU S, MELACHRINOU M *et al*.: Primary Sjögren syndrome complicated by autoimmune hemolytic anaemia and pure red cell aplasia. *Am J Med Sci* 2007; 334: 493-6.
5. RUBIN LA, NELSON DL: The soluble interleukin-2 receptor: biology, function, and clinical application. *Ann Intern Med* 1990; 113: 619-27.
6. ZHANG RJ, ZHANG X, CHEN J *et al*.: Serum soluble CD25 as a risk factor of renal impairment in systemic lupus erythematosus - a prospective cohort study. *Lupus* 2018; 27: 1100-6.
7. TOURNADRE A, DUBOST JJ, SOUBRIER M *et al*.: Soluble IL-2 receptor: a biomarker for assessing myositis activity. *Dis Markers* 2014; 2014: 472624.
8. CAROTTI M, SALAFFI F, FERRACCIOLI GF, BINCI MC, SARTINI A, CERVINI C: Soluble interleukin-2 receptor in sera and synovial fluids of rheumatoid patients: correlations with disease activity. *Rheumatol Int* 1994; 14: 47-52.
9. TISHLER M, YARON I, SHIRAZI I, LEVARTOVSKY D, YARON M: Salivary and serum soluble interleukin-2 receptor in primary Sjögren's syndrome. *Arch Oral Biol* 1999; 44: 305-8.
10. MANOUSSAKIS MN, PAPADOPOULOS GK, DROSOS AA, MOUTSOPOULOS HM: Soluble interleukin 2 receptor molecules in the serum of patients with autoimmune diseases. *Clin Immunol Immunopathol* 1989; 50: 321-32.
11. VITALI C, BOMBARDIERI S, JONSSON R *et al*.: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-8.
12. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69: 1103-9.
13. RETAMOZO S, ACAR-DENIZLI N, RASMUSSEN A *et al*.: Systemic manifestations of primary Sjögren's syndrome out of the ESSDAI classification: prevalence and clinical relevance in a large international, multi-ethnic cohort of patients. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S97-106.
14. WEN W, LIU Y, ZHAO C, SUN X, ZHANG C, LI Z: Clinical and serologic features of primary Sjögren's syndrome concomitant with autoimmune hemolytic anaemia: a large-scale cross-sectional study. *Clin Rheumatol* 2015; 34: 1877-84.
15. GODDARD AF, JAMES MW, MCINTYRE AS *et al*.: Guidelines for the management of iron deficiency anaemia. *Gut* 2011; 60: 1309-16.
16. WEISS G: Anaemia of chronic disorders: new diagnostic tools and new treatment strategies. *Semin Hematol* 2015; 52: 313-20.
17. NOCTURNE G, MARIETTE X: Advances in understanding the pathogenesis of primary Sjögren's syndrome. *Nat Rev Rheumatol* 2013; 9: 544-56.
18. DIAMANT M, TVEDE N, PRAUSE JU, OXHOLM P: Soluble interleukin-2 receptors in serum from patients with primary Sjögren's syndrome. *Scand J Rheumatol* 1991; 20: 370-2.
19. JANIK JE, MORRIS JC, PITTALUGA S *et al*.: Elevated serum-soluble interleukin-2 receptor levels in patients with anaplastic large cell lymphoma. *Blood* 2004; 104: 3355-7.
20. MAIER LM, ANDERSON DE, SEVERSON CA *et al*.: Soluble IL-2RA levels in multiple sclerosis subjects and the effect of soluble IL-2RA on immune responses. *J Immunol* 2009;182: 1541-7.
21. RUSSELL SE, MOORE AC, FALLON PG, WALSH PT: Soluble IL-2Ralpha (sCD25) exacerbates autoimmunity and enhances the development of Th17 responses in mice. *PLoS One* 2012; 7: e47748.
22. KLATZMANN D, ABBAS AK: The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. *Nat Rev Immunol* 2015; 15: 283-94.
23. ALUNNO A, CARUBBI F, BISTONI O *et al*.: T regulatory and T helper 17 cells in primary Sjögren's syndrome: facts and perspectives. *Mediators Inflamm* 2015; 2015: 243723.

24. MIYASAKA N, MUROTA N, YAMAOKA K *et al*.: Interleukin 2 defect in the peripheral blood and the lung in patients with Sjögren's syndrome. *Clin Exp Immunol* 1986; 65: 497-505.
25. CHOPRA M, LANGENHORST D, BEILHACK A, SERFLING E, PATRA AK: Interleukin-2 critically regulates bone marrow erythropoiesis and prevents anaemia development. *Eur J Immunol* 2015; 45: 3362-74.
26. HE J, ZHANG X, WEI Y *et al*.: Low-dose interleukin-2 treatment selectively modulates $CD4^{(+)}$ T cell subsets in patients with systemic lupus erythematosus. *Nat Med* 2016; 22: 991-3.
27. MIAO M, HAO Z, GUO Y *et al*.: Short-term and low-dose IL-2 therapy restores the Th17/Treg balance in the peripheral blood of patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2018; 77: 1838-40.
28. PAPADAKI HA, KRITIKOS HD, VALATAS V, BOUMPAS DT, ELIOPOULOS GD: Anaemia of chronic disease in rheumatoid arthritis is associated with increased apoptosis of bone marrow erythroid cells: improvement following anti-tumor necrosis factor-alpha antibody therapy. *Blood* 2002; 100: 474-82.
29. NOCTURNE G, MARIETTE X: B cells in the pathogenesis of primary Sjögren syndrome. *Nat Rev Rheumatol* 2018; 14: 133-45.
30. SUEGA K, MADE BAKTA I: Influence of Iron on Plasma Interleukin-2 and Gamma Interferon Level in Iron Deficiency Anaemia. *Acta Med Indones* 2010; 42: 147-51.