

Immune Status and Chemokine C Receptor 7 Expression in Primary in Patients with Immune Thrombocytopenia

Primer İmmün Trombositopeni Hastalarında İmmün Durum ve Kemokin C Reseptör Ekspresyonu

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Abstract

Objective: The present study investigated immune disorders and chemokine C receptor 7 (CCR7) expression in primary immune thrombocytopenia (ITP) patients and analyzed their changes and clinical significance before and after treatments.

Materials and Methods: Flow cytometry was used to detect the proportion of different immune cell subsets in the peripheral blood of 42 patients with ITP and 20 healthy controls at different time points. Treatments included first-line drugs, such as glucocorticoids and intravenous immunoglobulin, and second-line therapy, such as interleukin-11 and thrombopoietin receptor agonists.

Results: An elevated CD4/CD8 ratio and decreased natural killer (NK) cells and CD4⁺CD25⁺CD127^{low} regulatory T-cells (Tregs) were found in pretreatment ITP patients compared to healthy controls. The newly diagnosed group had a higher CD4/CD8 ratio and more NK cells than the relapsed group. Treg levels of the remission group were higher than those of the recurrence group. The CD4⁺CCR7⁺, CD8⁺CCR7⁺, and CCR7⁺ subsets of B cells and NK cells showed higher increases in the newly diagnosed and relapsed group compared to controls and the remission group. The values for the CD4⁺CCR7⁺ and CD8⁺CCR7⁺ subsets in the relapsed group were slightly higher than those in the newly diagnosed group. The CCR7⁺ subsets of CD4⁺ T-cells, CD8⁺ T-cells, NK cells, and B cells had lower values in the remission group compared to the relapsed group. Higher levels of the CD8⁺CCR7⁺ subset and lower levels of NK cells were found in the remission group compared to the controls. The ratio between the CD4⁺CCR7⁺ subset and CD8⁺CCR7⁺ subset was lower in ITP patients than in healthy controls. There was a negative correlation between the CD8⁺CCR7⁺ subset and platelet count in the ITP patients.

Conclusion: ITP patients with CCR7 had immune disorders and high heterogeneity, and CCR7 was found to be involved in the pathogenesis of ITP. Further studies are needed to investigate effective treatments for ITP by targeted regulation of CCR7.

Keywords: Primary immune thrombocytopenia, Immune state, Chemokine C receptor 7, T-cells

Öz

Amaç: Bu çalışmada, primer immün trombositopeni (ITP) hastalarında immün bozuklukları ve kemokin C reseptörü 7 (CCR7) ekspresyonu araştırıldı ve tedavilerden önce ve sonra değişiklikleri ve klinik önemi analiz edildi.

Gereç ve Yöntemler: Akım sitometri kullanılarak 42 ITP hastasının ve 20 sağlıklı kontrolün farklı zamanlarda periferik kanındaki farklı immün hücre alt gruplarının oranı saptandı. Tedaviler, glukokortikoidler ve intravenöz immüno globulin gibi birinci basamak ve interlökin-11 ve trombopoietin reseptör agonistleri gibi ikinci basamak ilaçları içeriyordu.

Bulgular: Sağlıklı kontrollerle karşılaştırıldığında tedavi öncesi ITP hastalarında CD4/CD8 oranı yüksek, ve doğal öldürücü (NK) hücreler ile CD4⁺CD25⁺CD127^{düşük} düzenleyici T hücreleri (Treg) düşük olarak bulundu. Yeni tanı grubunda, nüks eden gruba göre CD4/CD8 oranı daha yüksek ve NK hücresi ise daha fazlaydı. Remisyon grubunun Treg seviyeleri, nüks grubundan daha yüksekti. B hücrelerinin ve NK hücrelerinin CD4⁺CCR7⁺, CD8⁺CCR7⁺ ve CCR7⁺ alt kümeleri, yeni tanı ve nüks eden grupta kontrollere ve remisyon grubuna kıyasla daha fazla oranda artış gösterdi. Nüks eden gruptaki CD4⁺CCR7⁺ ve CD8⁺CCR7⁺ alt grupları için değerler, yeni tanı konan gruptan biraz daha yüksekti. CD4⁺ T hücrelerinin, CD8⁺ T hücrelerinin, NK hücrelerinin ve B hücrelerinin CCR7⁺ alt kümeleri, relaps grubuna kıyasla remisyon grubunda olanlarda daha düşüktü. Kontrollere kıyasla remisyon grubunda CD8⁺CCR7⁺ alt grubunun değerleri daha yüksek ve NK hücrelerinin ise daha düşük bulundu. CD4⁺CCR7⁺ hücrelerin CD8⁺CCR7⁺ hücrelere oranı, ITP hastalarında sağlıklı kontrollere göre daha düşüktü. ITP hastalarında CD8⁺CCR7⁺ alt grubu ile trombosit sayısı arasında negatif korelasyon vardı.

Sonuç: CCR7 ekspresyonu olan ITP hastalarında immün bozukluklar ve yüksek heterojenite vardı ve CCR7'nin ITP'nin patogenezinde rol oynadığı bulundu. CCR7'nin hedeflenen regülasyonu ile ITP'nin etkin tedavisini araştırarak daha fazla çalışmaya ihtiyaç vardır.

Anahtar Sözcükler: Birincil immün trombositopeni, İmmün durumu, Kemokin C reseptörü 7, T hücreler



Introduction

Primary immune thrombocytopenia (ITP) is an autoimmune disease characterized by increased platelet destruction and reduced platelet production. ITP has an increased bleeding risk depending on disease severity [1]. The complex pathogenesis of ITP has not yet been fully elucidated. The presence of autoreactive antibodies against platelet glycoproteins, such as GP IIb/IIIa and GP Ib/IX, is considered to play a central role in the cause of ITP. However, dysfunctional cellular immunity has also recently been found to be important in the pathogenesis of ITP [2]. Treatments for ITP are difficult due to the diversity seen in the pathogenesis of ITP. Therefore, it is of vital importance to identify commonalities in the diversity of the pathogenesis of ITP for more precise treatment in the future.

It is well known that a variety of immune cells are involved in the destruction and generation of platelets in ITP. The immune system is involved in ITP pathogenesis together with the production of cytokine mediators and certain cellular subpopulations participate in many different immune phenomena [3]. Due to the heterogeneity of ITP, the results of various studies addressing these topics are contradictory.

Chemokines are a group of small (8-14 kDa) structurally related molecules that regulate the trafficking of various cells by binding to chemokine receptors, which are also structurally related and contain seven transmembrane domains coupled to a G protein [4]. Chemokine C receptor 7 (CCR7 or CD197) and its ligation with chemokines CCL19 and CCL21 participate in T-cell homeostasis and trafficking as well as amplifying antigen-specific T-cell responses and T-cell homeostatic proliferation, suggesting a relationship with autoimmunity [5]. Research has shown that

CCR7 signaling delivers costimulatory signals during early T-cell receptor activation, leading to enhanced IFN- γ production and Th1 polarization. Thus, active CCR7 signaling favors Th1 polarization of T-cells [6]. It has also been reported that the expression of CCR7 on CD8⁺ T-cells in peripheral blood inhibits the apoptosis of CD8⁺ T-cells [7]. Whether the above effects are also present in ITP needs further exploration. Moreover, CCR7 plays an important role in autoimmune diseases mediated by T-cell subsets, such as experimental dry eye disease [8], multiple sclerosis (experimental autoimmune encephalomyelitis) [9], and arthritis [10]. Based on the functional importance of CCR7 in immune cells and autoimmune diseases, we measured the proportion of peripheral blood (PB) mononuclear cells and the expression of CCR7 in PB mononuclear cells within a cohort of ITP patients before and after treatment, and we analyzed differences in that relationship between different groups according to curative effect.

Materials and Methods

Study Participants

Approval of the Institutional Review Board was obtained from Shaoxing People's Hospital for the present study, and informed consent was obtained from patients and healthy donors to allow their information to be used in this study in accordance with the Declaration of Helsinki.

All patients met the diagnostic criteria reported by the 2010 International Consensus of Primary Immune Thrombocytopenia, including "newly diagnosed ITP" referring to a disease present for fewer than 3 months. Forty-two patients newly diagnosed with primary ITP were enrolled in this study (Table 1), and 20 healthy volunteers were examined as the control group.

Cell types	Controls	New patients	Relapsed	Remission
Platelet count (mean \pm SD)	214.90 \pm 30.02	12.21 \pm 8.60	20.46 \pm 16.91	158.15 \pm 34.96
CD3 ⁺ T-cells (mean \pm SD)	77.39 \pm 5.39	74.06 \pm 6.37	78.30 \pm 6.86	76.89 \pm 4.22
CD4 ⁺ T-cells (mean \pm SD)	40.01 \pm 4.42	45.79 \pm 8.51 [*]	46.02 \pm 10.35	41.58 \pm 4.43
CD8 ⁺ T-cells (mean \pm SD)	32.46 \pm 3.26	31.43 \pm 5.56 [*]	47.39 \pm 8.38 [#]	32.53 \pm 2.74 [*]
CD4 ⁺ /CD8 ⁺ T-cells (mean \pm SD)	1.29 \pm 0.12	1.48 \pm 0.26 [§]	1.00 \pm 0.32	1.29 \pm 0.18
B cells (mean \pm SD)	4.50 \pm 1.29	3.62 \pm 1.25	4.87 \pm 1.81	4.58 \pm 0.97
NK cells (mean \pm SD)	15.33 \pm 4.32	10.97 \pm 3.95 [§]	15.20 \pm 3.84	12.92 \pm 3.85
Tregs (mean \pm SD)	5.42 \pm 1.13	4.11 \pm 0.99 [*]	4.14 \pm 0.98 [*]	5.63 \pm 1.02 ^{§##}
CD4 ⁺ CD197 ⁺ T-cells (mean \pm SD)	26.64 \pm 5.66	48.33 \pm 6.45 [*]	54.87 \pm 6.96 ^{##}	28.16 \pm 5.75 ^{§##}
CD8 ⁺ CD197 ⁺ T-cells (mean \pm SD)	4.03 \pm 1.15	16.51 \pm 2.46 [*]	20.92 \pm 2.40 [*]	9.75 \pm 3.15 ^{§##}
CD19 ⁺ CD197 ⁺ B cells (mean \pm SD)	4.60 \pm 1.41	16.70 \pm 2.58 [*]	18.71 \pm 2.20 [*]	7.09 \pm 3.38 ^{§##}
CD197 ⁺ NK cells (mean \pm SD)	22.20 \pm 3.44	34.87 \pm 7.96 [*]	36.07 \pm 4.28 [*]	17.27 \pm 4.12 ^{§##}

NK: Natural killer; Tregs: regulatory T-cells; SD: standard deviation.
^{*}p<0.05 compared to healthy controls.
[§]p<0.05 compared to relapsed group.
^{##}p<0.05 compared to newly diagnosed patients.
[#]p<0.05 compared to newly diagnosed patients and remission group.

According to careful follow-up of the 42 newly diagnosed ITP patients, 13 patients were in relapse and 13 patients were in complete remission (CR); others were lost to follow-up. The newly diagnosed group, which had not received any initial treatment, consisted of 23 men and 19 women with a mean age of 46.50 ± 21.40 years. Thirteen patients, consisting of 6 men and 7 women with a mean age of 45.23 ± 20.23 years, were treated and evaluated for CR. The relapsed group ($n=13$) consisted of 7 men and 6 women with a mean age of 50.20 ± 14.60 years who lost CR or response (platelet count below $50 \times 10^9/L$, bleeding after CR, or less than a twofold increase in baseline platelet count from CR where "baseline platelet count" refers to the platelet count at the time of starting the initial treatment). The control group included 11 men and 9 women with a mean age of 40.20 ± 18.60 years whose physical examinations proved that they were healthy. Blood samples from the patients and the control group were processed and tested in the same assays.

Treatment

The patients in this retrospective study were all treated with intravenous immune globulin (400 mg/kg per day for 3-5 days) plus corticosteroids (dexamethasone at 40 mg per day for 4 days or prednisone at 1.0 mg/kg per day for 1-3 weeks and then tapered) according to first-line treatment standardization. Some patients with severe bleeding symptoms were also given platelet transfusions to increase platelet counts. In addition to standard therapy, cyclosporine A, rituximab, vincristine, azathioprine, danazol, interleukin-11, and thrombopoietin were used as salvage therapies for nonresponsive patients.

Treatment Responses

Treatment responses were evaluated according to the consensus definition of the International Working Group [4], including CR (complete remission) and R (relapse). In our study, the criteria to assess these responses were as follows: CR, platelet count of $\geq 100 \times 10^9/L$ and the absence of bleeding; R, platelet count of $< 30 \times 10^9/L$, bleeding, or less than a twofold increase in baseline platelet count or bleeding after CR. Blood samples were collected from the patients before they started treatment with thrombopoietic agents in the "newly diagnosed" and "relapsed" groups. After treatment, blood samples were collected from patients at the time of maximal response. The study observation period to assess the response was at least 6 months at the time of the last visit or until death (due to any cause) if death occurred before that date.

Flow Cytometry

The monoclonal antibodies (mAbs) specific for human surface antigens were purchased from BD Biosciences (San Jose, CA, USA). Peripheral blood mononuclear cells (PBMCs; 2-5 mL) were isolated from whole blood by Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden) gradient centrifugation.

Mononuclear cells were washed in AIM-V medium (Life Technologies, Grand Island, NY, USA), counted in the presence of trypan blue dye, and immediately used for experiments.

Detection of T-Cell Subsets, B Cells, NK Cells, and Regulatory T-Cells

The T-cell subsets ($CD3^+$ T-cells, $CD3^+CD4^+$ helper T-cells, and $CD3^+CD8^+$ cytotoxic T-cells), $CD19^+$ B cells, and $CD16^+CD56^+$ NK cells in PB were stained with two- and three-color mAbs using PercpCy5.5-conjugated anti-CD3 mAbs, CD16-specific mAbs, PE-conjugated CD8-specific mAbs, FITC-conjugated CD4-specific mAbs, CD19-specific mAbs, CD56-specific mAbs, and APC-conjugated CD45-specific mAbs. The isolated PBMCs (1×10^6) were stained with the above antibodies for 15 min at $4^\circ C$ in the dark according to the manufacturer's protocol and then the PBMCs (300-500 μL) were analyzed by multiparameter flow cytometry within 1 h.

Samples were anticoagulated with heparin and examined within 4 h. Approximately 100 μL of PB mononuclear cells (6×10^5) from PB was incubated in the dark for 20 min with FITC-conjugated CD25-specific mAbs, PE-conjugated CD127-specific mAbs, and PercpCy5.5-conjugated anti-CD4 mAbs. Antihuman immunoglobulin G was used as an isotype-matched negative control for all samples. After incubation, red blood cell lysis, and washing with phosphate-buffered saline, a fixation solution was added for 10 min. Cells were then assessed by flow cytometry and analyzed using FlowJo 7.6.1 software. Within the $CD4^+$ T-cell gate on lymphocytes, $CD4^+$ regulatory T-cells (Tregs) were identified as $CD25^+CD127^{low}$.

Statistical Analysis

The R language (version 4.0.4) was used for data analysis and visualization. The dplyr and ggstatsplot packages were used for data and statistical processing while ggplot2 packages were used for data visualization. The differences in the percentages of cell types between control subjects and ITP patients were analyzed by Games-Howell test. The Pearson correlation coefficient was used to evaluate the correlation between variables in different groups. Values of $p < 0.05$ were considered statistically significant.

Results

Immune Status in ITP

All patients involved in this study had newly diagnosed ITP, and they were all subjected to autoimmune screening and had no history of lymphoproliferation or severe/recurrent/atypical infections. Based on the follow-up of these newly diagnosed ITP patients, they were divided into the following two groups: relapse and complete remission. Hemorrhagic symptoms were present in 10 patients (23.8%) in the form of petechial hemorrhage and some bruising. None of the patients had

enlarged liver, enlarged spleen, or lymphadenopathy. There were no significant differences in age or sex between the ITP and control groups. Platelet count was significantly decreased in the ITP patients compared to the controls. No significant differences were detected in the proportions of CD3⁺ T and B lymphocytes between the ITP patients and controls. The overall p-value was 0.012, showing a statistical difference, but there was no statistical difference between the ITP patients and controls in terms of B lymphocytes (p>0.05). Perhaps our small sample size led to a reduction in the statistical effect. In addition, the number of groups that we compared was relatively large, which may also lead to lower statistical effect and overall differences after correction (Figures 1A and 2A). CD4⁺ T-cells were increased in the newly diagnosed patients compared to the controls (p=0.027, Figure 1B), and CD8⁺ T-cells showed the highest increase in relapsed patients compared to the other groups

(p<0.0001, Figure 1C). An elevated CD4/CD8 ratio, a decreased proportion of NK cells, and low CD4⁺CD25⁺CD127 Tregs were observed in pretreated ITP patients compared to controls (p<0.02, Figures 1D, 2B, and 2C). A higher CD4/CD8 ratio and lower levels of NK cells were observed in the newly diagnosed group compared to the relapsed group (both p<0.05, Figures 1D and 2B). More Tregs were observed in the remission group compared to the relapsed group and newly diagnosed patients (p=0.009, Figure 2C). No difference in CD4/CD8 ratio or NK cells was observed between the relapsed and remission groups (both p>0.05, Figures 1D and 2B).

CCR7 in Primary Immune Thrombocytopenia

The CD4⁺CCR7⁺ subset, CD8⁺CCR7⁺ subset, and CCR7⁺ subset of B cells and NK cells showed higher increases in the newly diagnosed group and the relapsed group compared to the

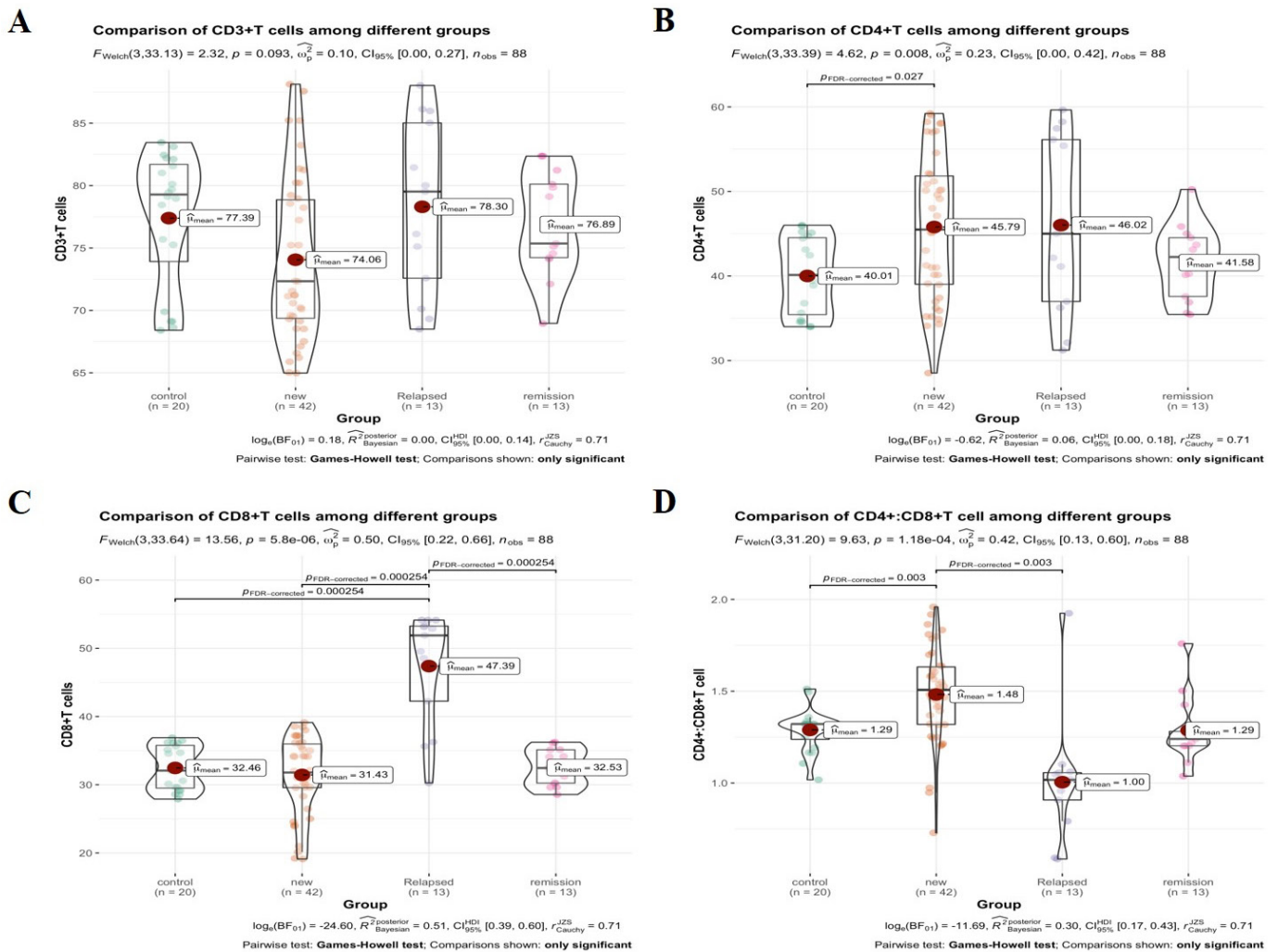


Figure 1. CD3⁺ T-cells, CD4⁺ T-cells, CD8⁺ T-cells, and CD4⁺/CD8⁺ T-cell ratio in the control, newly diagnosed, relapsed, and remission groups after treatment. (A) Mean values of CD3⁺ T-cells in the control, newly diagnosed, relapsed, and remission groups were 77.39, 74.06, 78.30, and 76.89, respectively. (B) Mean values of CD4⁺ T-cells in the control, newly diagnosed, relapsed, and remission groups were 40.01, 45.79, 46.02, and 41.58, respectively. (C) Mean values of CD8⁺ T-cells in the control, newly diagnosed, relapsed, and remission groups were 32.46, 31.43, 47.39, and 32.53, respectively. (D) The CD4⁺/CD8⁺ T-cell ratios in the control, newly diagnosed, relapsed, and remission groups were 12.9, 1.48, 1.00, and 1.29, respectively.

controls and the remission group ($p < 0.05$, Figures 3A-3D), but there was no difference between the newly diagnosed group and the relapsed group in the CCR7⁺ subset of B cells and NK cells ($p > 0.05$, Figures 3C and 3D). The levels of the CD4⁺CCR7⁺ subset and CD8⁺CCR7⁺ subset in the relapsed group were slightly higher than those in the newly diagnosed group (both $p < 0.05$, Figures 3A and 3B). The levels of CCR7⁺ subsets of CD4⁺ T-cells, CD8⁺ T-cells, NK cells, and B cells were lower in the remission group compared to the relapsed group ($p < 0.001$, Figures 3A-3D). Higher levels of the CD8⁺CCR7⁺ subset and lower levels of NK cells were found in the remission group compared to the controls ($p < 0.05$, Figures 3B and 3D). No difference in the CD4⁺CCR7⁺ subset or CCR7⁺ subset of B cells was found between the controls and the remission group ($p < 0.001$, Figures 3A and 3C).

The ratio between the CD4⁺CCR7⁺ subset and CD8⁺CCR7⁺ subset was lower in the ITP patients than the controls ($p < 0.001$,

Figure 4A), but no differences were found among the newly diagnosed group, the relapsed group, and the remission group ($p > 0.05$, Figure 4A). There was a negative correlation between the CD8⁺CCR7⁺ subset and platelet count for all groupings of ITP patients (both $p < 0.001$, Figure 4B).

Discussion

ITP is an autoimmune disease, and although various mechanisms involved in ITP, including cytokines [11,12,13], immune cells [14,15,16,17,18,19,20], T-cell receptor excision circles [21], and microRNAs [22], have been investigated, the pathogenesis of ITP still needs further study. Currently, drug treatments include steroid therapy in combination with other immunosuppressive drugs and thrombopoietin receptor agonists (e.g., thrombopoietin, eltrombopag, and romiplostim), which have shown not only limited efficacy [23] but also high toxicity or high cost. Thus, a more thorough understanding

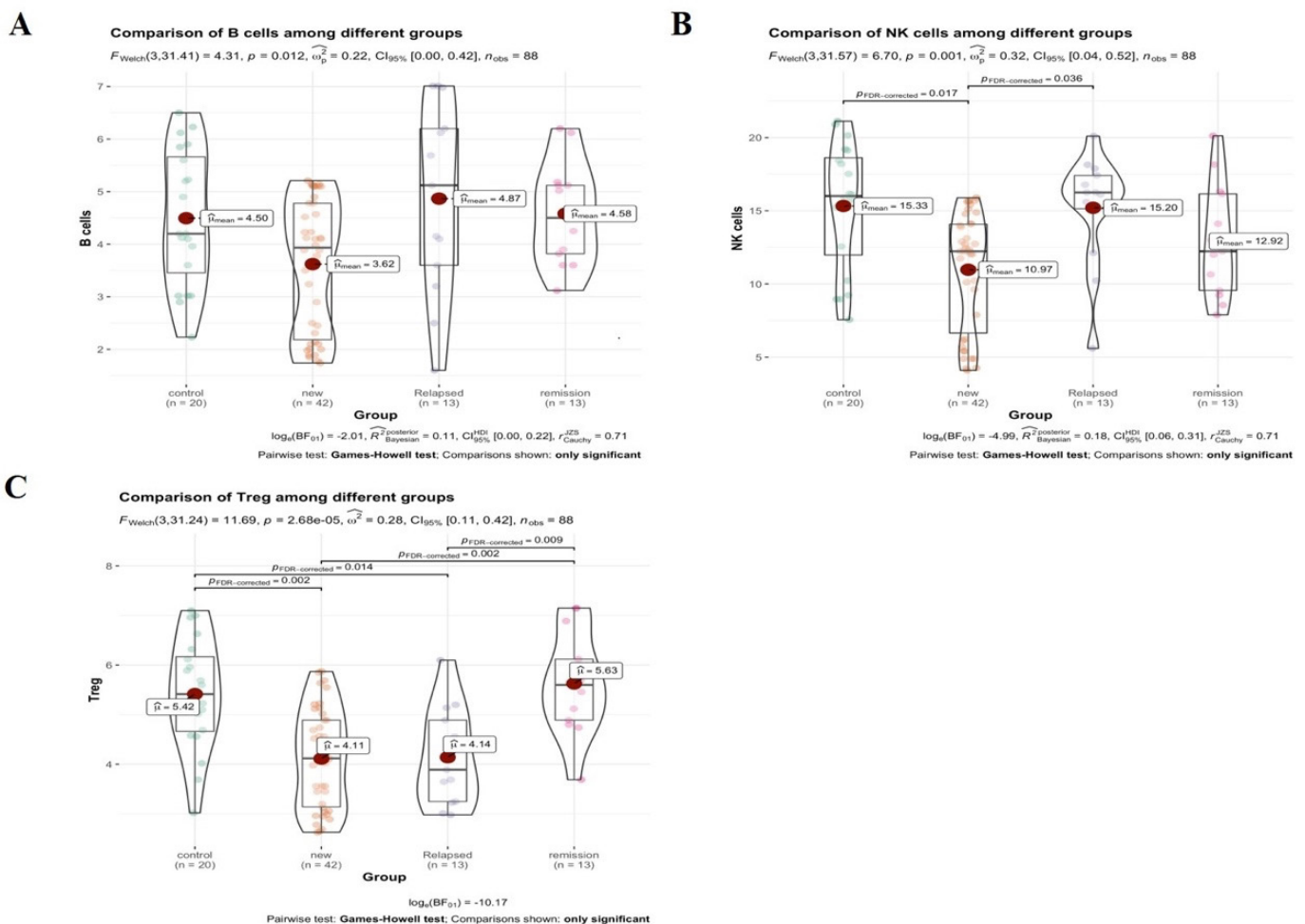


Figure 2. B cells, NK cells, and CD4⁺ Treg cells in the control, newly diagnosed, relapsed, and remission groups after treatment. (A) Mean values of B cells in the control, newly diagnosed, relapsed, and remission groups were 4.50, 3.62, 4.87, and 4.58, respectively. (B) Mean values of NK cells in the control, newly diagnosed, relapsed, and remission groups were 15.33, 10.97, 15.20, and 12.92, respectively. (C) Mean values of CD4⁺ regulatory T-cells (Tregs) in the control, newly diagnosed, relapsed, and remission groups were 5.42, 4.11, 4.14, and 5.63, respectively.

of the immunopathogenesis of ITP is needed. Despite studies showing that increased levels of autoantibodies against self-antigens play a crucial role in platelet destruction, antiplatelet autoantibodies are detectable in only half of all ITP patients and other mechanisms are likely to be involved [20]. The pathophysiology of ITP remains complicated and requires further study. In this retrospective study, we analyzed PBMCs, including T-lymphocyte subsets, B lymphocytes, and NK cells, as well as the expression of CCR7 in 42 patients diagnosed with primary ITP before and after treatment in our department. We analyzed immune statuses and the relationship between the expression of CCR7 on lymphocyte subsets and therapeutic outcomes. Changes in CCR7 expression before and after treatment may be helpful for clinicians in selecting alternative treatments for ITP patients.

Abnormal T lymphocyte subpopulations in patients with ITP have been previously reported. CCR7 expression, which plays a critical role in T-cell maturation, differentiation, and function, has been reported on T-cells [24] and CCR7+ central memory T-cells

were associated with disease severity and immunoglobulin E in bronchial asthma. However, the function of CCR7 has not been elucidated in ITP.

Recent research has shown that the relative numbers of CD4+ and CD8+ T-cells are normal in ITP and that CD8+ T-cells increase potential cytotoxicity in ITP [19]. However, other reports have indicated increased CD8+ T lymphocytes and decreased CD4/CD8 ratios in acute and chronic patients [25].

The present study found that CD4+ T-cells were increased in newly diagnosed patients and that CD8+ T-cells were increased in relapsed patients. CD4/CD8 ratios were increased in newly diagnosed patients and decreased in relapsed patients. There were no significant differences between the patients with CR and the controls. The differences in CD4/CD8 ratios may be related to the complex pathogenesis of ITP. According to treatment responses, the CD4/CD8 ratios almost returned to normal in the CR group even though there was no significant difference due to the small sample size. We also found that the CCR7+

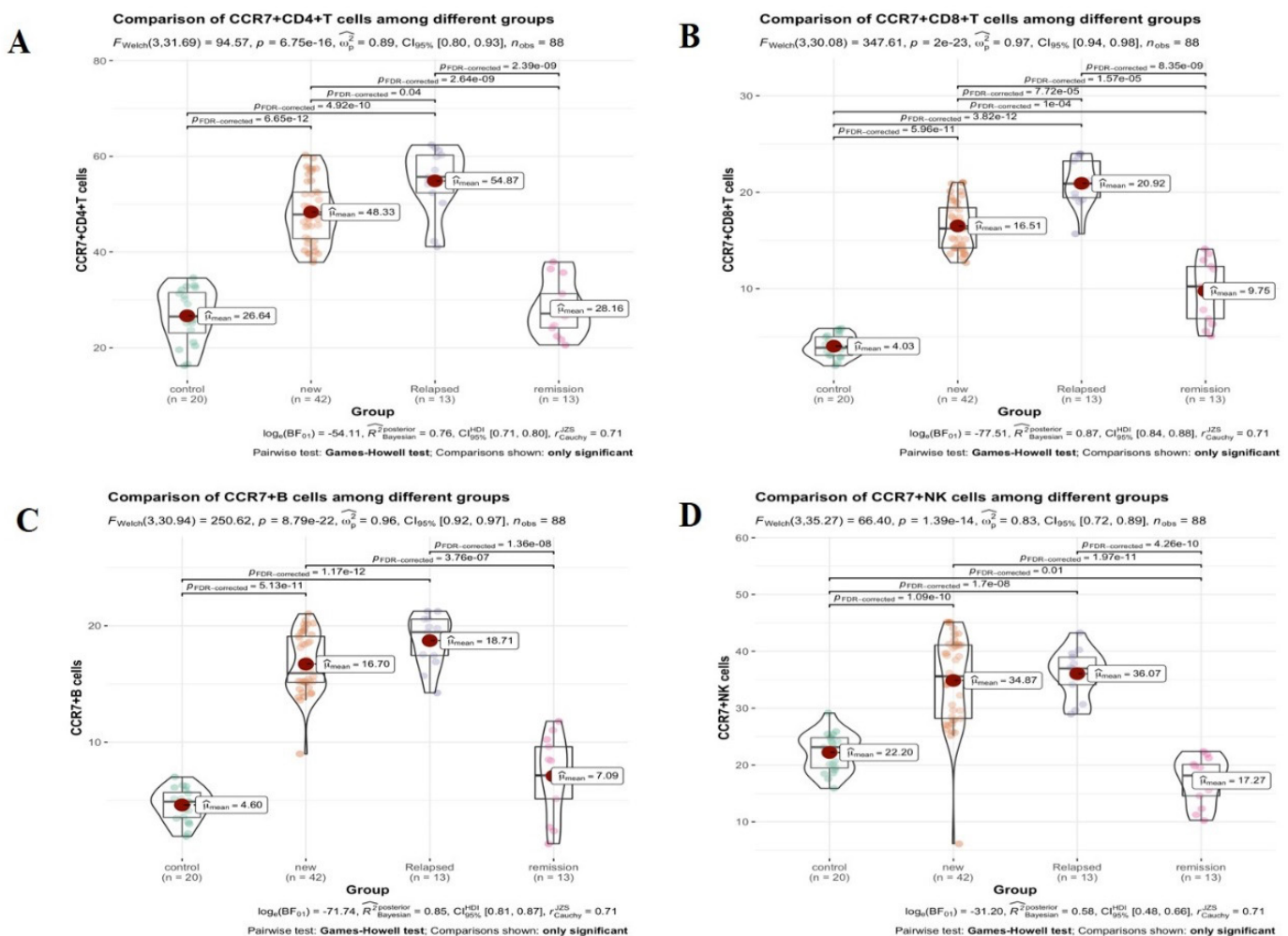


Figure 3. Expression of CCR7 on CD4+ T-cells (A), CD8+ T-cells (B), B cells (C), and NK cells (D) in the control, newly diagnosed, relapsed, and remission groups after treatment.

subset of circulating CD4⁺ T-cells was significantly increased when patients were newly diagnosed before treatment. After treatment, the CCR7⁺ subset of circulating CD4⁺ T-cells was significantly decreased in the CR group, and this subset was significantly increased in the relapsed group. The change in the CCR7⁺ subset of circulating CD4⁺ T-cells may be an indicator of effective treatment. However, it is not clear whether change in the proportion of CCR7⁺CD4⁺ T-cells was caused by effective treatment or whether change in the proportion of CCR7⁺CD4⁺ T-cells led to effective treatment. Psarras et al. [26] reported that CCR7 promotes human plasmacytoid dendritic cell maturation by upregulating chemokine receptors, thereby inducing CD4⁺ T-cell proliferation and activation. CCR7 expression on CD4⁺ T-cells in two cases of Blau syndrome, an inflammatory disorder characterized by uveitis, was reported to be increased [5]. CCR7 promotes T helper 17 (Th17)-mediated autoimmune disease [27], and the increased differentiation of Th17 cells is a vital promoter of ITP progression [28]. Whether the increased expression of CCR7 on CD4⁺ T-cells also promotes the differentiation of Th17 cells in ITP remains to be further studied. Moschovakis et al. [6] reported that a lack of CCR7 signaling favors the development of Th2 cells in immunodeficient mice, suggesting that CCR7 signaling affects the Th1/Th2 polarization potential of CD4⁺ T-cells. Some studies [29,30] have shown that increased Th17 cells are consistent with Th1 polarization in ITP. It remains unknown whether CCR7 signals affect Th1/Th2 polarization through Th17 cells to participate in the occurrence and development of ITP. Gupta et al. [31] reported that CD8⁺CCR7⁺CD45RA⁻ Tregs inhibited B cell proliferation and immunoglobulin production in healthy PB. In the present study, the CCR7⁺ subset of circulating CD8⁺ T-cells was significantly increased when patients were newly diagnosed before treatment, and this subset was significantly decreased in the CR group but significantly increased in the relapsed group after treatment. We

did not further classify CCR7⁺CD8⁺ T-cells, but we found that the proportion of CCR7⁺CD8⁺ T-cells was inversely correlated with platelet count in ITP patients. Kim et al. [32] reported that CCR7 expression on circulating CD8⁺ T lymphocytes protects those T lymphocytes from apoptosis in squamous cell carcinoma of the head and neck. However, the presence of this phenomenon in ITP needs to be further explored.

B cells play a key role in the pathogenesis of ITP by producing platelet autoantibodies. Many studies have shown that patients with ITP may have more B cells in PB than healthy individuals [33]. In the present study, there was no significant difference in the number of B cells of newly diagnosed and relapsed patients compared to the controls and the CR group. Compared to healthy controls, Li et al. [34] reported that the proportions of CD19⁺ B cells in ITP patients without treatment were comparable. In the present study, we did not analyze the B-cell subsets and antiplatelet antibody (IgG, IgA, and IgM) levels, but further research on these aspects will be conducted. The CCR7⁺ subset of circulating CD19⁺ B cells was significantly increased when patients were newly diagnosed before treatment. After treatment, the CCR7⁺ subset of circulating CD19⁺ B cells was significantly decreased in the CR group but significantly increased in the relapsed group. CCR7 regulates the trafficking and retention of B cells. Moschovakis et al. [6] reported that CCR7 signaling is involved in B-cell activation in vivo. It remains to be further explored whether CCR7 also participates in the activation and proliferation of B cells in ITP patients.

Several studies on NK cells have been conducted in ITP, but the results are contradictory. The present study found that NK cells were significantly decreased in newly diagnosed patients compared to controls but significantly increased in the relapsed group compared to newly diagnosed patients. However, no significant difference was found between ITP patients who were

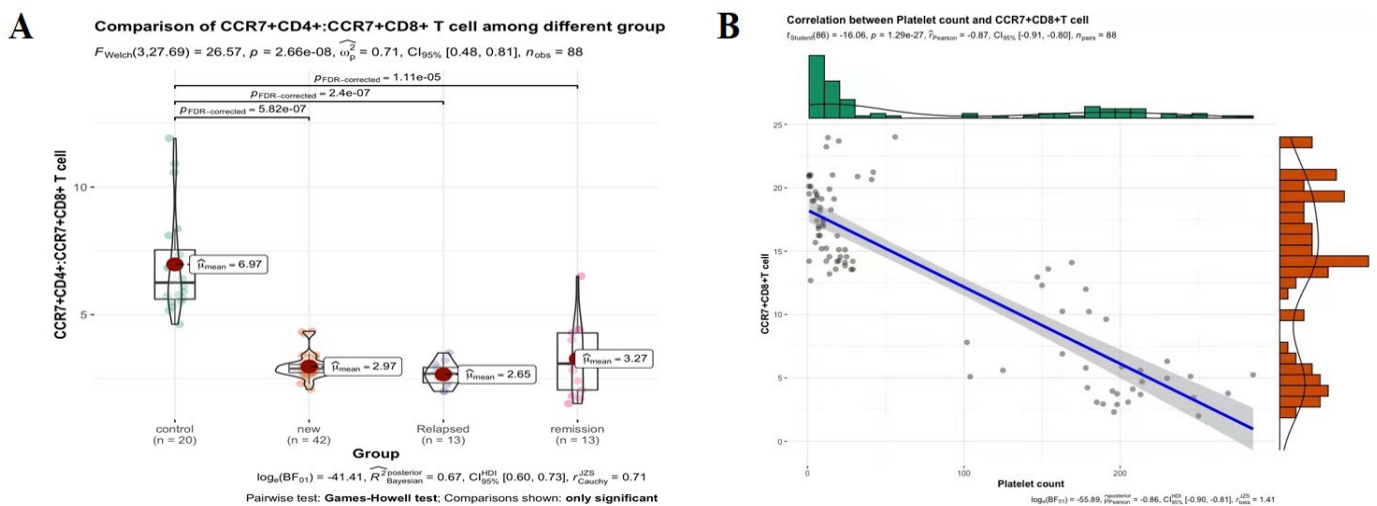


Figure 4. (A) The CD4⁺CCR7⁺ T-cell/CD8⁺CCR7⁺ T-cell ratio in the control, newly diagnosed, relapsed, and remission groups after treatment. (B) Correlation between platelet count and CCR7⁺CD8⁺ T-cells.

effectively treated or not treated in the present study, which may be due to the small sample size, and we will perform more studies in the future. In contrast to CD8⁺ T-cells, NK cells were not involved in thrombocytopenia [35]. Our study also showed that NK cells were not involved in thrombocytopenia. The CCR7⁺ subset of NK cells was significantly increased when patients were newly diagnosed before treatment. After treatment, the CCR7⁺ subset of NK cells was significantly decreased in the CR group but significantly increased in the relapsed group. The CCR7⁺ subset of NK cells was similar to the CCR7⁺CD8⁺ T-cell subset and CCR7⁺CD19⁺ B-cell subset. Further research is needed to explore this correlation. Even if the ITP patients were in CR after treatment, the expression of CCR7 did not return to a normal level, which may account for the existing immune function deficiency in ITP patients who do not recover or for recurrence in some patients with ITP. Moreover, the expression of CCR7 was the highest in the relapsed group. It remains to be further explored whether CCR7 is also involved in disease recurrence or outcome after recurrence.

Tregs are a T-cell subset with immunomodulatory properties that can inhibit effector cells, such as CD4⁺ T-cells, CD8⁺ T-cells, and B-cells, and induce tolerance to dendritic cells [36]. Many studies have reported a low frequency of circulating Tregs in ITP patients. Our study has similarly shown that Tregs were reduced in newly diagnosed and relapsed patients, and these patients recovered following treatment in the CR group, suggesting that an approach involving the restoration of Tregs may be another treatment route in ITP.

Conclusion

ITP is a heterogeneous disease and a syndrome in which multiple pathways leading to thrombocytopenia are involved. Identification of common key molecules among different pathways may aid in identifying an effective therapeutic strategy. CCR7 may be such a molecule, and its specific mechanism in ITP still needs further exploration. Because our sample size was small and the test results were not comprehensive, we plan to expand the sample size and further explore the specific mechanism of CCR7 in the pathogenesis of ITP in future studies.

Ethics

Ethics Committee Approval: Ethics clearance was granted from Institutional Review Board of Shaoxing People's Hospital for the present study.

Authorship Contributions

Concept: D.L.; Design: D.L.; Data Collection or Processing: G-Z.Z.; Analysis or Interpretation: L.Z.; Literature Search: W-Y.F.; Writing: D.L.

Conflict of Interest: No conflict of interest was declared by the authors.

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