The abnormal expression of peripheral blood CD4+ T lymphocyte subsets are correlated with primary Sjögren's syndrome complicated with haematological involvement

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Abstract

Objective

Complicated primary Sjögren's syndrome (pSS) with haematological involvement (HI) is not uncommon; however, the aetiology of this condition remains obscure. The clinical characteristics, cytokine levels, and expression of peripheral blood lymphocyte subsets (CD4+ T lymphocyte subsets in particular) of patients with pSS-HI were investigated in this study.

Methods

The pSS-HI group (n= 3), the pSS complicated without HI (pSS-non-HI) group (n=94), and the healthy controls (HCs) group (n=40) were enrolled in the Second Hospital of Shanxi Medical University. The clinical data were gathered, and cytokines and peripheral blood lymphocyte subsets were quantified using flow cytometry and the Cytometric Bead Array (CBA), respectively.

Results

Patients with pSS-HI were more likely than those without pSS-HI to develop skin involvement, had a higher positive rate of anti-SSA antibody, and had elevated levels of IgA, IgG, and ESR. Compared to the pSS-non-HI group, the number of all lymphocyte subsets was lower in the pSS-HI group. However, the proportion of Th2 cells in the pSS-HI group was higher than those in the pSS-non-HI group. In contrast to the pSS-non-HI group, the pSS-HI group exhibited elevated levels of IL-10 and decreased levels of IL-4. A significant correlation was observed between IL-10 and the number of total T cells, CD4+ T cells, CD8+ T cells, NK cells, Th1 cells, Th2 cells, and Th17 cells. In the context of pSS-HI, protective factors may include the number of Treg cells and CD4+ T cells, whereas risk factors may include IgA and the number of Th2 cells.

Conclusion

An immunological mechanism potentially implicated in the development of pSS-HI may be the elevation of IL-10 and the reduction of peripheral blood CD4+ T cell subsets (particularly Treg cells) and serum IL-4 levels.

Key words

primary Sjögren's syndrome, haematological involvement, regulatory T cells, interleukin-4, interleukin-10

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Introduction

Primary Sjögren's syndrome (pSS) is a persistent autoimmune disorder that primarily affects the lacrimal and salivary glands; the most frequent clinical symptoms are parched mouth and eyes (1). There has been a gradual rise in the incidence of pSS in recent years. This condition typically manifests in women around the age of 50 (2).

pSS has the potential to impact not only the salivary and lacrimal glands but also various exocrine glands, tissues, and organs. This can lead to harm across multiple systems and organs, including anaemia, thrombocytopenia, pulmonary interstitial fibrosis, and renal tubular acidosis (3). Research has indicated that an estimated one-third of patients diagnosed with pSS exhibit reduced blood cell counts, which may present as thrombocytopenia, leukopenia, or anaemia (4). Furthermore, pSS is more likely to develop lymphoma, which is an additional sign of haematological involvement of pSS (pSS-HI), than other connective tissue diseases. Furthermore, the incidence of lymphoma substantially amplifies the morbidity and mortality associated with pSS (5). Despite its prevalence, pSS-HI patients are typically asymptomatic. In addition, the majority of patients with pSS-HI have severe disease, a high rate of recurrence, and high treatment costs; some patients even have a dismal prognosis following active treatment (6). As a result, investigating the pathogenesis, clinical characteristics, and effective biomarkers is crucial in the pursuit of developing efficacious medications for patients with pSS-HI.

An incompletely understood inflammatory process involving lymphocytic infiltration into exocrine glands and other tissues contributes to the pathogenesis of pSS. The pathogenesis of pSS is extraordinarily complex, requiring the participation of multiple subsets of T cells in addition to hyperactive B cells (7, 8). Recently, it has been discovered that $CD4^+$ T cell subsets in particular play a crucial role in the pathogenesis of pSS (9-11).

In pSS, the mechanism underlying $CD4^+$T cell subsets has become a focal point of research. T helper cell 1 (Th1), T helper cell 2 (Th2), T helper cell 17 (Th17), regulatory T (Treg) cells, and additional subsets of $CD4^+$T cells are possible. Th1 and Th17 cells may instigate SS, with Th2 and follicular helper T (Tfh) cells becoming more prevalent as the disease advances, according to research by Maehara *et al*. (12). Several studies have demonstrated that by secreting cytokines including IFN-γ, tumour necrosis factor-α (TNF-α), and IL-17, Th1 and Th17 cells can stimulate the migration and overactivation of T cells; these cytokines are involved in the pathogenesis of pSS (13, 14). It is believed that Th2 cells may modulate the initial responses of B cells (12). Moreover, we discovered an increase in Tfh cells in the peripheral blood of patients with pSS and a correlation between activated Tfh cells and pSS disease activity (15, 16). As a distinct subset of T cells responsible for negative regulatory effects, Treg cells involvement in pSS is contentious. Several studies have yielded conflicting results regarding whether or not pSS increases the expression of Treg cells (17, 18). Moreover, in the pathogenesis of pSS, the signature cytokines of $CD4^+$T cell subsets, including IFN-γ, IL-4, IL-17, and IL-10, play a significant role (19).

It remains unknown whether the aforementioned immune system disorders may manifest similarly in pSS-HI. Presently, research on pSS-HI focuses primarily on clinical characteristics, disease risk factors, and the correlation with auto-antibodies; however, lymphocyte subsets, particularly $CD4^+$T lymphocyte subsets, and the expression of related cytokines are the subject of few studies. The investigation of subsets of peripheral blood lymphocytes and associated cytokines contributes to a better understanding of the pathogenesis of pSS-HI and to the development of more dependable therapeutic targets for future clinical practice.

Materials and method

Patients

The Rheumatology Department of the Second Hospital of Shanxi Medical University admitted a cohort of 137 patients with newly diagnosed pSS between January 2023 and December 2023. All pSS patients were enrolled from hospitalised

patients. Among these, 128 were female and 9 were male. Of these, 94 pSS patients complicated without haematological involvement (pSS-non-HI) in addition to 43 pSS-HI. All patients with pSS were diagnosed using the criteria established by the American-European Consensus Group in 2002 (20) or the American College of Rheumatology/ European League Against Rheumatism in 2016 for the classification of pSS (21). Additionally, pSS-HI patients must satisfy one or more of the subsequent criteria: (i) haemoglobin (Hb) <120 g/L for adult males, Hb <110 g/L for adult females; (ii) white blood cells (WBC) <3.5 $\times 10^9$/L; (3) platelets (PLT) <125$\times 10^9$/L. Pregnancy, severe infections, severe haematologic diseases, other autoimmune diseases, drug-induced cytopenias, anaemia resulting from gastrointestinal haemorrhage, and a prior history of radiation therapy were all deemed ineligible for inclusion among the pSS patients. pSS skin involvement is defined as the presence of vasculitis, annular erythema, photosensitivity, the Raynaud's phenomenon, and other similar symptoms in patients. Manifestations of liver involvement in pSS include jaundice, hepatomegaly, impaired liver function, and elevated aminotransferase levels, among others. Six males and thirty-four females comprise the forty healthy controls (HCs) from the centre of health examination in the Second Hospital of Shanxi Medical University, who were matched with the pSS group in terms of age and gender. The Ethics Committee of the Second Hospital of Shanxi Medical University granted approval for this research (no. 2016KY-007). All participants consented to the investigation with full knowledge.

Table I. Clinical and laboratory characteristics, as well as fundamental information about the pSS-HI and pSS-non-HI groups.

	pSS-HI group n=43	pSS-non-HI groupc n=94	*p*-value
Basic information			
Gender (male/female)	2/41	7/87	0.809
Age (year)	56.63 ± 13.59	55.40 ± 11.62	0.589
Disease duration (year)	2.00 (0.25, 5.00)	3.00 (0.50, 8.25)	0.288
The corrected ESSDAI	3.00 (1.00, 10.00)	2.00 (0.00, 5.00)	0.089
Clinical characteristics			
Dry mouth, n (%)	34 (79.07)	83 (88.30)	0.156
Dry eyes, n (%)	20 (46.51)	47 (50.00)	0.705
Enlargement of parotid gland, n (%)	2 (4.65)	6 (6.38)	0.993
Teeth fell off in clumps, n (%)	15 (34.88)	23 (24.47)	0.206
Fever, n (%)	2 (4.65)	8 (8.51)	0.651
Joint pain, n (%)	19 (44.19)	45 (47.87)	0.688
Skin involvement, n (%)	18 (41.86)	16 (17.02)	0.002
Respiratory involvement, n (%)	4 (9.30)	10 (10.64)	1.000
Liver involvement, n (%)	8 (18.60)	10 (10.64)	0.200
Renal involvement, n (%)	2 (4.65)	2 (2.13)	0.789
Laboratory characteristics			
WBC ($\times 10^9$/L)	4.18 (2.81, 5.86)	5.26 (4.35, 6.74)	< 0.001
Hb (g/L)	112.00 (98.00, 126.00)	131.00 (121.75, 138.25)	< 0.001
PLT ($\times 10^9$/L)	157.00 (90.00, 246.00)	233.00 (199.00, 279.25)	< 0.001
LY ($\times 10^9$/L)	0.81 (0.60, 1.37)	1.57 (1.23, 1.91)	< 0.001
ALT (U/L)	21.70 (15.30, 32.40)	20.70 (13.75, 27.17)	0.775
AST (U/L)	28.70 (21.60, 41.80)	24.35 (19.03, 32.25)	0.027
GGT (U/L)	48.10 (19.50, 79.10)	22.90 (15.68, 52.37)	0.007
ALP (U/L)	99.00 (68.00, 113.79)	83.50 (66.75, 102.01)	0.045
ESR (mm/h)	55.00 (26.00, 96.00)	30.00 (15.00, 51.50)	0.001
CRP (mg/L)	3.20 (1.45, 4.49)	2.80 (1.33, 7.56)	0.898
IgA (g/L)	3.81 (3.21, 4.64)	3.08 (2.27, 3.49)	< 0.001
IgG (g/L)	23.25 (16.07, 26.48)	17.80 (14.55, 20.89)	0.001
IgM (g/L)	1.85 (0.95, 1.99)	1.55 (1.01, 1.89)	0.197
Anti-SSA, n (%)	32 (74.42)	46 (48.94)	0.005
Anti-SSB, n (%)	15 (34.88)	20 (21.28)	0.09
RF-IgA, n (%)	23 (53.49)	40 (42.55)	0.233
RF-IgG, n (%)	17 (39.53)	34 (36.17)	0.705
RF-IgM, n (%)	23 (53.49)	51 (54.26)	0.933

pSS-HI: pSS complicated with haematological involvement; pSS-non-HI: pSS without haematological involvement; ESSDAI: EULAR Sjögren's syndrome disease activity index; the corrected ESSDAI: remove the haematological domain from ESSDAI; WBC: white blood cells; Hb: haemoglobin; PLT: platelets; LY: lymphocyte; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase; ALP: alkaline phosphatase; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Ig: immunoglobulin.

Clinical data collection

We collected the basic information (name, gender, age, duration of disease, disease activity score, etc.), clinical characteristics (dry mouth, dry eyes, fever, joint pain, etc.), and laboratory characteristics of all participants, including routine blood tests (WBC,Hb, PLT, lymphocyte (LY), alanine aminotransferase (ALT), etc.), inflammatory biomarkers, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), immune globulin (Ig) (IgA, IgM, and IgG), antibodies (anti-SSA, anti-SSB, RF-IgA, RF-IgG, RF-IgM), etc. The pSS disease activity was assessed using the ESSDAI, which is the EULAR Sjögren's syndrome disease activity index (22). The corrected ESSDAI was constructed by eliminating the haematological domain.

Detection of lymphocyte subsets

Venous blood was extracted peripherally from every participant. Peripheral blood mononuclear cells (PBMCs) were isolated via centrifugation, and flow cytometry was used to determine the number and proportion of lymphocyte subsets. Using various antibody combinations to stain the cell surface and intracellular compartments, the lymphocyte subsets were distinguished. The lymphocyte subsets were defined as $CD3^+CD19^-$ total T cells, $CD3^-CD19^+$total B cells, $CD3^+CD4^+$T cells, $CD3^+CD8^+$T cells, and $CD3^-CD16^+CD56^+$NK cells. Similarly, the $CD4^+$T cell subsets were categorised as follows: Th1 ($CD4^+IL\text{-}2^+$) cells, Th2 ($CD4^+IL\text{-}4^+$) cells, Th17 ($CD4^+IL\text{-}17^+$) cells, and Treg ($CD4^+CD25^+Foxp3^+$) cells.

Detection of cytokine levels

Within one hour of collection, blood

samples from all pSS patients were centrifuged at 3000g for twenty minutes; the resulting serum was then used for cytokine assays. Using a Cytometric Bead Array (CBA), the concentrations of IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α, and INF-γ in the serum were determined.

Statistical analysis

The data were evaluated using SPSS 26.0. For quantitative data that followed a normal distribution, the mean ± standard deviation was used as a measure. The differences between the two groups were examined using a two-tailed Student's t-test. When quantitative data failed to conform to the normal distribution, the median (P25, P75) was used to depict it. A Mann-Whitney test was used to compare the two groups, while the Kruskal-Wallis H test – both non-parametric tests – was used to compare multiple groups. The qualitative data were expressed numerically. To compare two groups, the χ^2 test was applied. Furthermore, Spearman correlation analysis was used to conduct the correlation analysis. $p<0.05$ (two-sided) was deemed to indicate statistical significance.

Results

Comparison of the basic information and clinical and laboratory characteristics between the pSS-HI group and the pSS-non-HI group

Initially, we examined the distinctions in the basic information (*e.g.* age, gender, the corrected ESSDAI), clinical characteristics (*e.g.* dry mouth, dry eyes, fever, joint pain), and laboratory characteristics (*e.g.* ESR, CRP, IgA, IgM, and IgG) between the pSS-HI and pSS-non-HI groups. Gender, age, duration of disease, and the corrected ESSDAI did not differ between the two cohorts. In contrast, with the exception of skin involvement [18 (41.86%) *vs.* 16 (17.02%), $p=0.002$] in pSS, no statistically significant differences were observed in the other clinical characteristics, including dry mouth, dry eye, and parotid gland enlargement, between the two groups (Table I).

A multitude of significant laboratory characteristic differences were discovered between the pSS-HI and pSS-non-HI groups. Furthermore, the pSS-HI group exhibited significantly higher expressions of aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) compared to the pSS-non-HI group ($p<0.05$). ESR was significantly higher in the pSS-HI group than in the pSS-non-HI group [55.00 (26.00, 96.00) *vs.* 30.00 (15.00, 51.50), $p=0.001$], whereas CRP did not differ significantly between the two groups. Concurrently, the IgA and IgM levels of pSS-HI patients are greater than those of pSS-non-HI patients [3.81 (3.21, 4.64) *vs.* 3.08 (2.27, 3.49), $p<0.001$; 23.25 (16.07, 26.48) *vs.* 1.55 (1.01, 1.89), $p=0.001$]. Anti-SSA positivity was significantly higher in pSS-HI than in pSS-non-HI [32 (74.42%) *vs.* 46 (48.94%), $p=0.005$]. However, anti-SSB, RF-IgA, RF-IgG, and RF-IgM were not significantly differentiating factors (Table I).

Table II. The absolute number and proportion of CD4+ T lymphocyte subsets in the peripheral blood in the pSS-HI and pSS-non-HI groups.

	pSS-HI group n=43	pSS-non-HI group n=94	*p*-value
The absolute number of lymphocyte subsets (cells/μl)			
Total T cells	572.04 (340.05, 911.15)	1075.10 (828.22, 1388.93)	< 0.001
Total B cells	134.61 (85.21, 225.46)	201.92 (139.17, 302.76)	0.002
CD4+ T cells	360.03 (179.16, 505.60)	599.22 (468.78, 749.86)	< 0.001
CD8+ T cells	221.70 (139.40, 350.88)	398.74 (314.09, 548.54)	< 0.001
NK cells	97.08 (45.19, 164.98)	152.74 (100.67, 243.51)	< 0.001
Th1 cells	52.92 (31.16, 99.00)	104.2 (61.37, 136.42)	< 0.001
Th2 cells	6.00 (3.00, 9.00)	9.91 (6.08, 11.81)	< 0.001
Th17 cells	7.00 (3.98, 10.31)	10.71 (7.38, 14.11)	< 0.001
Treg cells	16.11 (10.65, 28.00)	33.67 (24.17, 40.52)	< 0.001
The proportion of lymphocytes subsets (%)			
Total T cells	70.71 (58.76, 75.79)	71.91 (65.33, 77.92)	0.150
Total B cells	16.68 (12.26, 25.08)	15.02 (9.75, 18.83)	0.179
CD4+ T cells	37.9 (29.19, 45.10)	39.35 (34.36, 45.89)	0.138
CD8+ T cells	26.41 (20.26, 32.83)	27.33 (21.80, 35.35)	0.621
NK cells	12.30 (7.41, 16.61)	10.31 (7.44, 14.91)	0.605
Th1 cells	19.13 (12.55, 24.37)	17.69 (12.46, 21.77)	0.397
Th2 cells	1.82 (1.30, 2.40)	1.61 (1.18, 1.90)	0.031
Th17 cells	2.03 (1.23, 2.52)	1.95 (1.34, 2.36)	0.549
Treg cells	5.21 (3.84, 6.77)	5.63 (4.11, 6.23)	0.827
CD4+ T/CD8+ T	1.41 (1.01, 1.91)	1.52 (1.04, 1.85)	0.594
Th1/Th2	9.35 (7.00, 12.83)	12.13 (7.78, 15.02)	0.176
Th17/Treg	0.37 (0.26, 0.53)	0.38 (0.25, 0.45)	0.341

pSS-HI: pSS complicated with haematological involvement; pSS-non-HI: pSS without haematological involvement.

Comparative analysis of peripheral lymphocyte subsets, particularly CD4+ T lymphocyte subsets, between the pSS-HI group and the pSS-non-HI group

The absolute number and proportion of lymphocyte subsets in the pSS-HI and pSS-non-HI groups were investigated in greater detail. There was a statistically significant difference in the number of all lymphocyte subsets between the two groups ($p<0.05$) (Table II). Compared to the pSS-non-HI group, the number of all lymphocyte subsets was lower in the pSS-HI group (Fig. 1). The statistically significant data on the proportion of lymphocyte subsets between two groups are shown in Table II. The proportion of Th2 cells in the pSS-HI group was higher than those in the pSS-non-HI group [1.82 (1.30, 2.40) *vs.* 1.61 (1.18, 1.90), $p<0.05$; 0.36 (0.20, 0.54) *vs.* 0.32 (0.19, 0.38), $p<0.05$] (Fig. 1).

The number and proportion of lymphocyte subsets in pSS-HI group, pSS-non-HI group and HCs group were also further explored. (Suppl. Table S1, Suppl. Fig. S1 and S2).

Cytokine concentrations in the serum of the pSS-HI group vs. the pSS-non-HI group

To determine whether the pSS-HI group and the pSS-non-HI group differ in the serum concentrations of cytokines.

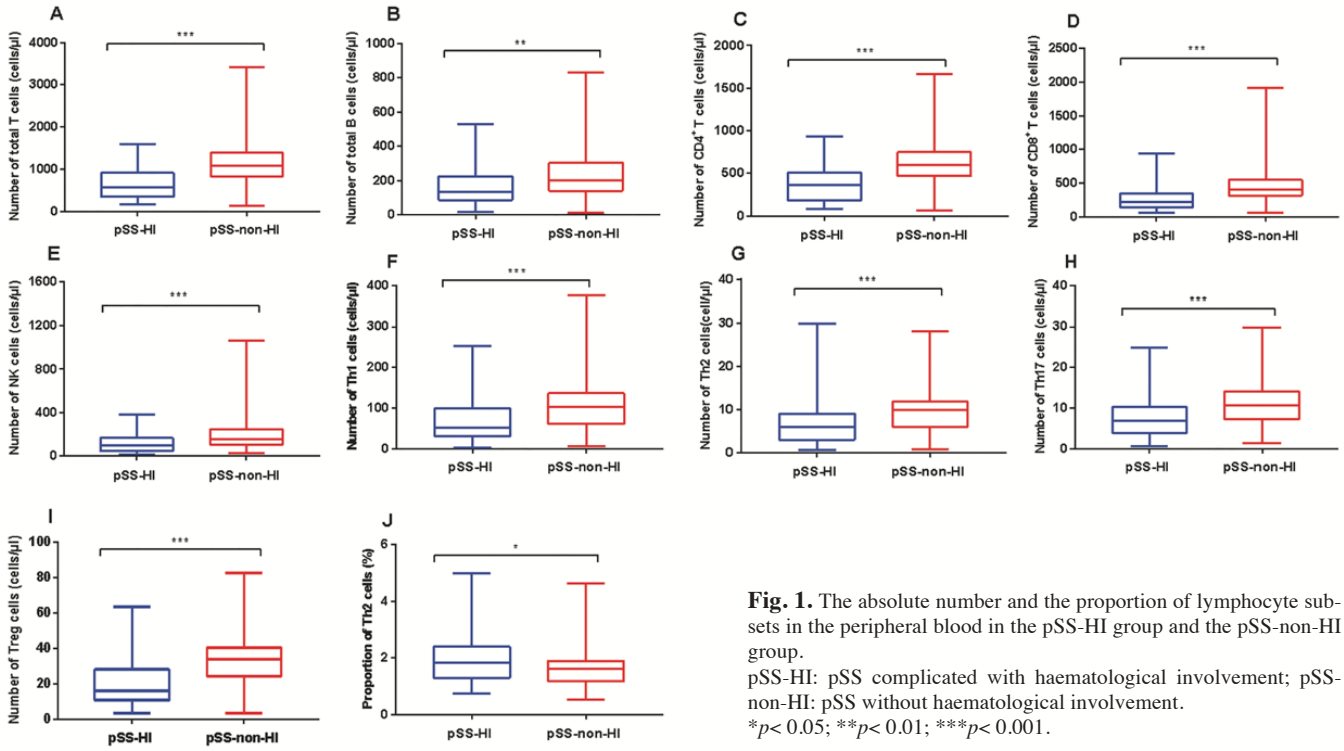

Fig. 1. The absolute number and the proportion of lymphocyte subsets in the peripheral blood in the pSS-HI group and the pSS-non-HI group.
pSS-HI: pSS complicated with haematological involvement; pSS-non-HI: pSS without haematological involvement.
*p< 0.05; **p< 0.01; ***p< 0.001.

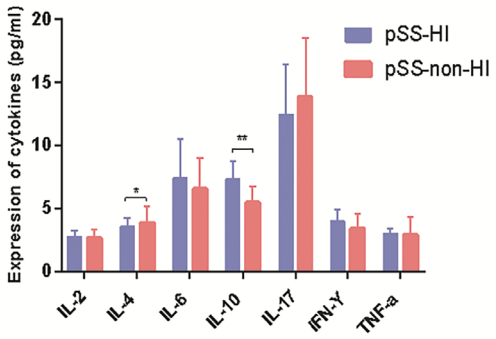

Fig. 2. The differences in the expression of cytokines between the pSS-HI group and the pSS-non-HI group.
IL-2: interleukin-2; IL-4: interleukin-4; IL-6: interleukin-6; IL-10: interleukin-10; IL-17: interleukin-17; IFN-γ: interferon-γ; TNF-α: tumour necrosis factor-α; pSS-HI: pSS complicated with haematological involvement; pSS-non-HI: pSS without haematological involvement.
*p< 0.05; **p< 0.01; ***p< 0.001.

Remarkably, compared with pSS-non-HI group, the serum level of IL-10 [7.34 (4.62, 8.71) *vs*. 5.47 (4.06, 6.75), p=0.006] was significantly higher and the serum level of IL-4 [3.57 (2.18, 4.27) *vs*. 3.85 (2.70, 5.17), p=0.042] was significantly lower in pSS-HI group. While no significant differences were observed in the levels of the remaining cytokines (IL-2, IL-6, IL-17, IFN-γ, and TNF-α), the pSS-HI group exhibited elevated serum concentrations of IL-6 and IFN-γ and decreased expression of IL-17 compared to the pSS-non-HI patients (Fig. 2).

Correlation between IL-10 and laboratory data and lymphocyte subsets

Positive correlations were found between IL-10 and laboratory data, specifically ESR and IgG; negative correlations were observed with Hb (p<0.05). In addition, the data demonstrated that the correct ESSDAI exhibited a progressive rise as serum IL-10 expression increased. Upon examining the correlation between IL-10 level and the number and proportion of lymphocyte subsets, it was found that IL-10 level exhibited a negative association with the following lymphocyte subsets: the number of total T cells (r= -0.217, p=0.011), the number of CD4+ T cells (r= -0.229, p=0.007), the number of CD8+ T cells (r= -0.202, p=0.018), the number of NK cells (r= -0.246, p=0.004), the number of Th1 cells (r= -0.218, p=0.011), the number of Th2 cells (r= -0.206, p=0.016), and the number of Th17 cells (r= -0.258, p=0.002) (Fig. 3).

Multivariate logistic regression analysis associated with pSS-HI

Univariable logistic regression demonstrated that skin involvement, ESR, IgA, IgG, anti-SSA, lymphocyte subsets (such as CD4+ T cells, Th1 cells, Th2 cells, etc.) were significantly associated with the presence of pSS-HI (Suppl. Table S2).

Subsequently, we conducted multivariate logistic regression to assess the independent effect of the occurrence of pSS-HI (Table III). Our results demonstrated that four factors were associated with the occurrence of the patients with pSS-HI, including IgA (OR=1.601, 95% CI 1.129–2.269, p=0.008), the number of Th2 cells (OR=1.140, 95% CI 1.015–1.281, p=0.027), the number of Treg cells (OR=0.938, 95% CI 0.893–0.986, p=0.011), and the number of CD4+ T cells (OR=0.995, 95% CI 0.992–0.998, p=0.005).

Discussion

A minority of pSS-HI patients may manifest myelodysplastic syndrome, cryoglobulinemia, lymphoma, or other malignancies, while cytopenia (throm-

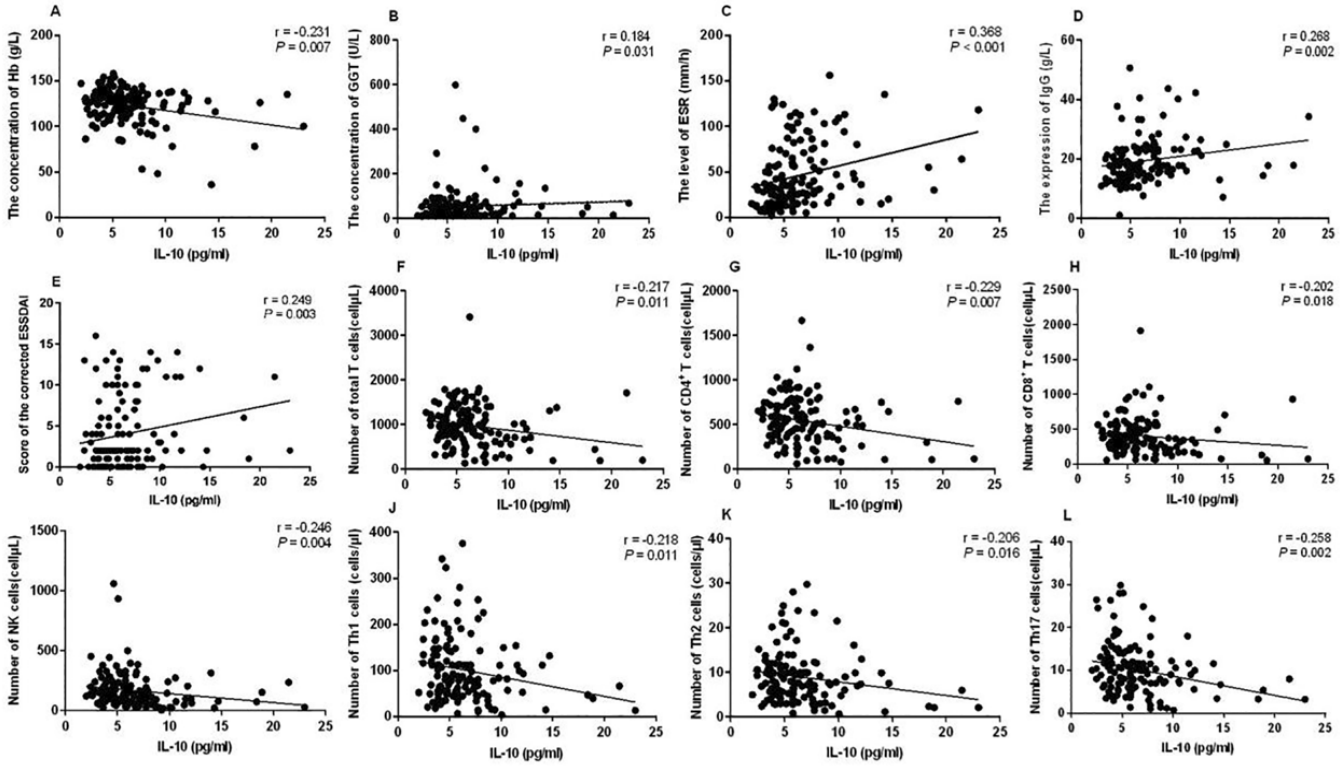

Fig. 3. Correlation analysis between IL-10 and the corrected ESSDAI score, laboratory data, and the number and proportion of lymphocyte subsets.
IL-10: interleukin-10; Hb: haemoglobin; GGT: gamma-glutamyltransferase; ESR: erythrocyte sedimentation rate; IgG: immunoglobulin G; ESSDAI: EULAR Sjögren's syndrome disease activity index; the corrected ESSDAI: remove the haematological domain from ESSDAI.

Table III. Multivariate logistic regression analysis associated with pSS-HI.

Factor	B	SE	Wald	*p*-value	*OR* (95% *CI*)
IgA (g/L)	0.470	0.178	6.989	0.008	1.601 (1.129-2.269)
Th2 cells (cells/μl)	0.131	0.059	4.880	0.027	1.140 (1.015-1.281)
Treg cells (cells/μl)	-0.064	0.025	6.398	0.011	0.938 (0.893-0.986)
CD4+ T cells (cells/μl)	-0.005	0.002	7.957	0.005	0.995 (0.992-0.998)

IgA: immunoglobulin A; Th2 cells: T helper cell 2 cells; Treg cells: regulatory T cells; OR: odds ratio; 95% *CI*: 95% confidence interval.

bocytopenia, anaemia, leukopenia, etc.) is the most common symptom. This study focuses on the exploration of pSS-HI, with cytopenia as the principal symptom. According to studies, anaemia affects 20% of patients with pSS, leukopenia affects 12%-22% of patients, and thrombocytopenia complicates 5%-13% of patients (23, 34). The incidence of pSS-HI patients in our department was 31.39%. There are typically no clinical manifestations of pSS-HI. Certain patients may develop life-threatening complications, including severe autoimmune haemolytic anaemia and severe thrombocytopenia. Despite this, patients are often disregarded. A specific recurrence rate is observed among patients with pSS-HI. In addition, certain patients lack sensitivity to a number of medications and respond poorly to them. Consequently, investigating the pathogenesis, clinical characteristics, and risk factors of pSS-HI patients is crucial.

With the exception of skin involvement, this study found no significant difference in clinical manifestations between the pSS-HI and pSS-non-HI groups; therefore, clinical manifestations do not differentiate the two groups. In line with certain findings from prior research, patients with pSS-HI exhibited elevated levels of ESR, IgA, and IgG, a higher positive rate of anti-SSA antibody, and a greater proportion of total B cells compared to patients with pSS-non-HI. This disparity may be attributed to the participation of B cells in the synthesis of autoantibodies, which leads to the formation of antigen-antibody complexes and the development of HI in pSS patients (25, 26).

To further elucidate the immune cell status of patients with pSS-HI, newly diagnosed pSS patients were included in this study; this allows for a clearer illustration of the role that lymphocyte subsets and cytokines play in the occurrence and development of pSS. Our results showed that a compromised immune system was present in patients with pSS. Patients with pSS exhibited a significant reduction in the number of total T cells, CD4+T cells, and NK cells in peripheral blood when compared to the HCs group. It has been established that aberrant activation of CD4+T cells, in addition to B cells, is a significant factor in the pathogenesis of pSS (8-10). Consistent with prior research, our findings revealed that the number of Th17 cells and the ratio of Th1/Th2 and Th17/Treg in the peripheral blood of pSS patients were higher than those in HCs patients, while the number of Th2 cells was comparatively lower (27-29).

Recently, Treg cells, as a focal point of immunological research, have been found to inhibit an overly aggressive immune response and maintain autoimmune tolerance (30). Nevertheless, the involvement of Treg cells in the development of pSS remains inconclusive. Several studies have reported a reduction in the number of Treg cells in the peripheral blood and salivary glands of pSS patients (31-33). Conversely, some results demonstrated an increase in the numbers of Treg cells in pSS patients (17, 34). This could potentially be attributed to variations in the selection criteria for the study population or the distinct approaches used to identify Treg cells. In our study, there was no statistically significant distinction observed regarding the number of Treg cells between the HCs group and the pSS group. Interestingly, we found that the number of Treg cells was considerably reduced in the pSS-HI group as compared to healthy individuals and pSS-non-HI patients. We hypothesised that the presence of pSS-HI could be attributed to a reduction in the number and functionality of Treg cells, resulting in impaired anti-inflammatory capability and diminished immune tolerance, in conjunction with the elevated ESR levels observed in patients with pSS-HI. In addition, compared with the pSS-non-HI group, the number of lymphocytes, including total T cells, $CD4^+$T cells, NK cells, $CD8^+$T cells, Th1 cells, Th2 cells, Th17 cells, and so on, was also less abundant in the peripheral blood of pSS-HI patients. Lymphopenia and leukopenia were observed in pSS patients in relation to anti-lymphocyte antibodies (ALA), according to Jia *et al*. (35). Therefore, we speculate that the depletion of numerous lymphocyte subsets in pSS-HI patients may be related to the *in vivo* production of ALA. Additional research is required to determine whether ALA is significantly related to the incidence of pSS-HI. The observed elevation in IgA and IgG levels, as well as the positive rate of anti-SSA antibodies, could potentially be attributed to the substantially higher proportion of total B cells in the peripheral blood of pSS-HI patients.

IL-10, a cytokine of the Th2 type, is secreted primarily by activated T cells and macrophages but also by Treg cells, and exhibits anti-inflammatory properties (36). IL-10 has been demonstrated to have a proinflammatory effect in the presence of IFNγ as well (37). In addition, IL10 is an important activating factor for B cells, promoting the differentiation of B cells into plasma cells (38). Research has indicated that elevated levels of plasma IL-10 are significantly associated with the severity of salivary gland lymphocyte infiltration, susceptibility to pSS, and ESR (39). Our results suggested that a high level of IL-10 expression may be a crucial cytokine in the development of pSS-HI, as it stimulates the activation of B cells and the production of autoantibodies, and as it stimulates the prediction of pSS severity, according to our findings. Furthermore, the data revealed a negative correlation between IL-10 and NK cells, Th1 cells, Th2 cells, Th17 cells, and others. This suggests that IL-10 might exert an inhibitory influence on the development of the aforementioned lymphocyte subsets in pSS-HI patients. IL-4, akin to IL-10, functions as a cytokine that inhibits inflammation (38). Insufficient concentrations of serum IL-4 can disrupt the equilibrium between the anti-inflammatory and pro-inflammatory systems, ultimately precipitating pSS-HI.

Limited research has incorporated lymphocyte subsets into its analyses, despite the abundance of studies examining risk factors for pSS-HI (40). According to our study's findings, IgA and the number of Th2 cells may be risk factors for pSS-HI patients, whereas the number of Treg cells and $CD4^+$ T cells in peripheral blood may serve as protective factors. The results of the current studies regarding the screening of risk factors for pSS-HI may be slightly different, which may be related to the inclusion and exclusion criteria of the studies, the selection of multivariate logistic regression analysis methods and independent variables. Our research distinguishes itself from prior investigations by proposing that abnormal expression of peripheral blood lymphocyte subsets might serve as a predictor for the occurrence of pSS-HI. Additionally, it elucidates the significant role that $CD4^+$T cell subsets play in the immune mechanism of pSS-HI. Future efforts to improve $CD4^+$T lymphocyte subsets (particularly Treg cells) are anticipated to yield novel therapeutic agents and techniques for the treatment of pSS-HI.

Conclusion

Our study demonstrated that patients with pSS-HI were more likely to develop skin involvement, elevated IgA levels, and produced auto-antibodies than patients with pSS-non-HI. Additionally, the immunological mechanism leading to the development of pSS-HI may involve a reduction in peripheral blood $CD4^+$ T cell subsets (particularly Treg cells) and serum IL-4 levels, coupled with an increase in serum IL-10.

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References

1. NEGRINI S, EMMI G, GRECO M *et al*.: Sjögren's syndrome: a systemic autoimmune disease. *Clin Exp Med* 2022; 22(1): 9-25. https://doi.org/10.1007/s10238-021-00728-6
2. BROM M, MOYANO S, GANDINO IJ, SCOLNIK M, SORIANO ER: Incidence of cancer in a cohort of patients with primary Sjögren syndrome in Argentina. *Rheumatol Int* 2019; 39(10): 1697-1702. https://doi.org/10.1007/s00296-019-04433-2
3. WANG J, ZHOU L, LIU B: Update on disease pathogenesis, diagnosis, and management of primary Sjögren's syndrome. *Int J Rheum Dis* 2020; 23(6): 723-7. https://doi.org/10.1111/1756-185x.13839
4. BRITO-ZERÓN P, THEANDER E, BALDINI C *et al*.: Early diagnosis of primary Sjögren's syndrome: EULAR-SS task force clinical recommendations. *Expert Rev Clin Immunol* 2016; 12(2): 137-56. https://doi.org/10.1586/1744666x.2016.1109449
5. ZINTZARAS E, VOULGARELIS M, MOUTSOPOULOS HM: The risk of lymphoma development in autoimmune diseases: a meta-analysis. *Arch Intern Med* 2005; 165(20): 2337-44. https://doi.org/ 10.1001/archinte.165.20.2337
6. MANGANELLI P, FIETTA P, QUAINI F: Hematologic manifestations of primary Sjögren's syndrome. *Clin Exp Rheumatol* 2006; 24(4): 438-48.
7. DU W, HAN M, ZHU X *et al*.: The multiple roles of B cells in the pathogenesis of Sjögren's

syndrome. *Front Immunol* 2021; 12: 684999. https://doi:10.3389/fimmu.2021.684999
8. RÍOS-RÍOS WJ, SOSA-LUIS SA, TORRES-AGUILAR H: T cells subsets in the immunopathology and treatment of Sjögren's syndrome. *Biomolecules* 2020; 10(11): 1539. https://doi.org/10.3390/biom10111539
9. HONG X, MENG S, TANG D *et al*.: Single-cell RNA sequencing reveals the expansion of cytotoxic CD4+ T lymphocytes and a landscape of immune cells in primary Sjögren's syndrome. *Front Immunol* 2021; 11: 594658. https://doi.org/10.3389/fimmu.2020.594658
10. YAO Y, MA JF, CHANG C *et al*.: Immunobiology of T Cells in Sjögren's syndrome. *Clin Rev Allergy Immunol* 2021; 60(1): 111-31. https://doi.org/10.1007/s12016-020-08793-7
11. MING B, ZHU Y, ZHONG J, DONG L: Regulatory T cells: a new therapeutic link for Sjögren syndrome? *Rheumatology* (Oxford) 2023; 62(9): 2963-70. https://doi.org/10.1093/rheumatology/kead070
12. MAEHARA T, MORIYAMA M, HAYASHIDA JN *et al*.: Selective localization of T helper subsets in labial salivary glands from primary Sjögren's syndrome patients. *Clin Exp Immunol* 2012; 169(2): 89-99. https://doi.org/10.1111/j.1365-2249.2012.04606.x
13. ZHOU J, KAWAI T, YU Q: Pathogenic role of endogenous TNF-α in the development of Sjögren's-like sialadenitis and secretory dysfunction in non-obese diabetic mice. *Lab Invest* 2017; 97(4): 458-67. https://doi.org/10.1038/labinvest.2016.141
14. JIN JO, KAWAI T, CHA S, YU Q: Interleukin-7 enhances the Th1 response to promote the development of Sjögren's syndrome-like autoimmune exocrinopathy in mice. *Arthritis Rheum* 2013; 65(8): 2132-42. https://doi.org/10.1002/art.38007
15. LI XY, WU ZB, DING J *et al*.: Role of the frequency of blood CD4(+) CXCR5(+) CCR6(+) T cells in autoimmunity in patients with Sjögren's syndrome. *Biochem Biophys Res Commun* 2012; 422(2): 238-44. https://doi.org/10.1016/j.bbrc.2012.04.133
16. KIM JW, LEE J, HONG SM, LEE J, CHO ML, PARK SH: Circulating CCR7loPD-1hi follicular helper T cells indicate disease activity and glandular inflammation in patients with primary Sjögren's syndrome. *Immune Netw* 2019; 19(4): e26. https://doi.org/10.4110/in.2019.19.e26.
17. SARIGUL M, YAZISIZ V, BASSORGUN CI *et al*.: The numbers of Foxp3 + Treg cells are positively correlated with higher grade of infiltration at the salivary glands in primary Sjögren's syndrome. *Lupus* 2010; 19(2): 138-45. https://doi.org/10.1177/0961203309348234
18. LIN JC, PAN KL, LI CF *et al*.: Altered subgroups of regulatory T cells in patients with primary Sjögren's syndrome. *Heliyon* 2023; 9(5): e15565. https://doi.org/10.1016/j.heliyon.2023.e15565
19. TIAN Y, YANG H, LIU N, LI Y, CHEN J: Advances in pathogenesis of Sjögren's syndrome. *J Immunol Res* 2021; 2021: 5928232. https://doi.org/10.1155/2021/5928232
20. VITALI C, BOMBARDIERI S, JONSSON R *et al*.: European Study Group on Classification Criteria for Sjögren's syndrome. 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(6): 554-58. https://doi.org/10.1136/ard.61.6.554
21. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: International Sjögren's syndrome Criteria Working Group. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* 2017; 76(1): 9-16. https://doi.org/10.1136/ard.61.6.554
22. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's Task Force. EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome. *Ann Rheum Dis* 2010; 69(6): 1103-9. https://doi.org/10.1136/ard.2009.110619
23. MALLADI AS, SACK KE, SHIBOSKI SC *et al*.: Primary Sjögren's syndrome as a systemic disease: a study of participants enrolled in an international Sjögren's syndrome registry. *Arthritis Care Res* (Hoboken) 2012; 64(6): 911-18. https://doi.org/10.1002/acr.21610
24. RAMOS-CASALS M, FONT J, GARCIA-CARRASCO M *et al*.: Primary Sjögren syndrome: hematologic patterns of disease expression. *Medicine* (Baltimore) 2002; 81(4): 281-92. https://doi.org/10.1097/00005792-200207000-00004
25. TZIOUFAS AG, TATOULI IP, MOUTSOPOULOS HM: Autoantibodies in Sjögren's syndrome: clinical presentation and regulatory mechanisms. *Presse Med* 2012; 41(9 Pt 2): e451-60. https://doi.org/10.1016/j.lpm.2012.05.022
26. MAVRAGANI CP: Mechanisms and new strategies for primary Sjögren's syndrome. *Annu Rev Med* 2017; 68: 331-43. https://doi.org/10.1146/annurev-med-043015-123313
27. WU G, WU N, LI T, LU W, YU G: Total glucosides of peony ameliorates Sjögren's syndrome by affecting Th1/Th2 cytokine balance. *Exp Ther Med* 2016; 11(3): 1135-41. https://doi.org/10.3892/etm.2016.3016
28. HAO LR, LI XF, GAO C, CAO L, HAN ZY, GAO H: Th17/Treg cell level and clinical characteristics of peripheral blood of patients with Sjögren's syndrome complicated with primary biliary cirrhosis. *Medicine* (Baltimore). 2019; 98(24): e15952. https://doi.org/10.1097/md.0000000000015952
29. VERSTAPPEN GM, KROESE FGM, BOOTSMA H: T cells in primary Sjögren's syndrome: targets for early intervention. *Rheumatology* (Oxford) 2021; 60(7): 3088-98. https://doi.org/10.1093/rheumatology/kez004
30. EGGENHUIZEN PJ, NG BH, OOI JD: Treg enhancing therapies to treat autoimmune diseases. *Int J Mol Sci* 2020; 21(19): 7015. https://doi.org/10.3390/ijms21197015
31. BLINOVA VG, VASILYEV VI, RODIONOVA EB, ZHDANOV DD: The role of regulatory T cells in the onset and progression of primary Sjögren's syndrome. *Cells* 2023; 12(10): 1359. https://doi.org/10.3390/cells12101359
32. ALUNNO A, PETRILLO MG, NOCENTINI G *et al*.: Characterization of a new regulatory CD4+ T cell subset in primary Sjögren's syndrome. *Rheumatology* (Oxford) 2013; 52(8): 1387-96. https://doi.org/10.1093/rheumatology/ket179
33. LIU MF, LIN LH, WENG CT, WENG MY: Decreased CD4+CD25+bright T cells in peripheral blood of patients with primary Sjögren's syndrome. *Lupus* 2008; 17(1): 34-39. https://doi.org/10.1177/0961203307085248
34. GOTTENBERG JE, LAVIE F, ABBED K *et al*.: CD4 CD25high regulatory T cells are not impaired in patients with primary Sjögren's syndrome. *J Autoimmun* 2005; 24(3): 235-42. https://doi.org/10.1016/j.jaut.2005.01.015
35. JIA RL, LI J, HE J, LI C, LI ZG: The relevance of anti-lymphocyte antibody with primary Sjögren syndrome. *Chinese J Rheumatol* 2014; 18(4): 232-35. https://doi.org/10.3760/cma.j.issn.1007-7480.2014.04.004
36. WANG X, WONG K, OUYANG W, RUTZ S: Targeting IL-10 family cytokines for the treatment of human diseases. *Cold Spring Harb Perspect Biol* 2019; 11(2): a028548. https://doi.org/10.1101/cshperspect.a028548
37. SHARIF MN, TASSIULAS I, HU Y, MECKLENBRÄUKER I, TARAKHOVSKY, A, IVASHKIV LB: IFN-alpha priming results in a gain of proinflammatory function by IL-10: implications for systemic lupus erythematosus pathogenesis. *J Immunol* 2004; 172(10): 6476-81. https://doi.org/10.4049/jimmunol.172.10.6476
38. ROESCHER N, TAK PP, ILLEI GG: Cytokines in Sjögren's syndrome. *Oral Dis* 2009; 15(8): 519-26. https://doi.org/10.1111/j.1601-0825.2009.01582.x
39. BERTORELLO R, CORDONE MP, CONTINI P *et al*.: Increased levels of interleukin-10 in saliva of Sjögren's syndrome patients. Correlation with disease activity. *Clin Exp Med* 2004; 4(3): 148-51. https://doi.org/10.1007/s10238-004-0049-9
40. CHEN S, CHEN J, CHANG X, WU J: Analysis of risk factors for primary Sjögren's syndrome complicated with hematological involvement. *Chinese J Clin Med Pract* 2023; 27(3): 91-96. https://doi.org/10.7619/jcmp.20222505