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ORIGINAL RESEARCH

Immune Profile Differences between IgG4-Related Diseases and Primary Sjögren's Syndrome

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Purpose: Immunoglobulin G4-related disease (IgG4-RD) share clinical features with primary Sjögren's syndrome (pSS). This study aimed to identify altered serological parameters and potential biomarkers of IgG4-RD and pSS.

Methods: Forty IgG4-RD patients, 40 pSS patients, and 40 healthy controls (HC) were enrolled in this study. Routine serological parameters and clinical manifestations were assessed. IgG subclasses (IgGSc) were detected using a Siemens BN P, and lymphocyte subsets were analyzed using flow cytometry. Cytokines assays were performed using cytometric bead array.

Results: Compared to pSS, IgG4-RD patients had higher IgG4 (p <0.001) and lower IgG1 (p =0.014). The natural killer (NK) cells (p = 0.004), CD4+ T cells (p = 0.028), and TBNK cells (p = 0.040) were increased in IgG4-RD compared to pSS. IgG4 used to differentiate IgG4-RD from pSS produced an area under the curve (AUC) of up to 0.952. In addition, we compared serum parameters, immune cells, and cytokines of IgG4-RDwith mouth dryness or eye dryness with those of pSS with the same symptoms, and similar serological changes were observed. IgG4-RD patients with mouth dryness had higher IgG4 (p <0.001) and Th cells (p = 0.016) but lower IgG1 (p = 0.009) compared to pSS with dry mouth. IgG4-RD patients with eye dryness had higher levels of IgG4 (p <0.001), Treg cells (p = 0.037), and NK cells (p = 0.017) than pSS patients with eye dryness. Moreover, IgG4-RD patients with mouth and eye dryness had higher levels of B (p = 0.006), Th (p = 0.026), Th2 (p = 0.007), and Treg cells (p = 0.028) than IgG4-RD patients without mouth and eye dryness.

Conclusion: Immune system disorder is an outstanding feature of IgG4-RD, and its feature differ from pSS. Assessment of immune status is important in the diagnosis and differential diagnosis of IgG4-RD.

Keywords: IgG4-related disease, lymphocyte subsets, IgG subclasses, primary Sjögren's syndrome, differential diagnosis

Introduction

Immunoglobulin G4-related disease (IgG4-RD) was first described as a distinct clinicopathological entity in 2003.[1] It is a chronic, systemic fibroinflammatory disease of unknown etiology, characterized by uniform histopathological findings, mainly manifested as tumefactive lesions, dense lymphoplasmacytic infiltrates rich in IgG4-positive plasma cells, storiform fibrosis, and (usually but not always) elevated serum IgG4 concentrations.[2] Due to its relatively late discovery and generally indolent manifestations, it has not been fully recognized and reported. Therefore, the true prevalence of IgG4-RD remains unclear.[3] However, studies have shown that it is more common in males, and the mean age at diagnosis in large cohorts is between 50 and 60 years.[4] Nevertheless, typical cases have also been observed in pediatric patients.[5] IgG4-RD can affect almost every organ system. An epidemiological cohort study involving 3607 patients showed that IgG4-RD involved the lacrimal glands, salivary glands, pancreas, biliary tract, and retroperitoneal space.[6] Previous studies have reported the role of serum IgG4 levels in establishing the diagnosis, assessing prognosis, and monitoring the treatment response of IgG4-RD.[7,8] Elevated serum IgG4 levels have been demonstrated to correlate with IgG4-RD disease activity.[9,10] However, few studies have focused on other IgGScs, such as IgG1, IgG2, and IgG3.

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IgG4-RD has the same clinical features as Sjögren's syndrome (SS), such as swollen glands, dry mouth, dry eyes, and hyperimmunoglobulinemia.[11,12] Notably, lymphocytic infiltration which leads to the destruction of glandular tissue is also a common feature of IgG4-RD and primary SS (pSS). The similarity of manifestations between IgG4-RD and pSS makes the diagnosis or differential diagnosis with existing imaging or laboratory tests challenging.

The infiltrating CD4+ T helper (Th) cells play an important role in the pathogenesis of pSS.[13] Meanwhile, Th2-type cytokine interleukin (IL)-4 can induce naive B cells to produce antibodies that switch to IgG4, leading to the occurrence of IgG4-RD.[13] Therefore, Th cells are widely considered to be essential for the occurrence and development of IgG4-RD and pSS. However, the pathogenic or protective roles of other immune cells in IgG4-RD and pSS remain elucidate. It is unclear whether these cells can be used to distinguish between IgG and pSS. Whether the changes in immune cells are due to the disease itself or glandular involvement has not yet been investigated. Hence, exploring the changes in the distribution of lymphocyte subsets in IgG4-RD and pSS is a field of investigation to elucidate the pathogenesis of these diseases. Thereafter, we performed a comprehensive analysis of the role of IgGScs and immune function in the etiopathogenesis of IgG4-RD.

In the current study, we attempted to explore the IgGSc and immune profiles of IgG4-RD and pSS and compare whether the distribution of IgGSc and lymphocyte subsets in IgG-RD is different from that in pSS and normal healthy individuals, whether the profile could aid in distinguishing between the two diseases, and whether IgGSc is correlated with lymphocyte subsets by detecting the overall level of serum IgGSc and lymphocyte subsets.

Materials and Methods

Enrolled Individuals

In total, 40 inpatients who were newly diagnosed with IgG4-RD and admitted to the Second Hospital of Shanxi Medical University from 2018 to 2021 were included in this study. Forty age- and sex-matched patients with pSS and 40 healthy controls (HC) were recruited. The patients met the 2019 American College of Rheumatology/European League Against Rheumatism IgG4-RD classification criteria and the 2016 American College of Rheumatology/European League Against Rheumatism pSS classification criteria, respectively.[14,15] Peripheral venous blood samples from the patients were collected immediately after admission and stored at −80°C for IgGSc analysis. All patients had been off medication for more than 3 months at the time of enrollment.

Traditional Indexes

Clinical and laboratory parameters including age, sex, and organ involvement were recorded. Routine laboratory data were recorded, including routine blood counts, biochemical indicators, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Serum levels of immunoglobulins (Ig) G, IgM, and IgA were measured using immunoassays.

IgGSc Assay

The Siemens human IgGSc assay (Siemens Healthcare Diagnostics, Marburg, Germany) was based on an immunoturbidimetric assay and performed using the BN ProSpec system® (Siemens Company, Marburg, Germany).

Flow Cytometry

Venous blood samples were collected using ethylenediaminetetraacetic acid (EDTA) vacutainers. Peripheral lymphocyte subgroups ($CD3^+CD19^-$ T cells, $CD3^-CD19^+$ B cells, $CD3^+CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, $CD3^-$ $CD16/CD56^+$ natural killer (NK) cells) and $CD4^+IFN\text{-}\gamma^+$ T-helper (Th) 1 cells, $CD4^+IL\text{-}4^+$ Th2 cells, $CD4^+IL\text{-}17^+$ Th17 cells, and $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells were acquired using FACS Canto II (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). The detailed method has been described in our previous study.[16]

Cytometric Bead Array

Venous blood samples were collected in test tubes containing a separation gel and centrifuged at 2000 rpm for 20 min. The human Th1/Th2/Th17 cytokine kit (Jiangxi Cellgene Biotechnology Co., Ltd China) was used to measure the serum levels of IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α, and IFN-γ. Sample processing were performed according to the manufacturer's specification and the FCAP ArrayTM v 3.0.1 software returned data as Median Fluorescence Intensity (MFI) and concentration (pg/mL). A standard calibration curve was established for each cytokine.

Statistical Analysis

Statistical comparisons between the IgG4-RD and pSS groups were performed using SPSS 22.0 and GraphPad Prism 8.0.1. Continuous variables were presented as mean ± SD or median (Q25, Q75). Data from the three groups were analyzed using One-way Analysis of Variance (ANOVA) or the Independent-samples Kruskal–Wallis test. Comparison between the two groups was achieved using Mann–Whitney *U*-test. Categorical variables were compared using the Chi-squared test. MedCalc 15.2.0 software was used to draw the receiver operating characteristic (ROC) curves. The area under the curve (AUC) from ROC analysis was used to evaluate the diagnostic performance of indexes in differentiating IgG4-RD from pSS. P-values were calculated to determine the significance of differences ($p < 0.05$ was considered statistically significant).

Results

Higher Levels of Eosinophils and Basophils, and Lower IgA and IgM, in IgG4-RD

Patients with IgG4-RD displayed higher white blood cell (WBC, $p = 0.042$), platelet count (PLT, $p = 0.021$), monocyte (MONO, $p = 0.017$), eosinophil (EO, $p = 0.001$), and basophil (BASO, $p < 0.001$) counts than pSS patients did. The red blood cell (RBC, $p < 0.001$) and hemoglobin (Hb, $p = 0.001$) levels in IgG4-RD patients were lower than those in the HC (Table 1). No significant differences in erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total protein (TP), albumin (ALB), globulin (GLO), or IgG levels were found between the IgG4-RD and pSS groups. IgA ($p < 0.001$) and IgM ($p = 0.023$) of IgG4-RD patients were lower than in those with pSS (Table 2). Table 2 summarizes the clinical characteristics of the patients with IgG4-RD and pSS. IgG4-RD patients exhibited a lower positive rate of dry eye than in those with pSS (42.50% vs 67.50%, $p = 0.042$). However, regarding clinical manifestation of dry mouth, the positive rate of IgG4-RD patients was 67.50%, which did not differ from 82.50% in pSS.

Increased IgG1, IgG3, IgG4, and Altered Lymphocyte Subsets in IgG4-RD

The serum levels of IgG1, IgG2, IgG3, and IgG4 in the IgG4-RD, pSS, and HC groups were measured. IgG1 and IgG3 levels were higher in IgG4-RD ($p < 0.001$ and $p = 0.001$, respectively) and pSS patients ($p < 0.001$ and $p = 0.001$, respectively) than in the HC group. IgG4-RD exhibited higher IgG4 levels than the pSS ($p < 0.001$) and HC groups ($p < 0.001$) (Figure 1).

We found differences in the absolute counts of lymphocyte subsets among the different groups. In the IgG4-RD group, the absolute number of NK cells was higher than that in the pSS ($p = 0.023$) (Figure 2A); that of increased Th1 ($p = 0.009$) and decreased Th2 ($p = 0.016$) cells as compared to HCs (Figure 2B), resulting in an elevated Th1/Th2 ratio ($p < 0.001$) (Figure 2C). The Th1/Treg ratio in IgG4-RD was elevated compared with that in HC ($p < 0.001$) (Figure 2C). Moreover, the levels of IL-4 ($p < 0.001$), IL-6 ($p = 0.021$), IL-10 ($p < 0.001$), IL-17 ($p < 0.001$), IFN-γ ($p < 0.001$), and TNF-α ($p < 0.001$) in patients with IgG4-RD were significantly higher than those in the HC (Figure 2D). A similar trend was observed in the patients with pSS.

Increased Th, Th2, and Treg Cells in IgG4-RD Patients with Dry Mouth-Eyes

We further divided the IgG4-RD patients into two subgroups: those with and without dry mouth-eye. In IgG4-RD patients with dry mouth-eye, the number of B cells ($p = 0.006$) (Figure 3A), Th cells ($p = 0.026$), Th2 cells ($p = 0.007$), and Treg cells ($p = 0.028$) was higher than in IgG4-RD without dry mouth-eyes (Figure 3B). The cytokines and IgGSc levels did not differ between the two subgroups (Figure 3C and D).

Table 1 Detailed Laboratory Data of All Individuals

	IgG4-RD (n = 40)	pSS (n = 40)	HC (n = 40)	*p* value	*p* value		
					IgG4-RD vs pSS	IgG4-RD vs HC	pSS vs HC
Age, years	59.18 ± 9.30	57.83 ± 10.18	57.00 ± 7.37	0.555	–	–	–
Female/male	23/17	29/11	24/16	0.329	–	–	–
WBC (*10^9/L)	6.10 (4.72, 7.65)	4.67 (3.46, 6.81)	5.26 (4.40, 6.06)	**0.041**	**0.042**	0.259	1
RBC (*10^{12}/L)	4.15 (3.89, 4.54)	4.24 (3.82, 4.46)	4.66 (4.46, 4.99)	**<0.001**	1	**<0.001**	**<0.001**
Hb (g/L)	131.50 (119.00, 141.75)	129.00 (118.50, 136.00)	141.50 (138.00, 150.75)	**<0.001**	0.870	**0.001**	**<0.001**
PLT (*10^9/L)	231.50 (169.50, 287.75)	188.00 (125.50, 238.00)	228.00 (189.50, 259.25)	**0.007**	**0.021**	1	**0.017**
(*10^9/L)	1.67 (1.22, 2.07)	1.35 (1.02, 1.97)	1.69 (1.43, 1.96)	**0.035**	0.226	1	**0.034**
MONO (*10^9/L)	0.45 (0.38, 0.62)	0.35 (0.26, 0.47)	0.36 (0.27, 0.43)	**0.001**	**0.017**	**0.003**	1
NEUT (*10^9/L)	3.52 (2.33, 4.31)	2.93 (1.80, 3.75)	2.93 (2.60, 3.65)	0.449	–	–	–
EO (*10^9/L)	0.22 (0.07, 0.28)	0.06 (0.04, 0.12)	0.09 (0.06, 0.15)	**0.001**	**0.001**	0.064	0.434
BASO (*10^9/L)	0.03 (0.03, 0.06)	0.02 (0.01, 0.04)	0.03 (0.02, 0.04)	**0.001**	**<0.001**	0.212	0.100
LYMPH (%)	28.35 (23.65, 33.80)	30.4 (24.50, 35.85)	33.85 (27.63, 37.93)	**0.050**	0.933	**0.044**	0.502
MONO (%)	7.65 (6.70, 10.05)	8.20 (6.20, 10.75)	6.65 (5.68, 7.75)	**0.008**	1	**0.018**	**0.033**
NEUT (%)	57.50 (46.15, 65.50)	58.40 (51.95, 64.15)	57.30 (53.20, 62.18)	0.856	–	–	–
EO (%)	2.80 (1.65, 5.28)	1.30 (0.80, 2.60)	1.65 (1.10, 2.63)	**0.006**	**0.008**	0.054	1
BASO (%)	0.65 (0.40, 0.98)	0.50 (0.30, 0.60)	0.55 (0.40, 0.80)	**0.043**	**0.037**	1	0.319
BUN (mmol/L)	4.95 (3.92, 6.33)	5.30 (4.03, 6.15)	5.25 (4.45, 6.15)	0.764	–	–	–
Cr (umol/L)	58.00 (50.25, 80.94)	55.00 (49.25, 63.25)	67.00 (59.50, 77.00)	**<0.001**	0.165	0.064	**<0.001**
ALT (U/L)	21.15 (12.08, 31.50)	17.50 (13.48, 30.58)	17.55 (13.8022.05)	0.286	–	–	–
AST (U/L)	19.45 (16.13,28.68)	21.90 (18.73, 29.20)	21.25 (18.33, 25.38)	0.573	–	–	–
AST/ALT	1.16 (0.81, 1.34)	1.20 (0.92, 1.53)	1.21 (0.99, 1.61)	0.188	–	–	–

Notes: All data are expressed as median (interquartile range), except age and sex, which were compared using the Kruskal–Wallis one-way ANOVA test. Differences in sex were compared using the chi-square test. An independent samples *T* test was used to compare age differences. Bold indicates P value <0.05, which is statistically significant.
Abbreviations: WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelet count; LYMPH, lymphocyte; MONO, monocyte; NEUT, neutrophile; EO, eosinophil; BASO, basophil; BUN, blood urea nitrogen; Cr, creatinine; ALT, alanine aminotransferase; AST, aspartate transaminase.

Distinct Serum IgGSc and Immune Cell Profiling Between IgG4-RD and pSS

All parameters in IgG4-RD were compared with those in the pSS group, and the results are shown in the volcano map (Figure 4A). To further reveal the role of multiple differential indicators in identifying IgG4-RD, ROC curves were constructed (Figure 4B–D). The AUC of IgG4, IgA, BASO, B/Treg, and EO were 0.952 [95% confidence interval (CI) 0.879–0.987], 0.766 (95% CI 0.648–0.860), 0.741 (95% CI 0.622–0.839), 0.732 (95% CI 0.613–0.831), and 0.724 (95% CI 0.603–0.825), respectively. The sensitivity and specificity of IgG4 were 87.50% and 95.00%, respectively. In addition, NK cell, MONO, PLT, IgM, IgG1, Th2/Treg, Th, WBC, and TBNK cell were also valuable in differentiating IgG4-RD from pSS, with an AUC greater than 0.6 (all $p < 0.05$).

Potential Biomarkers Distinguish Between IgG4-RD with Dry Mouth/Eyes and pSS with Dry Mouth/Eyes

Considering that IgG4-RD and pSS patients have the same symptoms of dry mouth and dry eyes, we compared each shared symptom between the two groups of patients. Those with IgG4-RD with dry mouth evidenced increased IgG4 ($p < 0.001$), BASO ($p < 0.001$), EO ($p < 0.001$), MONO ($p = 0.029$), and Th cells ($p = 0.016$) but decreased IgA ($p = 0.001$), IgG1 ($p = 0.009$), and B/Treg ($p = 0.007$) than those with pSS with dry mouth (Figure 5A). Regarding dry eyes, in addition to the increase in IgG4 ($p < 0.001$), EO ($p = 0.009$), BASO ($p = 0.002$), and MONO ($p = 0.045$) levels, the levels of PLT ($p = 0.020$), NK cells ($p = 0.017$), and Treg cells ($p = 0.037$) were also higher than pSS with the dry eye group, and IgA ($p = 0.003$) and B/Treg ($p = 0.040$) levels were still decreased (Figure 5B). The corresponding AUCs of each difference index are shown in Figure 5C and D.

Table 2 The Data of Serological and Involved Organ from Patients with IgG4-RD and pSS

	IgG4-RD (n = 40)	pSS (n = 40)	*p* value
Serology (median, IQR)			
ESR (mm/h)	27.00 (13.00, 46.00)	23.00 (13.25, 51.50)	0.928
CRP (mg/L)	3.14 (3.13, 6.20)	3.14 (3.13, 13.65)	0.433
TP (g/L)	69.70 (63.40, 74.80)	70.30 (65.60, 75.30)	0.551
ALB (g/L)	37.70 (34.20, 41.10)	38.20 (33.23, 41.20)	0.941
GLO (g/L)	32.40 (26.90, 35.50)	31.70 (28.10, 38.20)	0.448
IgG (g/L)	15.60 (12.25, 19.00)	14.00 (11.65, 18.88)	0.377
IgA (g/L)	1.90 (1.30, 2.44)	2.91 (2.05, 4.14)	**<0.001**
IgM (g/L)	0.92 (0.55, 1.23)	1.05 (0.73, 1.62)	**0.023**
Organ involvements (n, %)			
Dry mouth	27 (67.50)	33 (82.50)	0.196
Dry eye	17 (42.50)	27 (67.50)	**0.042**
Salivary glands	4 (10.00)	8 (20.00)	0.348
Pancreas	6 (15.00)	0	–
Bile duct	2 (5.00)	0	–
Lung	11 (27.50)	5 (12.50)	0.099
Kidney	6 (15.00)	0	–
Retroperitoneum	4 (10.00)	0	–
Paranasal sinus	4 (10.00)	0	–
Lymph node	10 (25.00)	1 (2.50)	**0.007**
Thyroid	6 (15.00)	4 (10.00)	0.348

Notes: Data are reported as median (IQR) or number. Data between the groups were analyzed using the Mann–Whitney *U*-test. Differences in categorical variables were compared using the Chi-square test. Bold indicates P value <0.05, which is statistically significant.

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; TP, total protein; ALB, albumin; GLO, globulin; IgG, immunoglobulin G.

Correlations Between IgGSc and Immune Profiling

A correlation analysis was conducted to assess the relationship between IgGSc and the immune profile (Figure 6). Significant positive correlations between IgG4 and CD4+Th cells, Th17 cells, and the Th1/Th2 ratio and IgG2 also had a positive correlation with Th2 cells (p <0.05); however, negative correlations were observed between IgG1 and lymphocyte subsets, such as TBNK, NK, CD3+T, CD4+Th, Th2, and Treg cells (p <0.05). Meanwhile, there was a positive correlation between IgG1, IgG3, and IgG4 and cytokines, such as IL-4, IL-10, IL-17, and IFN-γ (p <0.001).

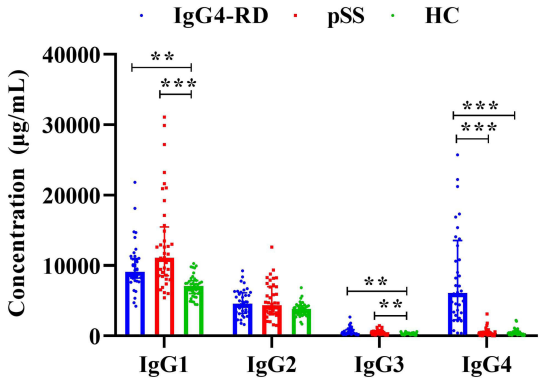

Figure 1 Serum levels of IgG subclasses among IgG4-RD, pSS, and healthy controls. ** p < 0.01, *** p < 0.001.

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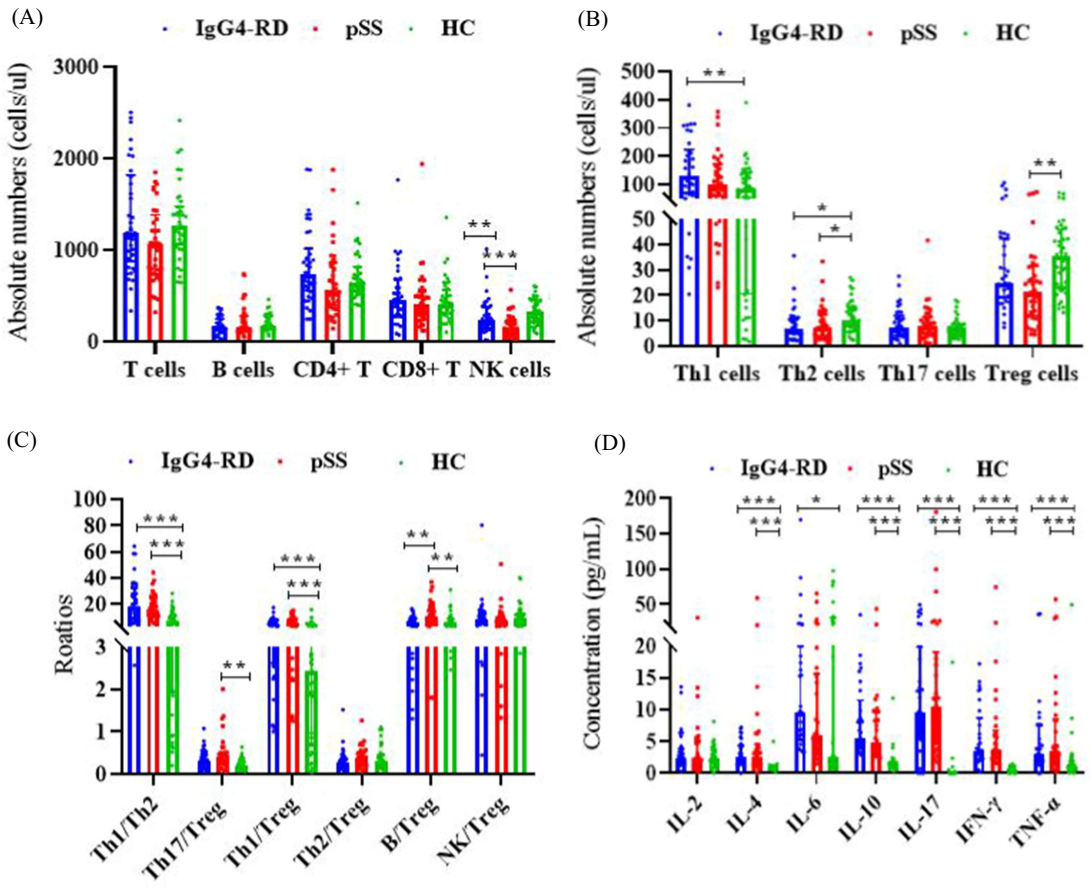

Figure 2 The altered lymphocyte profiling among IgG4-RD, pSS, and healthy controls. The levels of lymphocyte subsets (**A**), Th cell subsets (**B**), immune cell ratios (**C**), and cytokines (**D**) among IgG4-RD, pSS, and healthy controls. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

Serum IgG4 levels are elevated in multiple non-IgG4-RD inflammatory and malignant conditions. In a prospective UK cohort, Culver et al explore the role of serum IgG4 levels in diagnosis, treatment response, organ involvement, and recurrence of IgG4-related diseases in 2067 samples from 1510 patients.[17] Their study demonstrated that less than one-quarter of those with an elevated IgG4 meeting IgG4-RD diagnostic criteria. The similarity in clinical manifestations between IgG4-RD and pSS makes the differential diagnosis of the two disorders challenging. Given this diagnostic conundrum, our study focused on a more in-depth exploration of the immunological aspects. A comprehensive spectrum of T cells, B cells, NK cells, Th cell subsets, cytokines, and IgGSc in IgG4-RD patients and compared them with pSS were explored. We found that serological parameters such as IgGSc and immune spectrum in IgG4-RD differ from those in pSS. This finding provides more comprehensive information on how immune profiles account for the disease heterogeneity of IgG4-RD and pSS.

Basic laboratory indices of IgG4-RD differ from those of pSS. In this study, IgG4-RD patients exhibited higher EO and BASO than pSS patients, which is similar to previous studies.[18,19] And study has shown that approximately 20% to 40% of IgG4-RD patients presented with peripheral eosinophilia.[14] Also, it has been manifested that activation of TLRs in basophils can lead to an increase in IgG4 via B cell activating factors.[20] PLT was also elevated in IgG4-RD patients in this study, and a previous study found that PLT, as a mediator of immunity and inflammation, can regulate innate and adaptive immunity.[21] In addition, IgA and IgM were reduced in IgG4-RD, and a previous study also found low serum IgA levels in IgG4-RD.[19] Establish a comprehensive condition monitoring system that includes multiple indicators and regularly conduct tests on patients with IgG4-RD so as to better serve clinical practice.

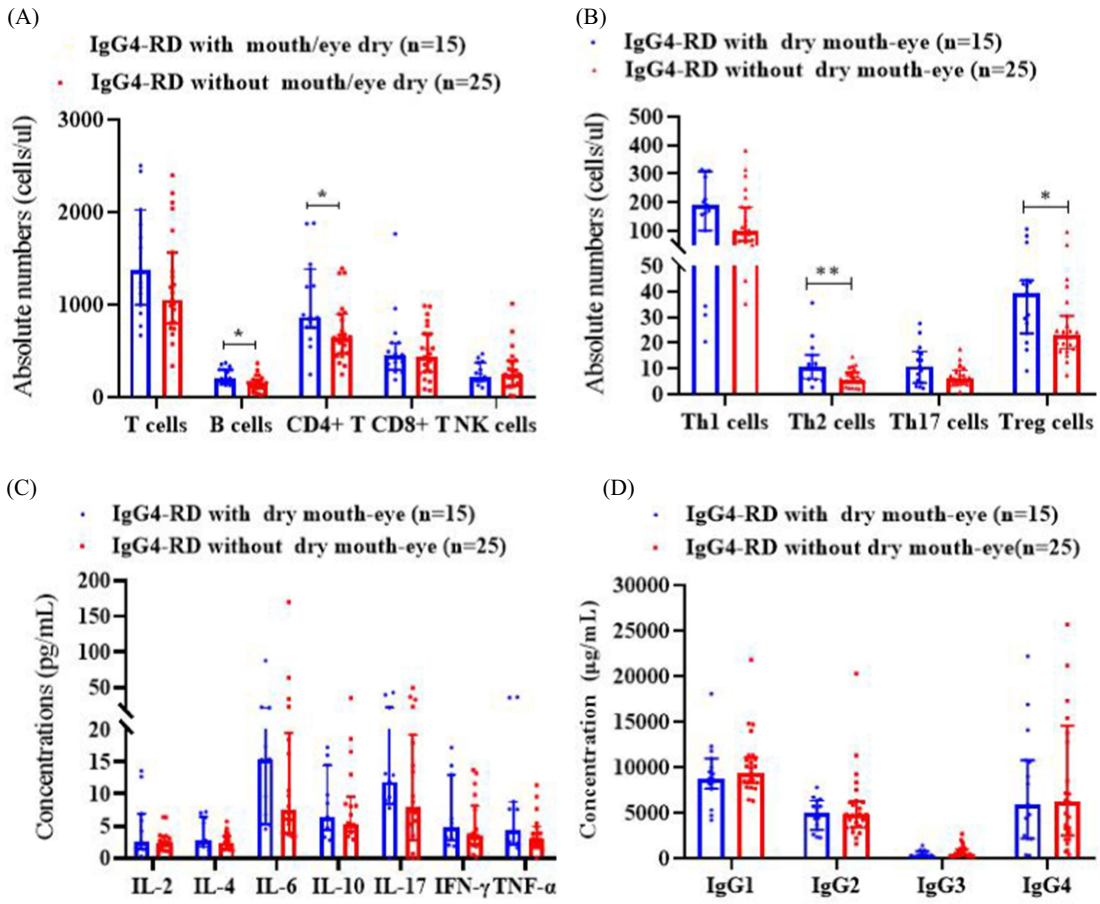

Figure 3 The altered immune profiling between IgG4-RD with dry mouth-eyes and IgG4-RD without dry mouth-eyes. The levels of lymphocyte subsets (**A**), Th cell subsets (**B**), cytokines (**C**), and IgGSc (**D**) between IgG4-RD with dry mouth-eyes and IgG4-RD without dry mouth-eyes. * $p < 0.05$, ** $p < 0.01$.

Human IgG can be divided into four subclasses based on textural and biological properties: IgG1, IgG2, IgG3, and IgG4. In our study, IgG4-RD patients exhibited higher IgG1, IgG3, and IgG4 levels than the HC group but did not have higher serum IgG2 levels. Meanwhile, owing to the different biological characteristics of IgGSc, its distribution and levels of IgGSc in various autoimmune diseases is also different. We further found that IgG1 was lower in the IgG4-RD group and could be used to distinguish between IgG4-RD and pSS. However, the reason for the elevated levels of IgG1 in pSS is unclear, there are still many issues regarding the mechanisms behind the changes of IgGSc to be explored in the field of autoimmune diseases. In previous studies, only IgG4 has been shown to have diagnostic value. To the best of our knowledge, the diagnostic value of other IgGSc has not been reported.[22] Multiple IgGSc may have potential roles in the diagnosis of different autoimmune diseases, broadening our understanding of the range of relevant biomarkers available for diagnosis. Therefore, the value of IgGSc in the diagnosis and prognosis of autoimmune diseases warrants further investigation.

Although the pathophysiological mechanism of IgG4-RD is indistinct, an immune response seems to be involved.[23,24] The importance of immunophenotyping in IgG4-RD has been reported.[25] However, the majority of previous studies focused on a single or a few cells, the overall state of the immune system in IgG4-RD patients is unknown. Hence, further studies on the identification of the immunophenotype contribute to a deeper comprehension of the pathogenesis of IgG4-RD. In our study, immune disorders have clearly occurred in IgG4-RD and pSS patients, but the immune status of both was diverse. Compared to pSS, NK cells were more abundant in the IgG4-RD group, which may explain the different pathological processes of the occurrence and development of various autoimmune diseases. IgG4-RD may have its specific abnormal patterns in the immune response, resulting in different tissue damage and disease progression patterns compared with pSS. This provides a theoretical basis for subsequent research on targeted therapies targeting the

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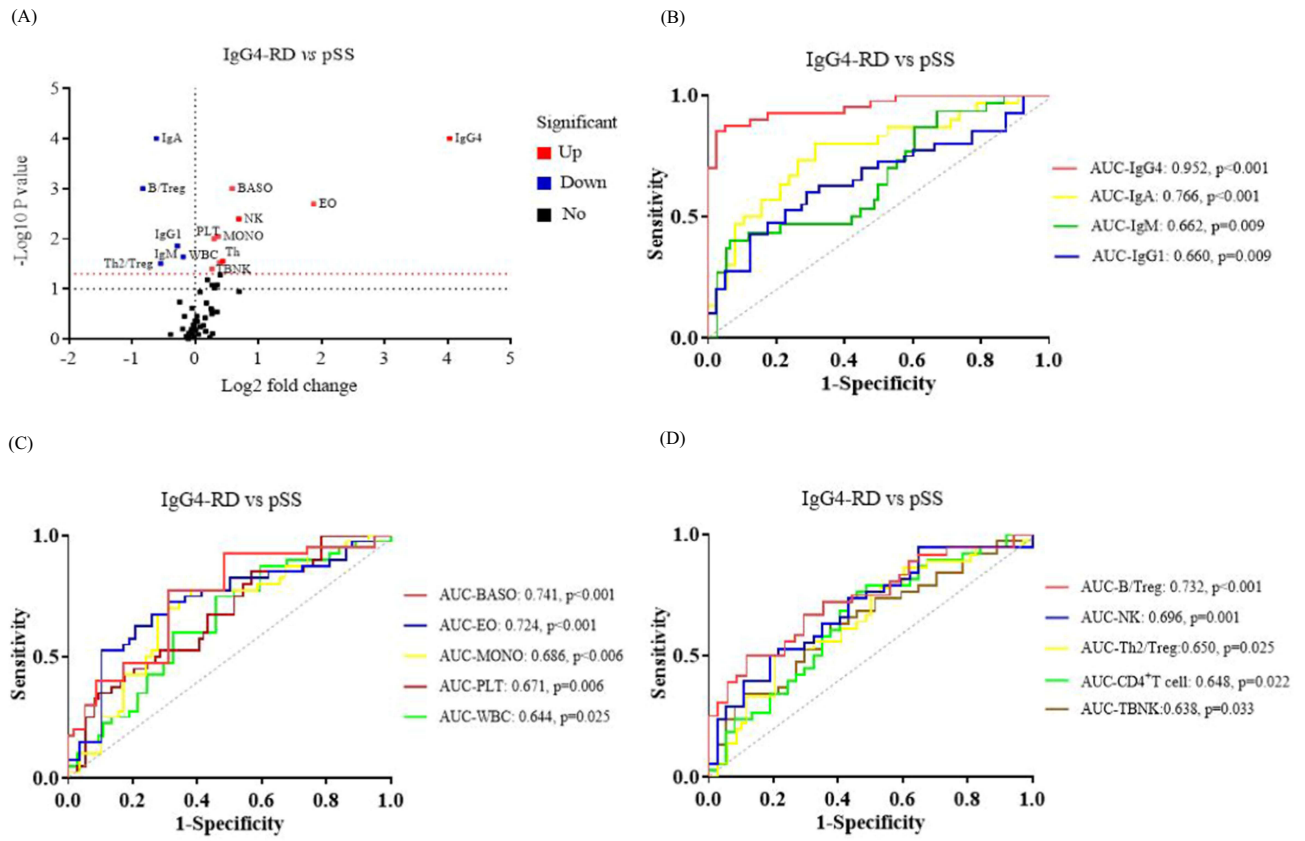

Figure 4 Identification of patients with IgG4-RD and pSS using volcano plots and ROC curves. (**A**) Volcano plots demonstrating the different indicates between IgG4-RD patients and pSS. $p < 0.05$ (horizontal red line), $p < 0.10$ (horizontal black line). A negative fold change indicates that the levels of indicates for IgG4-RD patients was lower than pSS, and vice versa. (**B**–**D**) The receiver operating characteristic (ROC) curves and area under the curve (AUC) of different indicators in distinguishing IgG4-RD and pSS.

key immune aspects of each disease. Meanwhile, a recent study on the immunophenotype of IgG4-RD showed a higher proportion of NK cells.[26] The function and mechanism of NK cells in IgG4-RD need to be further studied.

The Th cell subsets in IgG4-RD were also somewhat different from those in pSS. Overall, the absolute number of Th cells had increased. Subsequently, increased numbers of Th1 cells and elevated Th1/Th2 ratios were observed in patients with IgG4-RD. Ohta et al also reported elevated Th1 cells, but not Th2 cells.[27] IgG4-RD patients with dry mouth evidenced increased Th cells than those in pSS patients with dry mouth. The differences in the levels of Th cells between IgG4-RD patients and those with primary Sjögren's syndrome (pSS) provide a potential immunological marker basis for clinically differentiating these two autoimmune diseases with similar dry mouth symptoms. For some patients with atypical clinical manifestations, where dry mouth is the prominent symptom and it is difficult to make a definite diagnosis solely based on traditional diagnostic criteria (such as the dryness-related symptoms and autoantibody tests for pSS, the serum IgG4 level and characteristics of the affected organs for IgG4-RD), Th cell detection may serve as an important supplementary means of differentiation.

In addition, T follicular helper (Tfh) and peripheral T helper (Tph) cells are crucial subsets as key pathogenic players in IgG-RD and SS immunopathology. Tfh cells are a specialized CD4+ T cell subset that mainly reside in the germinal center (GC) and initiate and promote humoral immunity. Tfh cells provide critical "helper" functions in the processes of inducing activation and differentiation of B cells and in promoting B cell activation, clonal expansion, Ig heavy chain isotype switching, and somatic hypermutation.[28,29] Tph cells can migrate to the B cell follicular area or inflammatory sites in peripheral lymphoid organs, make direct contact with B cells and provide helper signals, and may regulate other B cell functions, such as cytokine production.[30,31] In Sjögren's syndrome, numerous studies have shown that B cells attack exocrine glands by producing autoantibodies, and Tfh cells play an important role in this process.[32] They promote

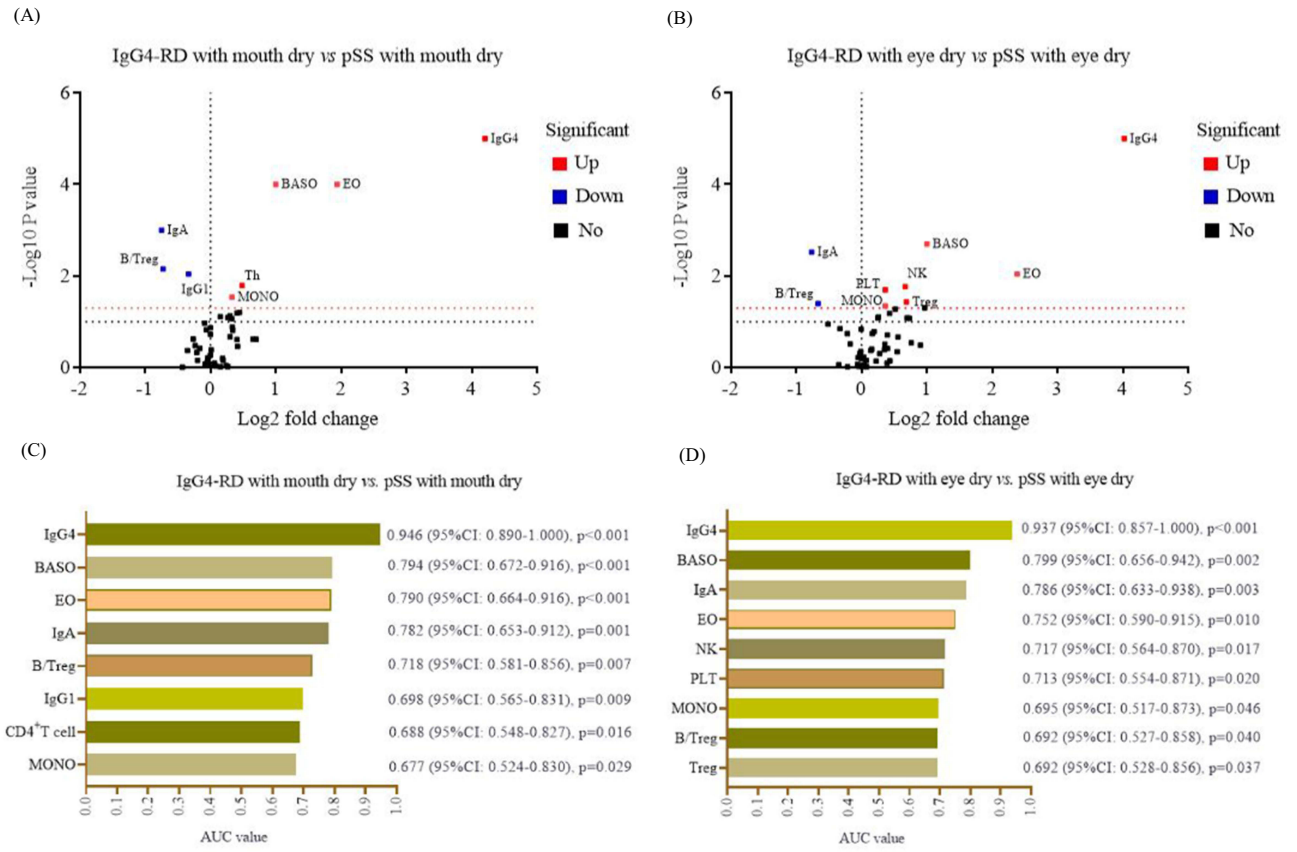

Figure 5 Volcano plots and ROC curves were used to identify IgG4-RD patients with different glandular involvement and pSS patients with corresponding glandular involvement. (**A** and **B**) Volcano plots demonstrating the different indicators between IgG4-RD patients with dry mouth vs pSS with dry mouth and IgG4-RD patients with dry eyes vs pSS with dry eyes. (**C** and **D**) The AUCs of different indicators in distinguishing IgG4-RD patients with dry mouth from pSS with dry mouth and IgG4-RD patients with dry eyes from pSS with dry eyes.

the survival, proliferation, and antibody class switching of B cells and induce the formation of germinal centers, thereby exacerbating the inflammatory response and functional damage of exocrine glands. For IgG4-RD, the production of a large amount of IgG4 antibodies by B cells is one of its important pathological features. Tfh cells play a crucial regulatory role in the process of IgG4 antibody class switching and production.[33] In the affected tissues of patients with IgG4-RD, the quantity and functional status of Tfh cells are closely related to the severity and progression of the disease, and their distribution in tissues has a certain correlation with fibrotic areas. CD4+CD8+Tfh cells were abundantly present in the fibroinflammatory lesions of patients with IgG4-RD.[34] Meanwhile, the importance of Tph cells has become increasingly prominent. Tph cells can secrete multiple cytokines such as IL-21, which cooperate with Tfh cells to promote the proliferation, differentiation of B cells and the production of IgG antibodies.[35,36]

IgG4-RD is a heterogeneous disease, and we further explored the immune status of patients with organ involvement.[37] The higher numbers of B cells, Th cells, and Th2 cells were found in IgG4-RD patients with dry mouth-eyes than in IgG4-RD patients without dry mouth-eyes. CD4+ Th cells are found in large numbers in the affected organs and are considered to trigger the amplification of B cells and tissue fibrosis. Understanding this mechanism could help develop therapeutic pathways that target the regulation of CD4+Th cells to prevent tissue damage and fibrosis. A previous study indicated that Th1 and Th2 activation contributed to IgG4-RD.[38,39] This may mean that skewing of the Th1/Th2 balance is associated with organs affected of IgG4-RD. In addition, our findings are consistent with studies showing that Treg cells are increased in IgG4-RD, and increased Treg cells were found in IgG4-RD patients with dry mouth-eyes, suggesting that Tregs can infiltrate and function in target organs.[39,40] It is possible that they may either have a protective role by suppressing excessive immune responses or, in some cases, may contribute to the disease progression if the function is dysregulated. For dry eyes, in addition to the increase in NK, Treg cells were also higher than those in

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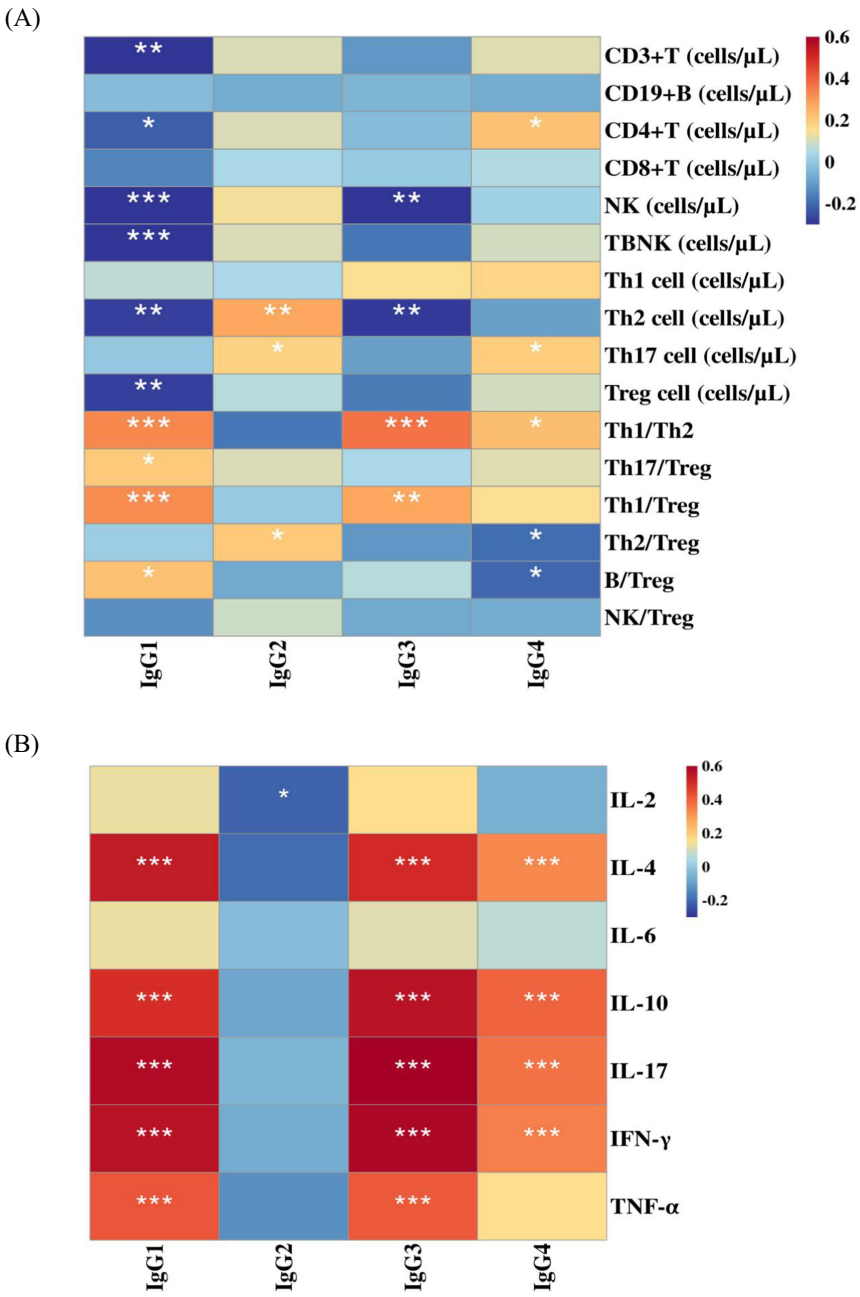

Figure 6 Spearman's rank correlation analysis of serum IgG subclass level with lymphocyte subsets/cytokines. (**A**) The correlation between IgGSc and lymphocyte subsets. (**B**) The correlation between IgGSc and cytokines. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the pSS with dry eyes group. The difference in immune cell profiles can be used as an additional diagnostic criterion to distinguish between these two diseases. These results suggest that immune cells are not only related to disease heterogeneity but also have an effect on gland involvement during the progressive stage of the disease.

Our study has several limitations. First, IgG4-RD can involve multiple organs and has various manifestations; however, because of the low incidence and small number of samples involving specific organs, such as cholangitis and retroperitoneal fibrosis, we could not further explore the immune profile of each subgroup. Thus, further exploration of the immune status in a large cohort may reveal the pathophysiological mechanisms of IgG-RD. Second, this study was limited to one center or area with similar genetic or ethnic backgrounds. The outcomes of this research need to be followed up in other projects, or similar study. Third, controls with dry mouth and dry eyes were not included in the study

to determine the diagnostic value of IgG4. It is crucial to include controls with symptoms of dry mouth and dry eyes to ensure a more accurate diagnostic assessment in future studies. Last but not least, the cost factors of serological testing may affect the accessibility of testing and the feasibility of large-scale screening.

Conclusion

In conclusion, the immune system disorder is apparent in patients with IgG4-RD, which differs from that in pSS. This study explored novel biomarkers based on the immune profile and found that IgG4 is undoubtedly an excellent biomarker for the differential diagnosis between IgG4-RD and pSS. Meanwhile, some immune characteristics, such as $CD4^+$ T cell and NK cell, exhibit a certain discriminatory ability and can serve as potential biomarkers for IgG4-RD, providing new ideas for the differential diagnosis of IgG4-RD. Detecting peripheral lymphocyte subsets, serum cytokines, and combining with serum IgG4 levels may contribute to the diagnosis of pSS and IgG4-RD. In addition, as our understanding of the pathogenesis of pSS and IgG4-RD increases, many candidate biomarkers may be identified in the future. The discovery of these biomarkers will be beneficial to clinical practice and further deepen our understanding of the immunopathogenesis of pSS and IgG4-RD.

Data Sharing Statement

The original data can be obtained by contacting the corresponding author.

Ethics Approval and Consent to Participate

This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Second Hospital of Shanxi Medical University (2016KY007). Written informed consent was obtained from all the participants.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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