



# Diminished natural killer T-like cells correlates with aggravated primary Sjögren's syndrome

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## Abstract

**Objective** To reveal the characteristics and potential role of natural killer T-like cells (NKT-like cells) in the pathogenesis of primary Sjögren's syndrome (pSS).

**Methods** Forty-six patients with pSS and 30 healthy subjects were enrolled in the study. The frequencies and cell count of NKT-like cells as well as other lymphocyte subsets were analyzed by flow cytometry. The clinical and laboratory indicators of pSS patients were also collected. Then, the correlation between NKT-like cells and pSS patient manifestations was analyzed by Spearman's rank test. In addition, NKT-like cells before and after therapy were also compared.

**Results** Both the number and the frequencies of NKT-like cells were significantly decreased in pSS patients. The counts of NKT-like cells were positively correlated with CD4<sup>+</sup> T cells ( $r=0.464$ ,  $P=0.001$ ), CD8<sup>+</sup> T cells ( $r=0.363$ ,  $P=0.013$ ), NK cells ( $r=0.488$ ,  $P=0.001$ ), and IgM levels ( $r=0.443$ ,  $P=0.002$ ), while negatively correlated with the disease duration ( $r=-0.33$ ,  $P=0.027$ ). Moreover, after effective therapy, NKT-like cells were recovered both in the cell counts and frequencies.

**Conclusion** In pSS, NKT-like cells were fundamentally decreased, potentially contributing to the disease pathogenesis. Modulating the status of NKT-like cells might provide a novel strategy for treating the disease.

## Key Points

- NKT-like cells were significantly decreased in pSS patients.
- NKT-like cells were correlated with pSS patient manifestations.
- NKT-like cells might be served as a new marker for assessing the status of pSS.

**Keywords** Lymphocytes · NK cells · NKT-like cells · Primary Sjögren's syndrome

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## Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disease accompanied with dry mouth, dry eyes, and multiple organ and system damage. The pathogenesis of pSS is still not fully understood, but lymphocytes infiltrating into exocrine glands may play an important role in this disease. T cells are found to be a large part of the lymphocytes which infiltrate in salivary and lacrimal gland tissues, especially in the early stage of pSS [1]. In pSS, B cells are all known as an important element that participated in the activation of autoimmunity and the formation of autoantibodies [2]. NK cells are the first-line defense against exogenous and endogenous molecules, whose dysfunction also contributes to the initiation of the inflammatory process in exocrine glands of pSS patients [3].

Natural killer T-like cells (NKT-like cells), firstly named by Godfrey [4] in 2004, are a distinct lineage of lymphocytes which express the marker of both CD3 + T cells and CD56 + NK cells [5]. Different from classical NKT cells and non-classical NKT cells, NKT-like cells are a distinct lineage of NKT cells which are CD1d-independent and produce abundant IFN- $\gamma$  [4].

NKT-like cells have the potential function of both T cells and NK cells [5, 6], including regulating the Th1 and Th2 balance and against infection and tumor, and serve as a bridge of innate immune and adaptive immune [7, 8]. Recently, dysfunction of NKT-like cells was reported in autoimmune diseases. In SLE, the number of circulating NKT-like cells was decreased and these cells maintained the inflammation in active SLE by upregulating granzyme B and TNF- $\alpha$  [9, 10]. NKT-like cells were also found to be able to produce cytokines that related to systemic sclerosis [11]. In pSS, the level and role of NKT-like cells remain inconsistent [12–15].

In this study, we detected both the proportion and cell count of NKT-like cells in pSS patients, and analyzed the correlation of these cells with clinical and laboratory features, trying to explore the characteristics and potential involvement of NKT-like cells in pSS.

## Materials and methods

### Study subject

Forty-six patients with primary Sjögren's syndrome (pSS) were enrolled at the Department of Rheumatology and Immunology, Peking University People's Hospital, during August to December in 2019. All patients fulfilled the 2016 American College of Rheumatology classification

criteria for pSS [16] and had taken different corticosteroids immunosuppressants. Thirty healthy donors were selected as the healthy controls. All subjects were collected in accordance with a protocol approved by the ethical committee of Peking University People's Hospital.

### Sample preparation

Whole blood sample were collected from 46 pSS patients and 30 healthy donors for the flow cytometric analysis and routine blood test. Meanwhile, sera were collected from 46 pSS patients for other laboratory tests.

### Flow cytometric analysis

According to the instruction, 50 $\mu$ L whole blood were added into two flow tubes, followed by staining with 10 $\mu$ L anti-CD3/-CD8/-CD45/-CD4 with different fluorescence and anti-CD3/-CD16/-CD56/-CD45/-CD19 antibodies, respectively. After 15 min, the mixture was incubated with 500 $\mu$ L hemolysin (purchased from BD company) for 15 min at room temperature. Finally, flow cytometry instrument (Mindray BriCyte E6 flow meter) was used to collect and analyze cells.

### Other laboratory tests

The serum samples were collected for the following serologic parameters: C-reaction protein (CRP), immunoglobulins (IgG, IgA, IgM), complements (C3, C4), RF, D-dimer, erythrocyte sedimentation rate (ESR), ANA, SSA, SSB, and anti- $\alpha$ -fodrin. CRP, immunoglobulins, complements, and D-dimer were examined by immunonephelometry method. ESR and IgM-RF were measured by Westergren's method and the rate nephelometry, respectively. ANA, anti-SSA/SSB, and anti- $\alpha$ -fodrin were detected by indirect immunofluorescence, western blotting, and enzyme-linked immunosorbent assay, respectively. While, white blood cells (WBC) and plates (PLT) were obtained from whole blood and detected by blood routine measurement.

### Statistical analysis

SPSS 16.0 was applied to analyze the data. All data were analyzed by the test of normal distribution. For normally distributed data, results were expressed as mean  $\pm$  SD. Meanwhile differences between groups were analyzed by Student *t* test. For nonparametric data, results were expressed as median (range 25 to 75%) values, and Mann–Whitney *U* test was used for comparison between the two groups. Spearman's analysis was used for the correlation analysis. Moreover, multivariable analysis was

then used to compare variables that showed  $p < 0.05$  by single variable. For all statistical analyses, two-tailed  $p$  values less than 0.05 were considered statistically significant.

## Results

### Demographic and clinical features of the pSS patients

All the 46 pSS patients in the study were female (100%), with ages spanning from 29 to 77 years. The disease duration varied from 5 to 13 years (median, 7 years). These patients demonstrated different clinical manifestations, with 43 having dry mouth (93.48%), 40 having dry eyes (86.96%), 15 having salivary gland involvement (32.61%), 14 having arthritis (30.43%), 4 having liver disease (8.7%), and 19 having pulmonary fibrosis disease (41.3%). Different positive rates for ANA, anti-SSA, anti-SSB, and anti- $\alpha$ -fodrin were also shown in these patients. Approximately 36.9% of the patients were taking regular corticosteroids, while some were receiving methotrexate, azathioprine, or mycophenolate (Table 1).

**Table 1** Clinical and demographic features of the 46 pSS patients in the study

| pSS patients ( $n = 46$ )               |            |
|---|------------|
| Ages, median (range), years             | 55 (29-77) |
| Female, n (%)                           | 46 (100)   |
| Disease duration, median (range), years | 7 (5-13)   |
| Dry mouth, n (%)                        | 43 (93.48) |
| Dry eyes, n (%)                         | 40 (86.96) |
| Salivary gland enlargement, n (%)       | 15 (32.61) |
| Arthritis, n (%)                        | 14 (30.43) |
| Liver disease, n (%)                    | 4 (8.70)   |
| Pulmonary fibrosis, n (%)               | 19 (41.30) |
| ANA, n (%)                              | 43 (93.48) |
| anti-SSA, n (%)                         | 36 (78.26) |
| anti-SSB, n (%)                         | 22 (47.83) |
| anti- $\alpha$ -fodrin, n (%)           | 12 (26.09) |
| Glucocorticoid, n (%)                   | 17 (36.96) |
| Cyclophosphamide, n (%)                 | 3 (6.52)   |
| Mycophenolate mofetil, n (%)            | 5 (10.87)  |
| Azathioprine, n (%)                     | 4 (8.70)   |
| Tacrolimus, n (%)                       | 5 (10.87)  |
| Methotrexate, n (%)                     | 0 (0)      |

### Reduced frequency and number of NKT-like cells in pSS patients

The number and frequency of NKT-like cells in pSS patients and healthy individuals were analyzed by detecting a group of triple-positive cells for anti-CD3/CD16/CD56 cells. As shown in Fig. 1, the frequency of NKT-like cells was significantly decreased in pSS patients (pSS vs. HC:  $3.99\% \pm 0.37\%$  vs  $8.41\% \pm 1.10\%$ ,  $P < 0.001$ ). Since the number of lymphocytes in pSS patients was significantly decreased (Supplementary Fig. 1), the number of NKT-like cells was further dampened. The mean number of NKT-like cells in the 46 pSS patients was 51 cells/ $\mu$ L, which was fundamentally lower than that in the 30 healthy controls (196 cells/ $\mu$ L,  $P < 0.001$ ). All these indicate the diminished NKT-like cells in pSS patients.

### Correlation of NKT-like cells with other lymphocytes

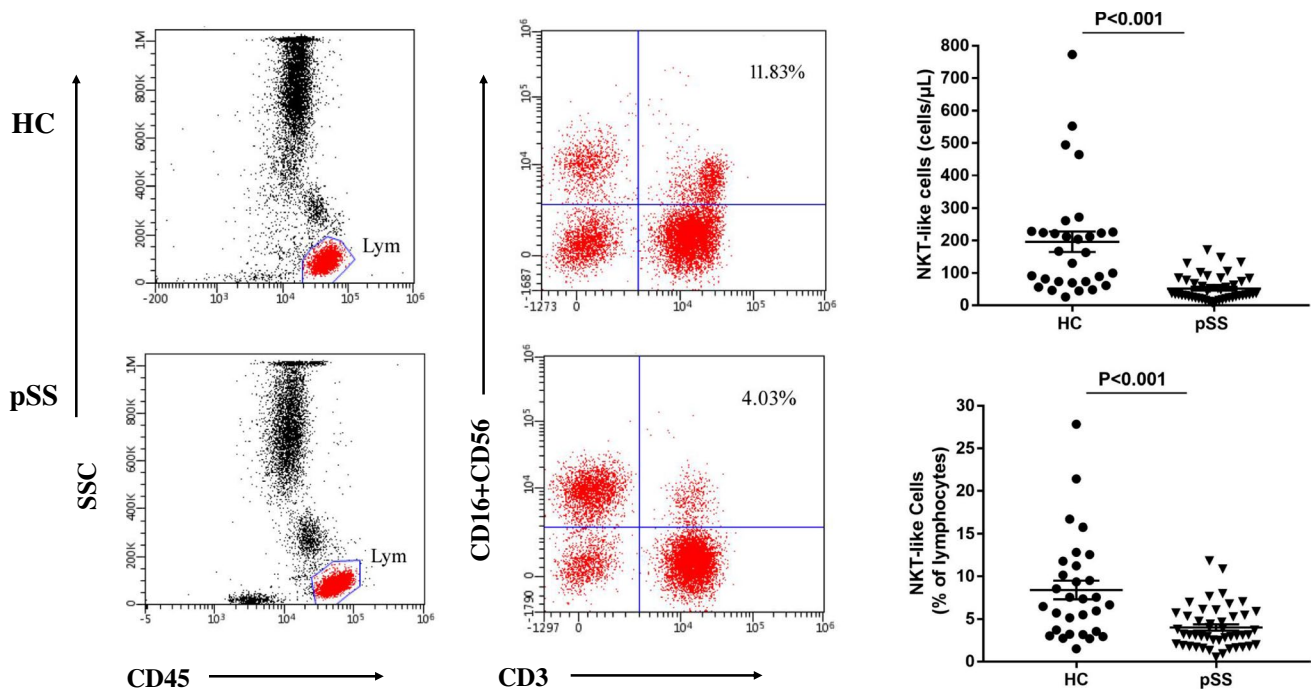
The correlation of NKT-like cells with other lymphocytes was then analyzed. The results showed that the cell count of NKT-like cells was positively correlated with that of CD4<sup>+</sup> T cells ( $r = 0.464$ ,  $P = 0.001$ ), CD8<sup>+</sup> T cells ( $r = 0.363$ ,  $P = 0.013$ ), and NK cells ( $r = 0.488$ ,  $P = 0.001$ ), respectively (Fig. 2). However, the correlation between the cell count of NKT-like cells and B cells was not statistically significant. Moreover, there was no significant correlation between NKT-like cells and other lymphocytes in the cell frequency.

### Correlation of NKT-like cells with laboratory features of pSS patients

The correlation between NKT-like cells and the clinical and laboratory parameters of pSS patients was further analyzed. Detailed results are shown in Table 2. The results showed that the cell count of NKT-like cells was significantly negatively correlated with the disease duration ( $r = -0.330$ ,  $P = 0.027$ ). Moreover, the cell count of NKT-like cells was positively related to IgM levels ( $r = 0.443$ ,  $P = 0.002$ ). In the cell proportion, there was no significant correlation between NKT-like cells and other laboratory features (data not shown).

### Recovery of NKT-like cells in pSS patients after therapy

We next sought to test whether NKT-like cells could be recovered in pSS patients after treatment. Whole blood was collected from 8 patients before and after medication for 3 to 6 months, and the proportion and cell number of NKT-like cells were analyzed. As shown in Fig. 3, in pSS patients,



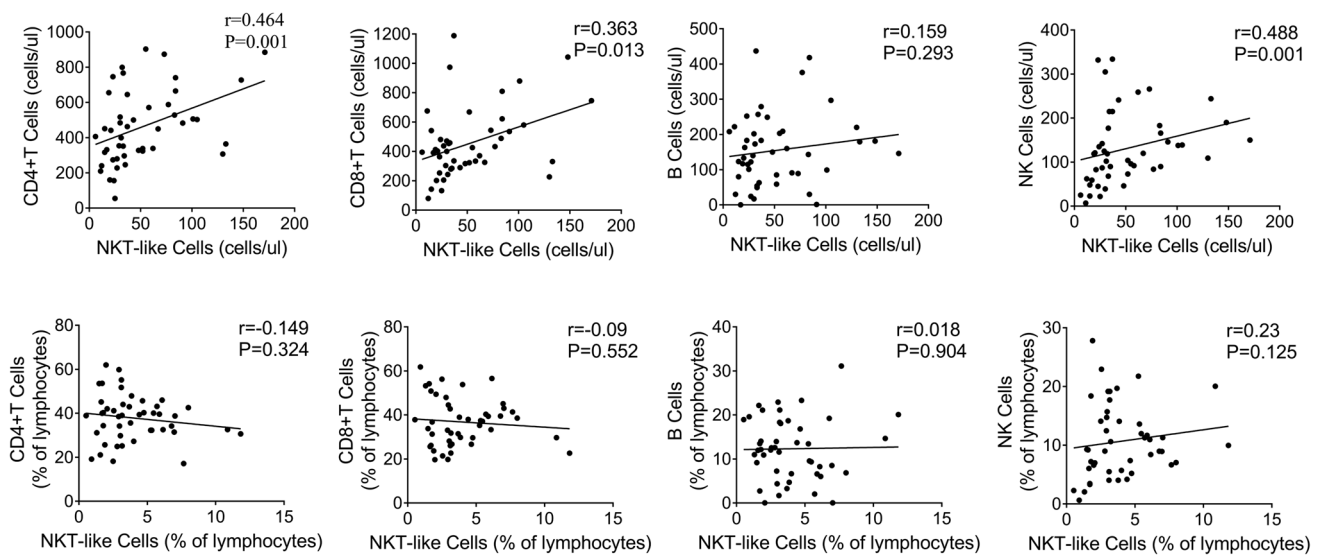
**Fig. 1** NKT-like cells were significantly decreased in pSS patients

NKT-like cells were increased significantly in both the proportion and cell count after therapy, recovering to almost normal levels.

**Discussion**

In this study, we revealed that the frequencies of NKT-like cells were significantly decreased in pSS patients, which was in line with Zhao’s [14] and Sudzius’s studies [15].

Moreover, we also analyzed the cell count of NKT-like cells and achieved even more obvious findings. The cell count of NKT-like cells was significantly lower in pSS patients than that in the HC group, which was in agreement with previous reports [15, 17]. However, some research teams reported that NKT-like cells were increased in pSS patients [12, 13]. The discrepancies among these studies might be potentially due to the patients studied with different disease courses and background therapies. Notably, in this study, we revealed that the cell count of NKT-like cells was significantly



**Fig. 2** The correlation of NKT-like cells with other lymphocytes in pSS patients

**Table 2** Correlation between the count of NKT-like cells and the laboratory parameters in patients with pSS

| Variables              | Spearman’s rank test |         |
|------------------------|----------------------|---------|
|                        | r                    | P value |
| Age                    | -0.056               | 0.712   |
| Disease duration       | -0.330               | 0.027   |
| ESR                    | -0.036               | 0.816   |
| CRP                    | -0.246               | 0.108   |
| IgG                    | 0.066                | 0.661   |
| IgA                    | 0.068                | 0.654   |
| IgM                    | 0.443                | 0.002   |
| C3                     | -0.080               | 0.596   |
| C4                     | -0.071               | 0.639   |
| RF                     | 0.261                | 0.083   |
| WBC                    | 0.083                | 0.582   |
| PLT                    | 0.116                | 0.443   |
| D-dimer                | 0.042                | 0.833   |
| anti- $\alpha$ -fodrin | 0.024                | 0.882   |

negatively related to the disease course. This indicates that the number of NKT-like cells would be further significantly decreased with the progression of pSS.

The correlation of NKT-like cells with other lymphocytes was conducted in this research. The result showed that the cell count of NKT-like cells was positively related to CD4T cells, CD8T cells, and NK cells, respectively. Nevertheless, there was no relationship between the cell count of NKT-like cells and B cells. These results were in line with the previous report [15] and suggested that NKT-like cells may originate from both NK cells and T cells [4, 5]. In the cell proportion, we found no significant correlation between NKT-like cells and any subpopulation of lymphocytes, which was not completely consistent with Sudzius’s [15] study.

We observed that the cell count of NKT-like cells was significantly positively correlated with IgM. As well known, IgM is an indicator of recent infection and it participates in both innate immunity and adaptive immunity [18–20].

Besides the potential function of T cells and NK cells, NKT-like cells may serve as a bridge between the innate and adaptive immunity through IgM, which needs further study.

In addition, NKT-like cells were further studied after therapy in pSS patients. The results showed that NKT-like cells can increase significantly after drug treatment for 3 or 6 months both in the cell number and the cell proportion. This indicates that NKT-like cells may be a potential marker for assessing the status of pSS.

Several limitations may be in this study. The results were from a single-center study with relatively small sample sizes, which may lead to bias. Further large-scale, multi-center studies will be required to confirm the alteration of NKT-like cells in pSS. In addition, the results only focused on the number and frequency of NKT-like cells. In-depth functional analyses of these cells would further reveal their role in the pathogenesis of pSS.

In summary, we demonstrated that the number and frequencies of NKT-like cells were decreased significantly in patients with pSS. These cells were negatively correlated with the disease duration, and could be recovered after treatment in pSS. NKT-like cells might be served as a new marker for assessing the status of pSS.

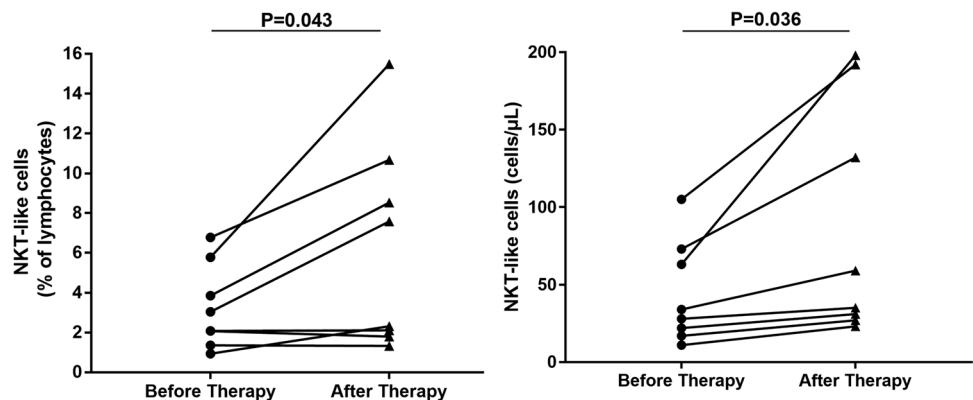
**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10067-021-06011-z>.

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**Declarations**

All procedures performed in studies involving human participants were in accordance with a protocol approved by the ethical committee of Peking University People’s Hospital. All the participants gave written informed consent.

**Fig. 3** NKT-like cells were increased significantly in pSS patients after effective therapy



**Disclosures** None.

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