Clin. Lab. 2019;65:XXX-XXX

ORIGINAL ARTICLE

Role of Th 1- and Th 2- Chemokine Receptor in the Diagnosis and Prognosis of Primary Immune Thrombocytopenia

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SUMMARY

Background: **The diagnostic and prognostic role of Th-1 chemokine receptor and Th-2 chemokine receptor in patients with primary immune thrombocytopenia has not been investigated extensively so far. In this study, our goal is to explore the diagnostic and prognostic role of C-C chemokine receptor 3 (CCR3) and C-C chemokine receptor 5 (CCR5) in patients with primary immune thrombocytopenia.**

Methods: **The expression levels of CCR3 and CCR5 were measured in peripheral blood mononuclear cells of patients with primary immune thrombocytopenia and healthy subjects. The relationship between the expression levels of CCR3 and CCR5 and clinicopathological characteristics was analyzed. The diagnostic accuracy of CCR3 and CCR5 as biomarkers to discriminate primary immune thrombocytopenia patients from healthy subjects was determined. Univariate and multivariate Cox regression analysis were performed to determine the prognosis value of CCR3 and CCR5 in primary immune thrombocytopenia. The outcome of primary immune thrombocytopenia patients was also evaluated.**

Results: **Compared to healthy subjects, the expression level of CCR3 was significantly downregulated and CCR5 was significantly upregulated ($p < 0.05$). The expression levels of CCR3 and CCR5 were significantly correlated with bleeding times and platelet counts at diagnosis ($p < 0.05$). CCR3 and CCR5 could act as a suitable biomarker for differentiating the primary immune thrombocytopenia patients from healthy subjects. CCR3 and CCR5 were independent prognostic factors. Overexpression of CCR5 and low expression of CCR3 lead to poor clinical benefits and indicated poor prognosis of primary immune thrombocytopenia.**

Conclusions: **To summarize, our results suggested that CCR3 and CCR5 could act as suitable biomarkers and indicated poor prognosis of primary immune thrombocytopenia.**

(Clin. Lab. 2019;65:xx-xx. DOI: 10.7754/Clin.Lab.2018.180915)

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Manuscript accepted December 29, 2018

KEY WORDS

diagnosis, prognosis, primary immune thrombocytopenia, C-C chemokine receptor 3 (CCR3), C-C chemokine receptor 5 (CCR5)

INTRODUCTION

The main clinical manifestations of primary immune thrombocytopenia are decreased platelet counts in peripheral blood, skin, mucosa or visceral bleeding caused by autoimmune abnormalities such as platelet dysfunction or excessive platelet destruction [1-3]. Abnormal immune cell subsets and cytokines are two important components of the pathogenesis in primary immune

thrombocytopenia [4]. At present, immunosuppressive agents are the main treatment for the disease, but in about 30% of the patients they are ineffective or recurrent, and long-term drug treatment side effects are obvious, while splenectomy is traumatic treatment, the effective rate is only 2/3 [5-7]. The occurrence of primary immune thrombocytopenia seriously affects the quality of life of patients and threatens the safety of patients. Primary immune thrombocytopenia is a T helper (Th) l dominant disease. The imbalance of Th 1/Th 2 can lead to platelet destruction [8]. Along with the differentiation of Th cells, some chemokine receptors will also be abnormally expressed. These chemokine receptors can specifically bind to chemokines and guide the migration of immune cells [9,10]. C-C chemokine receptor 5 (CCR5) is a member of the C-C chemokine receptor family. There are three kinds of ligands, which contain the C-C motif ligand (CCL): CCL3, CCL4, and CCL5. CCR5 and its ligands mainly mediate the chemotaxis and recruitment of $CCR5^+$ immune cells [11,12]. CCR5 is mainly expressed on cell membranes of monocytes, immature dendritic cells, and resting memory T lymphocytes [13]. CCR5 mediates intracellular signal transduction to the classical G protein-coupled receptor (GPCR) signaling pathway. Moreover, CCR5 tyrosine phosphorylation can be induced by CCL5 [14,15]. CCR5 tyrosine phosphorylation does not depend on the GPCR signaling pathway, activating the signaling pathways independent of GPCR [16]. Recent reports have shown that CCR5 is involved in the polarization of Th l cells and participates in primary immune thrombocytopenia, oral lichen planus, and other diseases [17,18]. CCR3 is selectively expressed in Th 2 cell subsets, and CCL11/CCR3 is a Th 2 response effector [19]. CCR3 with ligands participate in the homing of eosinophils and basophils in Th2 reaction [20]. The low expression of CCR3 in patients with primary immune thrombocytopenia is consistent with the fact that primary immune thrombocytopenia is a Th l predominant disease and may be the result of Th l/Th 2 imbalance [21,22]. When CCL11 expression is decreased, the competitive binding of CXCR3 to CCL may weaken the role of CCR3 and aggravate the Th l/Th 2 imbalance [23,24]. Therefore, CCR3 and CCR5 may be ideal diagnosis and prognosis markers in patients with primary immune thrombocytopenia. At present, mRNAs of CCR are used to diagnose many diseases with the development of real-PCR. Agic et al. reported that combination of CCR1 mRNA, MCP1, and CA125 measurements in peripheral blood could be a diagnostic test for endometriosis [25]. However, protein and mRNA levels are not equal due to post transcriptional regulation such as microRNA and long non-coding RNA [26, 27]. Furthermore, mRNA in serum is easily degraded during detection, which affects the accuracy of diagnosis and prognosis evaluation. Therefore, we detected the protein level of CCR3 and CCR5 to evaluate the diagnostic and prognostic role in patients with primary immune thrombocytopenia. In the present study, we investigated the expression levels of CCR3 and CCR5 in patients with primary immune thrombocytopenia and evaluated their diagnosis and prognosis power.

MATERIALS AND METHODS

Patients and clinical samples

In this study, we received the ethical approval from the ethics and scientific committees. To participate in our study, all primary immune thrombocytopenia patients provided their written informed consent. All patients met the diagnostic criteria reported by the international consensus of primary immune thrombocytopenia (2010), including (1) decreased platelet count and no abnormal blood cell morphology found by two assays; splenomegaly is not generally enlarged (2) bone marrow examination showed the number of megakaryocytes increased or normal, with maturation disorders. Exclusion criteria included the existences of secondary thrombocytopenia, active infections, diabetes mellitus, hyperthyroidism, viral hepatitis, hypertension, cardiovascular diseases, pregnant woman, and so on. From September 2015 and April 2016, 45 healthy subjects and 78 patients with primary immune thrombocytopenia were accepted to our study. The clinical and pathological data of 78 patients with primary immune thrombocytopenia and 45 healthy subjects listed in this study were described in Table 1. There was no significant difference in the distribution, including gender and age, between healthy subjects and patients with primary immune thrombocytopenia. The patients were given initial treatment: prednisone (Zhejiang Xianju pharmaceutical, China) 0.5 - 2.0 mg/kg/d (maximum dose not exceeding 60 mg/d) intravenous drip or fractional oral administration, and/or intravenous injection of gamma globulin (Shanxi Canbo biological products, China) 400 mg/(kg/d) for 3 - 5 d. Platelet counts were determined at diagnosis using a DxH 800 Coulter hematology analyzer. Follow-up was performed and ended on May 20, 2018. Patients were followed up regularly every month after therapy by telephone and outpatient follow-up.

Detection of the expression level of CCR3 and CCR5

Peripheral blood samples (5 mL) were obtained in heparin tubes at the time of diagnosis. Peripheral blood mononuclear cells were isolated using G2011 Monocyte Isolation Kit (Tianjin Hao Yang Biological Products Technology, China) and centrifuged as described previously [28]. After centrifugation, peripheral blood mononuclear cells were collected from the interphase layer and washed two times using 0.9% saline solution. Peripheral blood mononuclear cells were suspended in 5 mL of RPMI 1640 at the concentration 1 x 10^7 cells/mL. Protein lysing solution (Solarbio, China) was added to the cells for extraction of total protein content. To quantify the total protein extracted, the Bradford method (Thermo Fisher, USA) was performed. Fifty milligrams of total protein was separated using SDS-PAGE

and transferred to a PVDF membrane (Millipore, USA). Then the membrane was blocked with slim milk (5%) for 1 hour and then incubated with rabbit anti human monoclonal antibodies, including CCR3, CCR5, and GAPDH (Abcam, USA, 1:1000) at 4°C for 12 hours. After washing 3 times using phosphate buffered saline Tween, the membrane was incubated with horseradish peroxidase-labeled goat anti rabbit secondary antibodies (Abcam, USA, 1:3000) for 2 hours at 37°C and then washed again with phosphate buffered saline Tween. For the purposes of photography and analysis, luminol reagent was mixed with peroxide solution (Aladdin, China) at a ratio of 1:1. Using the ratio of target band to control band, the relative protein expression was calculated. Each experiment was conducted 3 times, and the average values were calculated.

Assessment of response after therapy

The curative effect was evaluated after therapy. Complete remission (CR) is defined as PLT more than 100 x 10^9/L for 2 months and bleeding symptoms disappearing after treatment; partial remission (PR) is defined as PLT at least 2 times higher than that of basal PLT for 2 months, and bleeding symptoms disappearing after treatment. Stable disease (SD) is defined as PLT less than 30 x 10^9/L or PLT 2 times less than that of basal PLT, still presenting bleeding symptoms after treatment. The objective response defined as CR or PR.

Statistics

SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Student's *t*-test and χ^2 test were used to estimate the significance of differences between groups. Receiver operating characteristic curve (ROC) was used to analyze the predictive value and optimal cutoff value of primary immune thrombocytopenia. The remission rates were calculated by the Kaplan-Meier method with the log-rank test applied for comparison. Therapy data were evaluated using univariate and multivariate Cox proportional hazards model. Variables with a value of $p < 0.05$ in univariate analysis were used in subsequent multivariate analysis. Twosided p-values < 0.05 was considered statistically significant.

RESULTS

Clinicopathological characteristics of study participants

The characteristics of 87 patients with primary immune thrombocytopenia and 45 healthy subjects were presented in Table 1. There was no significant difference in the distribution, including gender and age, between healthy subjects and patients with primary immune thrombocytopenia.

Seventy-two patients with bleeding history within 2 weeks and 5 patients with bleeding history of more than 2 weeks were observed in our study. Two patients with organ hemorrhage had gastrointestinal and urinary tract bleeding, and no intracranial hemorrhage cases. The platelet counts at diagnosis for the 71 patients was < 20 x 10^9/L. All patients received therapy including prednisone and/or gamma globulin. The level of CCR3 and CCR5 in peripheral blood mononuclear cells of patients with primary immune thrombocytopenia was compared to that of healthy subjects (Figure 1). Protein levels of patients vs. healthy subjects revealed a significantly lower level of CCR3 ($p < 0.01$), while protein levels of patients vs. healthy subjects revealed a significantly higher level of CCR5 ($p < 0.01$).

Diagnostic accuracy of CCR3 and CCR5 in primary immune thrombocytopenia

To determine whether other factors besides primary immune thrombocytopenia have an influence on expression levels of upregulated CCR5 and downregulated CCR3, we evaluated the statistical relationship between the expression level of CCR3 and CCR5 and clinical variables. As shown in Table 2, the expression level of CCR3 was significantly correlated with bleeding times and platelet counts at diagnosis ($p < 0.05$). However, there was no significant association between expression level of CCR3 and age, gender, and bleeding site ($p > 0.05$). CCR5 overexpression was significantly associated with bleeding times and platelet counts at diagnosis ($p < 0.05$). However, there was no significant association between expression level of CCR5 and age, gender, and bleeding site ($p > 0.05$). These data suggested that CCR3 and CCR5 may be good diagnostic and prognostic markers in primary immune thrombocytopenia. We further evaluated the diagnostic accuracy of CCR3 and CCR5 as biomarkers to discriminate primary immune thrombocytopenia patients from healthy subjects by plotting ROC curves. As shown in Figure 2, we found that CCR3 and CCR5 could discriminate patients with primary immune thrombocytopenia patients from healthy subjects with AUC values of 0.919 (95% CI: 0.872 - 0.965; $p < 0.05$) and 0.914 [95% Confidence Interval (CI): 0.865 - 0.962; $p < 0.05$], respectively. At the cutoff value of 0.825 for CCR3, the optimal sensitivity and specificity of CCR3 were 88.5% and 77.8%, respectively. At the cutoff value of 0.755 for CCR5, the optimal sensitivity and specificity of CCR5 were 75.6% and 93.3 %, respectively. The combination ROC curve analysis yielded the AUC value of 0.976 (95% CI = 0.0.955 - 0.997; $p < 0.05$). These data indicated that CCR3 and CCR5 showed the greatest ability for differentiating primary immune thrombocytopenia patients from healthy subjects and could act as a suitable biomarker for detecting primary immune thrombocytopenia.

Potential prognostic values of CCR3 and CCR5 in primary immune thrombocytopenia

We analyzed clinicopathological characteristics and expression level of CCR3 and CCR5 in the prognosis of primary immune thrombocytopenia. Multivariate Cox

Table 1. Clinical information of patients with primary immune thrombocytopenia.

	Clinical variable	PITP (n = 78)	HS (n = 45)	p-value
Gender	Male [n (%)]	31	20	> 0.05
	Female [n (%)]	47	25	
Age	≤ 60 [n (%)]	34	22	> 0.05
	> 60 [n (%)]	44	23	
Bleeding times	Bleeding history within 2 weeks [n (%)]	71	/	
	Bleeding history more than 2 weeks [n (%)]	5	/	
	No bleeding [n (%)]	2	/	
Bleeding symptoms	Skin hemorrhage [n (%)]	66	/	
	Mucous hemorrhage [n (%)]	10	/	
	Organ hemorrhage [n (%)]	2	/	
Platelet counts at diagnosis [n (%)] ($\overline{x} \pm s$)	Median (< 20 x 10^9/L)	71	0	< 0.05
	Range (≥ 20 x 10^9/L)	7	45	
	Lymphocyte count at diagnosis (x 10^9/L, $\overline{x} \pm s$)	3.70 ± 1.28	2.15 ± 1.04	< 0.05
	White blood cell counts at diagnosis (x 10^9/L, $\overline{x} \pm s$)	8.07 ± 2.41	6.84 ± 2.17	< 0.05
Initial treatment modalities	Prednisone [n (%)]	3	/	
	Gamma globulin [n (%)]	9	/	
	Prednisone and gamma globulin [n (%)]	66	/	

Table 2. Clinical variables of 78 primary immune thrombocytopenia patients with different levels of CCR3 and CCR5.

	Clinical variable	H-CCR3 (n = 39)	L-CCR3 (n = 39)	p-value	H-CCR5 (n = 39)	L-CCR5 (n = 39)	p-value
Gender	Male [n (%)]	15	16	> 0.05	17	14	> 0.05
	Female [n (%)]	24	23		22	25	
Age	≤ 60 [n (%)]	16	18	> 0.05	17	17	> 0.05
	> 60 [n (%)]	23	21		22	22	
Bleeding times	Bleeding history within 2 weeks [n (%)]	38	33	< 0.05	33	38	< 0.05
	Bleeding history more than 2 weeks [n (%)]	0	5		5	0	
	No bleeding [n (%)]	1	1		1	1	
Bleeding site	Petechiae [n (%)]	37	29	> 0.05	28	38	> 0.05
	Ecchymosis [n (%)]	1	9		10	0	
	Buccaltunica [n (%)]	1	1		1	1	
Platelet counts at diagnosed ($\overline{x} \pm s$)	Median (< 20 x 10^9/L)	32	39	< 0.05	38	33	< 0.05
	Range (≥ 20 x 10^9/L)	7	0		1	6	
	Lymphocyte count at diagnosed (x 10^9/L, $\overline{x} \pm s$)	2.56 ± 0.71	3.16 ± 0.89	< 0.05	3.22 ± 0.58	2.49 ± 0.95	< 0.05
	White blood cell count at diagnosed (x 10^9/L, $\overline{x} \pm s$)	7.86 ± 0.85	8.03 ± 0.72	< 0.05	8.11 ± 0.94	7.96 ± 0.51	< 0.05

Table 3. Univariate and multivariate unconditional logistic regression analysis of clinical variables of primary immune thrombocytopenia.

Clinical variable	OR	95% CI	p-value
Univariate			
Gender	1.265	0.783 - 2.143	> 0.05
Age	1.187	0.804 - 1.532	> 0.05
Bleeding times	0.362	0.121 - 0.898	< 0.05
Bleeding site	1.358	0.986 - 1.709	> 0.05
Platelet counts at diagnosis	0.317	0.113 - 0.634	< 0.05
Lymphocyte count at diagnosis	0.412	0.323 - 0.645	< 0.05
White blood cell count at diagnosis	2.152	1.485 - 2.798	> 0.05
CCR3	0.157	0.069 - 0.411	< 0.05
CCR5	0.163	0.060 - 0.457	< 0.05
Multivariate			
Bleeding times	0.214	0.102 - 0.487	< 0.05
Platelet counts at diagnosis	0.436	0.227 - 0.959	< 0.05
Lymphocyte count at diagnosis	0.241	0.098 - 0.614	< 0.05
CCR3	0.175	0.061 - 0.462	< 0.05
CCR5	0.169	0.053 - 0.423	< 0.05

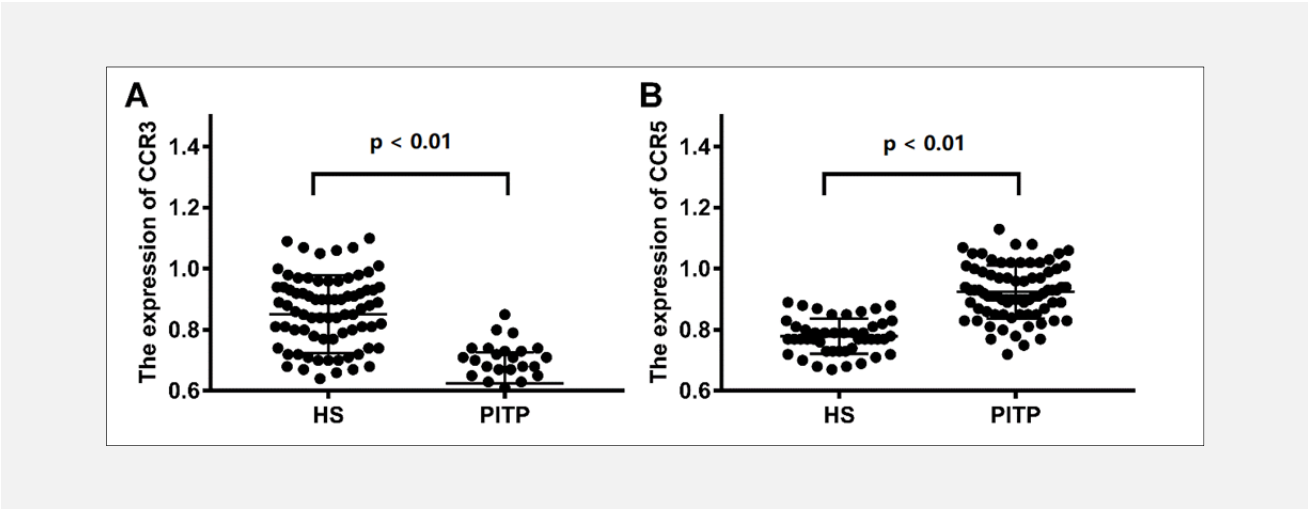

Figure 1. The level of CCR3 and CCR5 in healthy subjects and patients with primary immune thrombocytopenia.

regression analysis was performed to determine whether CCR3 and CCR5 could serve as prognostic factors. The univariate analysis showed that the bleeding times ($p < 0.05$), platelet counts at diagnosis ($p < 0.05$), CCR3 ($p < 0.05$), and CCR5 ($p < 0.05$), were independent factors (Table 3). Multivariate Cox regression analysis found that the CCR3 ($p < 0.05$) and CCR5 ($p < 0.05$) were the prognostic factors for primary immune thrombocytopenia patients (Table 3).

According to the median of CCR3, 78 primary immune thrombocytopenia patients were divided into groups: H-CCR3 group ($p > 0.915$) and L-CCR3 group ($p < 0.915$), and according to the median of CCR5, 78 primary immune thrombocytopenia patients were divided into groups: H-CCR5 group ($p > 0.865$) and L-CCR5 group ($p < 0.865$). After two weeks of therapy, object-

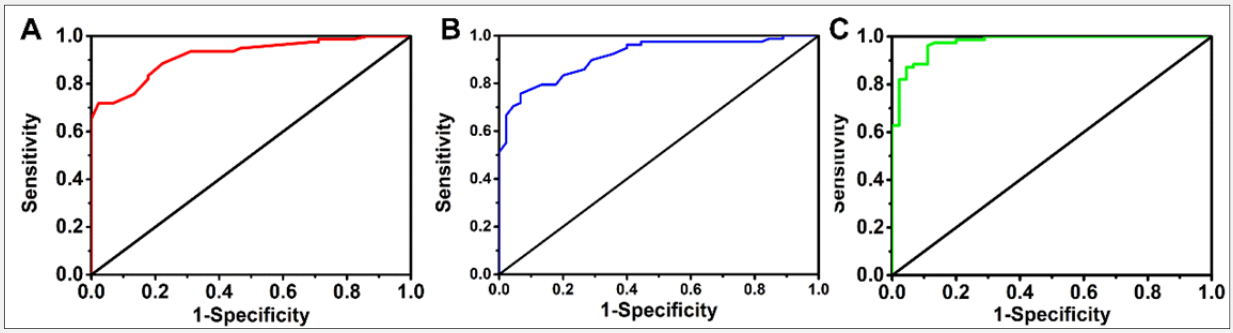

Figure 2. The ROC curve of CCR3 and CCR5 for diagnosing primary immune thrombocytopenia.

(A) CCR3, (B) CCR5, (C) Combination.

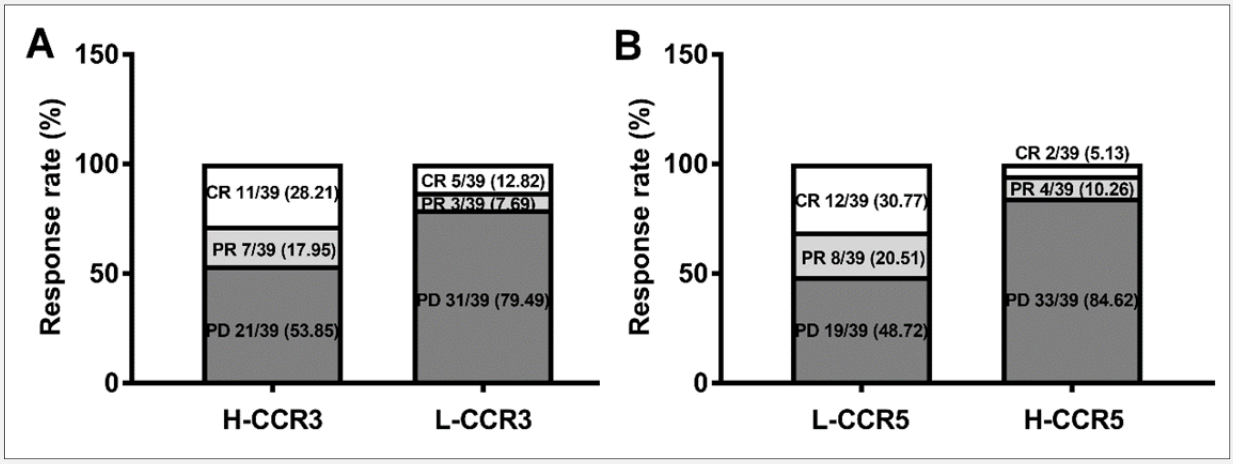

Figure 3. Response rates as a function of CCR3 (A) and CCR5 (B) levels.

CR - complete response, PR - partial response, SD - stable disease, PD - progressive disease.

tive analysis of therapy curative effect was assessed. A response rate after therapy was confirmed in 78 primary immune thrombocytopenia patients (Figure 3). Eighteen of thirty-nine (46.15%) patients with elevated expression of CCR3 had a significantly higher objective response (CR+PR) compared with 8/39 (20.51%) patients in L-CCR3 group. Of the patients with elevated expression of CCR5, 20/39 (51.28%) had a significantly lower objective response (CR+PR), compared with 6/39 (15.38%) patients in L-CCR5 group. A significant difference was also observed for PD rate between low expression group and high expression group ($p < 0.05$). Those results showed that overexpression of CCR5 and low expression of CCR3 reduced therapy sensitivity of primary immune thrombocytopenia patients that lead to poor clinical benefits.

We further examined whether the CCR3 and CCR5 expression level was correlated with the outcome of primary immune thrombocytopenia patients after therapy. Kaplan-Meier survival analysis and log-rank test were

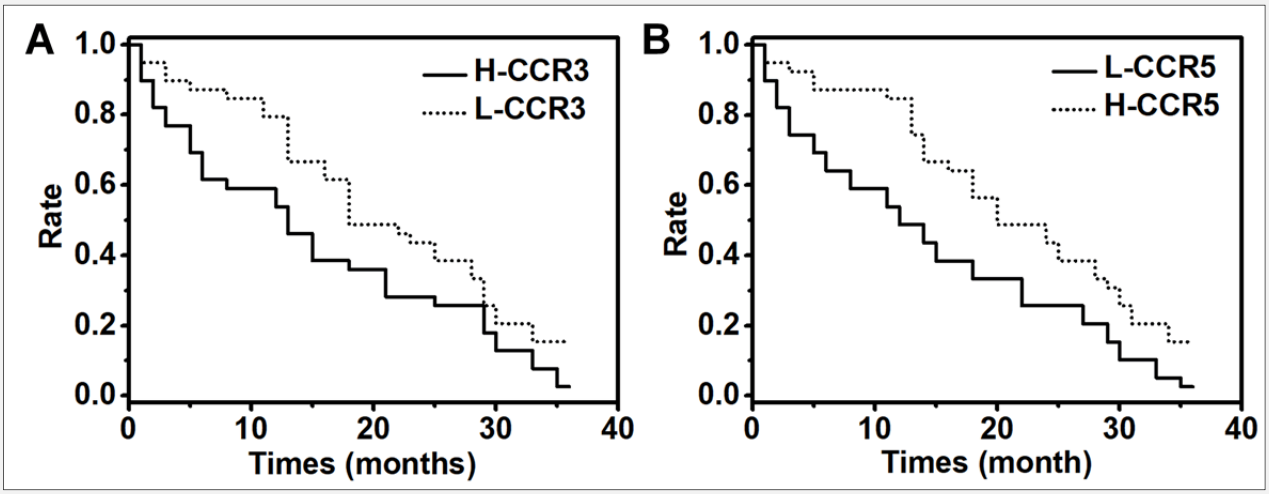

Figure 4. Kaplan-Meier analysis of overall survival according to CCR3 (A) and CCR5 (B) expression in patients with primary immune thrombocytopenia.

performed to compare the low and high level of CCR3 and CCR5 subgroups. The results presented in Figure 4 showed that the median therapy time of primary immune thrombocytopenia patients with high CCR5 expression is 21 months, which was significantly longer than patients with low CCR5 expression (14 months, $p < 0.05$), and the median therapy time of primary immune thrombocytopenia patients with high CCR3 expression is 15 months, which was significantly shorter than patients with low CCR3 expression (20 months, $p < 0.05$). These results suggested that patients with high CCR5 expression or low CCR3 expression had a longer therapeutic time of primary immune thrombocytopenia.

DISCUSSION

Primary immune thrombocytopenia is an autoimmune disease with multiple factors. At present, primary immune thrombocytopenia patients have a good remission rate and it rarely leads to death. The forecast of the remission time and degree of remission are still uncertain, which often affects the quality of life of patients and families [29,30]. Studies on the pathogenesis of primary immune thrombocytopenia have shown a Th l/Th 2 imbalance disorder in primary immune thrombocytopenia patients with high expression of Th l related chemokine receptor (CCR5 and CXCR 3) and low expression of Th 2 related chemokine receptor (CCR3 and CCR 4) [31,32]. Th l related chemokine receptor and Th 2 related chemokine receptor could act as a suitable biomarker for detection and prognosis evaluation on primary immune thrombocytopenia to help treatment. In the present study, we explored the diagnosis and prognosis value of CCR3 and CCR5 in patients with primary immune thrombocytopenia. We found that downregulated CCR3 and upregulated CCR5 was significantly correlated with bleeding times and platelet counts at diagnosis. Diagnostically, our study showed that CCR3 and CCR5 could discriminate patients with primary immune thrombocytopenia patients from healthy subjects. Prognosis, overexpression of CCR5, and low expression of CCR3 lead to poor clinical benefits and indicated poor prognosis of primary immune thrombocytopenia. Therefore, CCR3 and CCR5 were good diagnosis and prognosis markers in primary immune thrombocytopenia.

The spleen was the main site of platelet destruction in primary immune thrombocytopenia patients [33]. Many studies point out that the positive expression rate of CCR5 was 100% in primary immune thrombocytopenia patients [18,34]. Our study found the positive expression rate of CCR5 in primary immune thrombocytopenia patients was significantly higher than that in healthy subjects. However, we found that the expression of CCR3 was significantly decreased in primary immune thrombocytopenia patients. These data were consistent with previous reports. Accurate diagnosis and prognosis evaluation were beneficial to treatment of primary immune thrombocytopenia patients. We further found that the ectopic expression of CCR3 and CCR5 was correlated with bleeding times and platelet counts at diagnosis. Based on these research results, we speculated that

CCR3 and CCR5 may be ideal biomarkers for the diagnosis of primary immune thrombocytopenia. Therefore, ROC curve analysis was performed and revealed that CCR3 and CCR5 had higher resolution for detection of primary immune thrombocytopenia. This is conducive to early diagnosis and treatment of primary immune thrombocytopenia. The change of type and quantity in chemokine receptor is the key factor to regulate the polarization and activation of Th 1 and Th 2 cells. It can also affect the distribution, type, and quantity of T cells in spleen tissue and peripheral blood, leading to Th1 and Th 2 cells being predominant in reactive immune diseases. Hence, the abnormal expression of CCR3 and CCR5 may affect the prognosis of primary immune thrombocytopenia. Multivariate analysis confirmed CCR3 and CCR5 expression as independent prognostic factors for primary immune thrombocytopenia patients, suggesting that CCR3 and CCR5 have prognostic significance for primary immune thrombocytopenia patients treated by prednisone and/or gamma globulin. We also explore the relationship between the expression level of CCR3 and CCR5 and therapy curative effect. Our study showed that overexpression of CCR5 and low expression of CCR3 lead to poor clinical benefits. Furthermore, high CCR5 expression and low CCR3 expression led to long therapy time to obtain objective response, also suggesting poor prognosis. Therefore, according to the expression level of CCR3 and CCR5, different treatment strategies are conducive to personalized treatment of primary immune thrombocytopenia. Although the exact etiology of primary immune thrombocytopenia is unclear, more and more data show that thrombopoiesis inhibition and platelet destruction are direct factors for thrombocytopenia. The disorder of Th cells companied by abnormal expression of CCR3 and CCR5 in primary immune thrombocytopenia patients leads to the production and secretion of immunoglobulin G from autoreactive B cells, which not only destroys platelets, but also inhibits the maturation of megakaryocytes and the release of platelets [35-38].

CONCLUSION

In summary, the current study showed that the level of CCR3 was downregulated and level of CCR5 was upregulated in peripheral blood mononuclear cells of primary immune thrombocytopenia patients, which were associated with bleeding times and platelet counts at diagnosis. More importantly, CCR3 and CCR5 could distinguish primary immune thrombocytopenia from healthy subjects and predict poor clinical benefits and prognosis. Based on these results, CCR3 and CCR5 in peripheral blood mononuclear cells might serve as a reliable biomarker for detection and prognostic evaluation of primary immune thrombocytopenia.

Declaration of Interest:
Both authors state that they have no conflicting interest.

References:

1. Newland A, Godeau B, Priego V, et al. Remission and platelet responses with romiplostim in primary immune thrombocytopenia: final results from a phase 2 study. Br J Haematol 2016;172: 262-73 (PMID: 26537623).
2. Park YH, Yi HG, Kim CS, et al. Clinical Outcome and Predictive Factors in the Response to Splenectomy in Elderly Patients with Primary Immune Thrombocytopenia: A Multicenter Retrospective Study. Acta Haematol 2016;135:162-71 (PMID: 26771656).
3. Flint SM, Gibson A, Lucas G, et al. A distinct plasmablast and naïve B-cell phenotype in primary immune thrombocytopenia. Haematologica 2016;101:698-706 (PMID: 26969086).
4. González-López TJ, Alvarez-Román MT, Pascual C, et al. Eltrombopag safety and efficacy for primary chronic immune thrombocytopenia in clinical practice. Eur J Haematol 2016;97: 297-302 (PMID: 26709028).
5. Efficace F, Mandelli F, Fazi P, et al. Health-related quality of life and burden of fatigue in patients with primary immune thrombocytopenia by phase of disease. Am J Hematol 2016;91:995-1001 (PMID: 27351715).
6. Flint SM, Gibson A, Lucas G, et al. A distinct plasmablast and naive B-cell phenotype in primary immune thrombocytopenia. Haematologica 2016;101:698-706 (PMID: 26969086).
7. Steurer M, Quittet P, Papadaki HA, et al. A large observational study of patients with primary immune thrombocytopenia receiving romiplostim in European clinical practice. Eur J Haematol 2017;98:112-20 (PMID: 27557853).
8. Ebbo M, Audonnet S, Grados A, et al. NK cell compartment in the peripheral blood and spleen in adult patients with primary immune thrombocytopenia. Clin Immunol 2017;177:18-28 (PMID: 26598010).
9. Lee JY, Lee JH, Lee H, et al. Epidemiology and management of primary immune thrombocytopenia: A nationwide population-based study in Korea. Thromb Res 2017;155:86-91 (PMID: 2852 5829).
10. Li H, Hao Y, Zhang D, et al. Numerical and functional defects in CD8(+) CD28(-) T-suppressor lymphocytes from patients with primary immune thrombocytopenia. Br J Haematol 2017;178: 292-301 (PMID: 28466476).
11. Petti LM, Marlatt SA, Luo Y, Scheideman EH, Shelar A, DiMaio D. Regulation of C-C chemokine receptor 5 (CCR5) stability by Lys-197 and by transmembrane protein aptamers that target it for lysosomal degradation. J Biol Chem 2018;293:8787-801 (PMID: 29678881).
12. Thompson M, Saag M, DeJesus E, et al. A 48-week randomized phase 2b study evaluating cenicriviroc versus efavirenz in treatment-naive HIV-infected adults with C-C chemokine receptor type 5-tropic virus. Aids 2016;30:869-78 (PMID: 26636929).
13. Sabbe R, Picchio GR, Pastore C, et al. Donor- and Ligand-Dependent Differences in C-C Chemokine Receptor 5 Reexpression. J Virol 2001;75:661-71 (PMID: 11134280).
14. Costa C, Traves SL, Tudhope SJ, et al. Enhanced monocyte migration to CXCR3 and CCR5 chemokines in COPD. Eur Respir J 2016;47:1093-102 (PMID: 26965295).
15. Hu Q, Huang X, Shattock RJ. C-C chemokine receptor type 5 (CCR5) utilization of transmitted and early founder human immunodeficiency virus type 1 envelopes and sensitivity to small-molecule CCR5 inhibitors. J Gen Virol 2010;91: 2965-73 (PMID: 20810746).

16. Dogo-Isonagie C, Lam S, Gustchina E, et al. Peptides from Second Extracellular Loop of C-C Chemokine Receptor Type 5 (CCR5) Inhibit Diverse Strains of HIV-1. J Biol Chem 2012;287: 15076-86 (PMID: 22403408).

17. Sax MJ, Plummer PN, Gasch C, et al. Endothelial C-C chemokine receptor type 5 (CCR5) is required for tumor angiogenesis. Clinical and Experimental Metastasis 2015;32:223 (URL: http://hdl.handle.net/10536/DRO/DU:30089802).

18. Kindberg E, Mickiene A, Ax C, et al. A Deletion in the Chemokine Receptor 5 (CCR5) Gene Is Associated with Tickborne Encephalitis. J Infect Dis 2008;197:266-9 (PMID: 18179389).

19. Stellato C, Brummet ME, Plitt JR, et al. Expression of the C-C chemokine receptor CCR3 in human airway epithelial cells. J Immunol 2001;166:1457-61 (PMID: 11160184).

20. Zhang L, Soares MP, Guan Y, et al. Functional expression and characterization of macaque C-C chemokine receptor 3 (CCR3) and generation of potent antagonistic anti-macaque CCR3 monoclonal antibodies. J Biol Chem 2002;277:33799-810 (PMID: 121 01185).

21. Lloyd CM, Delaney T, Nguyen T, et al. CC Chemokine Receptor (CCR)3/Eotaxin Is Followed by Ccr4/Monocyte-Derived Chemokine in Mediating Pulmonary T Helper Lymphocyte Type 2 Recruitment after Serial Antigen Challenge *in Vivo*. J Exp Med 2000;191:265-74 (PMID: 10637271).

22. Efremov R, Truong MJ, Darcissac EC, et al. Human chemokine receptors CCR5, CCR3 and CCR2B share common polarity motif in the first extracellular loop with other human G-protein coupled receptors implications for HIV-1 coreceptor function. Eur J Biochem 1999;263:746-56 (PMID: 10469138).

23. Nibbs RJ, Salcedo TW, Campbell JD, et al. C-C chemokine receptor 3 antagonism by the beta-chemokine macrophage inflammatory protein 4, a property strongly enhanced by an amino-terminal alanine-methionine swap. J Immunol 2000;164:1488-97 (PMID: 10640766).

24. Wang K[1], Shen HH, Li W, Huang HQ. Human C-C chemokine receptor 3 monoclonal antibody inhibits pulmonary inflammation in allergic mice. Acta Pharmacol Sin 2010;28:1791-6 (PMID: 179 59030).

25. Agic A, Djalali S, Wolfler MM, Halis G, Diedrich K, Hornung D. Combination of CCR1 mRNA, MCP1, and CA125 measurements in peripheral blood as a diagnostic test for endometriosis. Reprod Sci 2008;15:906-11 (PMID: 19050323).

26. De Clara E, Gourvest M, Ma H, et al. Long non-coding RNA expression profile in cytogenetically normal acute myeloid leukemia identifies a distinct signature and a new biomarker in NPM1-mutated patients. Haematologica 2017;102:1718-26 (PMID: 2867 9652).

27. Si HB, Zeng Y, Liu SY, et al. Intra-articular injection of microRNA-140 (miRNA-140) alleviates osteoarthritis (OA) progression by modulating extracellular matrix (ECM) homeostasis in rats. Osteoarthritis Cartilage 2017;25:1698-707 (PMID: 28647 469).

28. Ferretti M, Gattorno M, Chiocchetti A, et al. The 423Q polymorphism of the X-linked inhibitor of apoptosis gene influences monocyte function and is associated with periodic fever. Arthritis Rheum 2009;60:3476-84 (PMID: 19877056).

29. Lozano ML, Revilla N, Gonzalez-Lopez TJ, et al. Real-life management of primary immune thrombocytopenia (ITP) in adult patients and adherence to practice guidelines. Ann Hematol 2016; 95:1089-1098 (PMID: 27098812).

30. Cooper SA, Huang AA, Raskin RE, Weng HY, Scott-Moncrieff JC. Scottmoncrieff. Clinical data, clinicopathologic findings and outcome in dogs with amegakaryocytic thrombocytopenia and primary immune-mediated thrombocytopenia. J Small Anim Pract 2016;57:142-7 (PMID: 26799165).

31. Hong J, Bang SM, Mun YC, et al. Efficacy and Safety of a New 10% Intravenous Immunoglobulin Product in Patients with Primary Immune Thrombocytopenia (ITP). J Korean Med Sci 2018; 33:e142 (PMID: 29736158).

32. Wang S, Guan Y, Wang Y, et al. Reduced PTEN involved in primary immune thrombocytopenia via contributing to B cell hyperresponsiveness. Mol Immunol 2018;93: 144-51 (PMID: 29175 594).

33. Tsuda H, Tsuji T, Tsuji M, Yamasaki H. Life-threatening bleeding episodes in primary immune thrombocytopenia: a single-center retrospective study of 169 inpatients. Ann Hematol 2017;96: 1915-20 (PMID: 28808759).

34. Qu MM, Liu XN, Liu XG, et al. Cytokine changes in response to TPO receptor agonist treatment in primary immune thrombocytopenia. Cytokine 2017;92:110-7 (PMID: 28142109).

35. Mezaache S, Derumeaux H, Ferraro P, et al. Validation of an algorithm identifying incident primary immune thrombocytopenia in the French national health insurance database. Eur J Haematol 2017;99:344-9 (PMID: 28692146).

36. Lakhwani S, Perera M, Fernández-Fuertes F, et al. Thrombopoietin receptor agonist switch in adult primary immune thrombocytopenia (ITP) patients: A retrospective collaborative survey involving four spanish centers. Eur J Haematol 2017;99:372-7 (PMID: 28759125).

37. Mulder DJ, Justinich CJ. Understanding eosinophilic esophagitis: the cellular and molecular mechanisms of an emerging disease. Mucosal Immunol 2011;4:139-47 (PMID: 21228772).

38. Lee NJ, Choi DY, Song JK, et al. Deficiency of C-C chemokine receptor 5 suppresses tumor development via inactivation of NF-κB and inhibition of monocyte chemoattractant protein-1 in urethane-induced lung tumor model. Carcinogenesis 2012;33: 2520-8 (PMID: 22907530).