Association between blood counts-related parameters and disease activity in patients with rheumatoid arthritis

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Abstract

Background: Erythrocytes and platelets have been demonstrated to play a critical role in inflammatory processes. However, little is known about the diagnostic value of these indices in RA patients. The aim of this study was to evaluate the clinical significance of blood counts-related parameters such as counts of red blood cells (RBCs) and platelets (PLTs), hemoglobin (Hb), red blood cells-platelet ratio (RPR) and hemoglobin-platelet ratio (HPR) in rheumatoid arthritis (RA) and their association with disease activity. Methods: Clinical and laboratory data from 178 RA patients and 164 healthy controls were collected and analyzed. RA patients were divided into inactive group and active group according to disease activity score in 28 joints based on C-reactive protein (DAS28-CRP). The relationship between blood RBC, Hb, PLT, RPR and HPR and DAS28-CRP was detected by Spearman correlation method. Receiver operating characteristic (ROC) curve was used to assess the diagnostic value of these parameters. The predictive role of these indices for RA disease activity was evaluated by logistical regression analysis. Results: Active RA patients exhibited lower levels of blood RBC counts, Hb, HCT, RPR and HPR but significantly higher level of PLT counts compared with those in inactive groups ($P < 0.01$). Spearman analysis showed that blood RBC counts, HCT, RPR and HPR were negatively but PLT counts were positively related with DAS28-CRP ($P < 0.001$) in RA. ROC curve analysis revealed that the AUC of RBC and Hb was higher than that of ESR, RF and CCP for distinguishing active RA from inactive group. Logistical regression analyses showed that PLT is an independent predictor for RA disease activity. Conclusion: Blood RBC counts, Hb, RPR and HPR were negatively but PLT counts were positively related with RA disease activity. Blood PLT may act as a novel inflammatory factor for predicting disease activity in RA.

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Abstract

Background: **Erythrocytes and platelets have been demonstrated to play a critical role in inflammatory processes. However, little is known about the diagnostic value of these indices in RA patients. The aim of this study was to evaluate the clinical significance of blood counts-related parameters such as counts of red blood cells (RBCs) and platelets (PLTs), hemoglobin (Hb), red blood cells-platelet ratio (RPR) and hemoglobin-platelet ratio (HPR) in rheumatoid arthritis (RA) and their association with disease activity.**

Methods: **Clinical and laboratory data from 178 RA patients and 164 healthy controls were collected and analyzed. RA patients were divided into inactive group and active group according to disease activity score in 28 joints based on C-reactive protein (DAS28-CRP). The relationship between blood RBC, Hb, PLT, RPR and HPR and DAS28-CRP was detected by Spearman correlation method. Receiver operating characteristic (ROC) curve was used to assess the diagnostic value of these parameters. The predictive role of these indices for RA disease activity was evaluated by logistical regression analysis.**

Results: **Active RA patients exhibited lower levels of blood RBC counts, Hb, HCT, RPR and HPR but significantly higher level of PLT counts compared with those in inactive groups ($P < 0.01$). Spearman analysis showed that blood RBC counts, HCT, RPR and HPR were negatively but PLT counts were positively related with DAS28-CRP ($P < 0.001$) in RA. ROC curve analysis revealed that the AUC of RBC and Hb was higher than that of ESR, RF and CCP for distinguishing active RA from inactive group. Logistical regression analyses showed that PLT is an independent predictor for RA disease activity.**

Conclusion: **Blood RBC counts, Hb, RPR and HPR were negatively but PLT counts were positively related with RA disease activity. Blood PLT may act as a novel inflammatory factor for predicting disease activity in RA.**

Keywords: Rheumatoid arthritis, Red blood cells, Hemoglobin, Platelets

What's known

Erythrocytes (RBC) and platelets (PLT) are involved in inflammatory processes. The level of blood hemoglobin (Hb) is decreased in rheumatoid arthritis (RA).

What's new

Blood RBC counts, RBC to PLT ratio (RPR) and HB to PLT ratio (HPR) were negatively but PLT counts are positively correlated with RA disease activity. Blood PLT may act as a novel predictor for RA disease activity.

Background

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial hyperplasia, pannus formation, and progressive damage of articular cartilage and bone[1]. RA patients are challenged to physical disabilities and a significant economic burden with disease progression. Previous studies revealed that early diagnosis and treatment could prevent bone destruction and joint deformities caused by RA, and thus contribute to a greater rate of remission in RA patient[2]. Therefore early diagnosis and treatment are vital in improving the prognosis of RA.

Currently, the 2010 American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) classification criteria for RA has been widely used for diagnosis in clinical practice. The 2010 ACR/EULAR RA classification criteria place more emphasis on circulating anti-cyclic citrullinated peptide (CCP), rheumatoid factor (RF) and other serological biomarkers, in comparison with the 1987 ARA

classification criteria[3]. It was reported that CCP rules are not useful in identifying a proportion of early unclassified RA patients [4]. Both CCP and RF are members of the autoantibodies family. CCP has high specificity but relatively low sensitivity for RA while RF is strongly sensitive but lack specificity for RA patients [5]. Therefore, there is a need for establishing new serological biomarkers to realize the early diagnosis of RA.

Recent data indicate that anemia could occur in the setting of autoimmune diseases in that bone marrow function and iron metabolism could be influenced by inflammation[6]. It is reported that inflammatory cytokines may suppress erythrocyte maturation [7, 8]. Therefore, it has been suggested that erythrocyte related parameters such as red blood cell distribution width (RDW) and hemoglobin contents may be used as inflammatory indicators for predicting the severity of several autoimmune diseases including systemic lupus erythematosus[9, 10], primary Sjögren's syndrome[11], and autoimmune hepatitis[12]. Recently accelerating studies have demonstrated the critical role of platelets in inflammatory processes. For instance, platelets could participate in the regulation of leukocyte recruitment by releasing numerous inflammatory mediators[13]. P-selectin and several adhesion molecules expressed on platelets could contribute to the interaction between platelets and other leukocytes such as neutrophils, monocytes, T cells and so on[14, 15]. However, little is known about the diagnostic value of peripheral blood erythrocytes and platelets related indices in RA patients. And there are few studies that have assessed the association of red cells to platelet ratio (RPR) and hemoglobin to platelet ratio (HPR) with the disease activity of RA patients. Therefore, this study aimed to investigate the diagnostic role of hematological indices including RBC, Hb, PLT, RPR and HPR in RA patients. We also evaluated the correlation of these indicators with RA disease activity.

Method

Study population

This study included one hundred and seventy-eight RA patients who were admitted to the Department of Rheumatology and Immunology of the Second Affiliated Hospital of Xi'an Jiaotong University during the period from September 2017 to April 2020. Patients fulfilled the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA [16]. Patients who had hematologic diseases, other autoimmune inflammatory diseases, infections, malignancies, or had any history of other chronic diseases such as diabetes mellitus, dyslipidemia, thyroid dysfunction, severe liver or kidney impairment as well as those receiving corticosteroids treatment within the last 3 months were excluded. One hundred and sixty-four healthy individuals were recruited from the health examination center of the same hospital, and matched with RA patients for age and gender. The study was approved by the Research Committee of Human Investigation of Xi'an Jiaotong University Health Science Center and the written informed consent was given by all subjects. All methods were performed in accordance with the relevant guidelines and regulations.

Assessment of disease activity

Using the Disease Activity Score in 28 joints based on C-reactive protein (DAS 28-CRP)[17, 18], disease activity of RA patients can be described as low (DAS 28-CRP [?] 2.7), moderate (2.7 $<$ DAS 28-CRP [?] 4.1) or high (DAS 28-CRP $>$ 4.1), respectively. We define patients with moderate and high disease activity as active RA, whereas those with low disease activity were defined as inactive RA.

Clinical and laboratory parameters

Patients' characteristics, including age, gender, medical history, symptoms and signs, diagnosis, treatment, laboratory testing results were gathered from their electronic medical records. The laboratory testing results included white blood cells (WBCs), lymphocytes, neutrophils, monocytes, red blood cells (RBCs), platelets (PLT), hemoglobin (Hb), red blood cell specific volume (HCT), red blood cell distribution width (RDW), platelet distribution width (PDW), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP). Red blood cells- platelet ratio (RPR) and hemoglobin- platelet ratio (HPR) was calculated.

Statistical analysis

Continuous variables with the normal distribution were presented as mean values +- standard deviation. Non-normally distributed data were presented as median (interquartile range). Categorical variables were expressed as frequencies or percentages. The differences of continuous variables were compared by The Student's t-test or Mann–Whitney U test, while the chi-square test was performed to compare the differences of categorical variables. Spearman's correlation analysis was used to detect the association between variables. Receiver operating characteristic (ROC) curves were plotted to distinguish RA patients from healthy individuals or to differentiate active RA from inactive group. The area under the curve (AUC) and 95% confidence interval (CI) were calculated to evaluate the diagnostic value of each parameter. The optimal cut-off value, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy (AC) of the indices were assessed. Binary logistical regression model was applied to explore the predictor of RA disease activity (inactive group VS. active group). After univariate regression analysis, variables with $P < 0.15$ were included in the further forward stepwise regression analysis. Statistical significance was considered as a two-tailed P value less than 0.05. All statistical analysis was performed by SPSS software (version 16.0, Chicago, IL, USA).

Results

Study population

Clinical and laboratory characteristics of RA patients and healthy controls were shown in Table 1. There were no differences in age and gender distribution, body mass index (BMI) and white blood cells (WBC) between the two groups. Besides, CRP, ESR, RF and CCP in RA patients were significantly higher than that in the control group ($P < 0.001$). Table 2 showed clinical and laboratory characteristics of RA patients. Age, gender, BMI, disease duration, CCP and WBC were similar between the active RA and inactive RA groups. The active RA patients had higher levels of ESR, CRP and RF compared with inactive RA groups ($P < 0.001$ or $P < 0.01$). The greater levels of Ig G, Ig A and Ig M were also observed in active RA groups as compared to that in inactive RA groups ($P < 0.05$).

Blood counts of RBC, Hb, PLT and levels of other related parameters

As shown in Table 1, blood counts of RBC as well as levels of Hb, HCT, RPR and HPR were decreased while PLT counts were elevated in RA groups compared with healthy controls ($P < 0.001$). There were no significant differences in RDW and PDW between the two groups ($P > 0.05$).

Patients with active RA showed lower levels of RBC counts, HCT, RPR and HPR but higher levels of PLT counts than that with inactive groups ($P < 0.001$ or $P < 0.01$, Table 2). No significant differences in RDW and PDW were observed between the active RA and inactive RA groups ($P > 0.05$, Table 2).

In order to evaluate the diagnostic performance of blood RBC, Hb, PLT, RPR and HPR, we further used ROC curve analysis. As shown in Table 4, the AUC of RBC, Hb, PLT, RPR and HPR was 0.785, 0.787, 0.648, 0.747 and 0.738 respectively (all $P < 0.001$) for RA patients versus healthy controls. There were significant differences in the AUC between RBC and PLT as well as between Hb and PLT for distinguishing RA patients from healthy controls ($P < 0.05$ or $P < 0.01$, Fig. 1). And no significant difference was observed in the AUC between RBC and Hb for distinguishing RA patients from healthy controls ($P > 0.05$, Fig. 1).

Table 5 showed the ROC curve analysis for the active and inactive group of RA patients. The AUC of RBC, Hb, PLT, RPR and HPR was 0.780, 0.786, 0.666, 0.744 and 0.727 for active RA versus inactive RA (all $P < 0.001$). The optimal cutoff values of RBC, Hb, PLT, RPR and HPR between active RA and inactive RA groups were 4.22×10^{12}/L, 121 g/L, 243×10^{9}/L, 0.48 and 0.017. As shown in Fig. 2, RBC and Hb had a higher AUC relative to ESR, RF and CCP in distinguishing active RA patients from inactive group ($P < 0.01$ or $P < 0.001$). There was no significant difference in the AUC among PLT, ESR, CRP, RF and CCP for distinguishing active RA patients from inactive group ($P > 0.05$).

Correlation of blood counts-related markers with disease activity and laboratory parameters

To further evaluate the relationship between blood counts-related markers and disease activity, we conducted a correlation analysis between blood levels of these markers and disease activity index DAS-28 CRP and laboratory parameters including CRP and ESR.

As shown in Table 3, RBC was negatively associated with DAS-28 CRP (r = -0.428, P < 0.001), CRP (r = -0.289, P < 0.001) and ESR (r = -0.481, P < 0.001). Hb was negatively associated with DAS-28 CRP (r = -0.489, P < 0.001), CRP (r = -0.341, P < 0.001) and ESR (r = -0.569, P < 0.001). PLT was positively associated with DAS-28 CRP (r = 0.327, P < 0.001), CPR (r = 0.284, P < 0.001) and ESR (r = 0.331, P < 0.001). RPR was negatively associated with DAS-28 CRP (r = -0.310, P < 0.001), CRP (r = -0.397, P < 0.001) and ESR (r = -0.329, P < 0.001). HPR was negatively associated with DAS-28 CRP (r = -0.293, P < 0.001), CRP (r = -0.402, P < 0.001) and ESR (r = -0.362, P < 0.001).

Notably, the correlation between Hb and disease activity parameters such as ESR and DAS-28 CRP was the strongest.

Binary logistic regression analysis of factors independently associated with disease activity in RA patients

In addition, we performed logistic regression analysis to assess the associated factors with RA disease activity. After univariate regression analysis, PLT, ESR and Hb were used for further multivariate regression analysis (P < 0.20). Results showed that only PLT is an independent predictor for RA disease activity (OR =2.348, 95% CI: (1.145-4.816), P < 0.05) (Table 6).

Discussion

In this retrospective study of one hundred and seventy-eight patients, we investigated the diagnostic value of RBC, Hb, PLT, RPR and HPR in a cohort of Chinese patients with RA. Moreover, we confirmed, for the first time, that PLT can serve as an indicator for the activity of RA patients.

The inflammatory milieu in rheumatoid arthritis has been demonstrated to modulate erythropoiesis. There are two main reasons responsible for inflammation-induced anemia in RA. Firstly, erythropoietin (EPO) gene transcription could be suppressed by proflammatory cytokines such as IL-1, IL-6 and TNF-α [19, 20]. Secondly, these proflammatory cytokines could inhibit the effect of EPO on erythroid progenitors in bone marrow [21]. RA patients with early stage were reported to present anemia caused by IL-6 suppression of erythropoiesis in bone marrow [22]. In addition, RBCs in RA patients have been shown to be affected by the presence of generated free radicals and excessive amounts of proteins circulating in the blood [23]. Notably, accelerating evidence revealed that RBC-derived microparticles (RMPs) could directly contribute to the pathogenesis of RA through their regulation on immune cell activation [24]. In our study, the Hb concentration was decreased in RA patients as compared to the health controls, and the level of Hb concentration was lower in active RA patients than that in the inactive group. A finding similar to ours was observed in the study by Padjen et al. [25] who identified that changes in Hb levels were strongly associated with disease activity of RA patients. Our results demonstrated that blood Hb concentration was negatively correlated with the indices of RA disease activity such as DAS 28-CRP, CRP and ESR.

Recently platelets have been demonstrated to be involved in the inflammatory process of RA [26]. The number of platelets was reported to be significantly higher in RA patients with high disease activity compared to that with low to moderate disease activity [27]. Platelet numbers were also negatively correlated with the levels of albumin and Hb. Moreover, a previous study revealed that mean platelet volume was significantly associated with RA disease activity [28]. Platelet derived growth factor was indicated to be participated in the invasion of synovial membranes and angiogenesis that are the characteristics of RA [29, 30]. Platelets could function as delivering vehicles carrying major amounts of cytokines, chemokines, and growth factors which were important to sustain autoimmune pathways [9, 31]. Notably, a study from Knijff-Dutmer *et al.* showed that platelet counts were similar in the three groups including active RA, inactive RA and healthy controls [32]. However, the levels of platelet-derived microparticles (PMPs) in RA patients were evidently higher than those in healthy controls. PMPs were also correlated with disease activity of RA [32]. Our results

showed that there were significant difference in the number of blood platelets between RA patients and the healthy controls. Moreover, in our study, high level of blood platelets was identified to be an independent predictor for RA disease activity by the regression analysis. Considering the significance of PMPs in RA inflammation, we will investigate the role of PMPs in RA patients in our further study.

The interaction between RBCs and platelets bears a helpful understanding of RA pathophysiology, as it has been shown that RMPs were capable of inducing platelet hyperstimulation following collagen activation in an*in vitro* study [33]. These RMPs were also confirmed to induce *ex vivo* platelet–platelet aggregates. Moreover, some adhesion proteins have been identified to be involved in the direct contact between platelets and RBCs. For example, as a family member of glycoproteins, adhesion molecule 4 (ICAM-4 or CD242) on RBC membranes could directly bind to the platelet's integrin αIIbβ3, illustrating the direct effect of RBC on the activation of thrombotic and inflammatory pathways [11, 34]. Knowing that interaction between RBCs and platelets plays a role in the pathogenesis of chronic inflammation of RA, we thus evaluated the diagnostic value of RPR and HPR as novel markers in RA. Our results showed that RPR and HPR were significantly related to the severity of patients with RA.

However, there were several limitations in this study. Firstly, this study was a retrospective analysis of the data on RA patients, and selection bias cannot be eliminated completely. Secondly, this study included only 178 patients with RA which were from a single center. Therefore, a multi-center prospective study with a large-scale sample is still required to confirm the accuracy of the results.

In summary, this study systematically investigated the role of RBC, Hb, PLT, RPR and HPR as biomarkers in determining the disease activity of RA. We found that RBC, Hb, RPR and HPR was decreased in RA patients and negatively correlated with RA disease activity. Moreover, PLT was found to be elevated in RA patients and positively correlated with RA disease activity. High PLT was revealed as an independent predictor for the disease activity in RA patients.

Abbreviations

RA, rheumatoid arthritis; DAS28-CRP, Disease Activity Score in 28 joints based on C-reactive protein; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; CCP, anti-cyclic citrullinated peptide; Ig G: immunoglobulin G; Ig A: immunoglobulin A; Ig M: immunoglobulin M; WBC, white blood cells; RBC, red blood cells; Hb: hemoglobin; HCT: Red blood cell specific volume; RDW: red blood cell volume distribution width; PLT: platelet; PDW: platelet volume distribution width; RPR: red blood cells-platelet ratio; HPR: hemoglobin-platelet ratio.

Authors' contributions

LX, ML and YG designed the experiment strategy and wrote the manuscript. LT, HFS, YPZ, and ZSL assessed patients and collect data. YW, NL WL and HM performed the experiments. HZ, LJ and TZ conducted statistical analyses and interpreted the data. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to data protection policies, but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Research Committee of Human Investigation of Xi'an Jiaotong University Health Science Center and the written informed consent was obtained by all subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figure legend

Fig. 1 Performance of blood RBC, Hb, and PLT in discriminating RA patients from healthy controls. (A) The ROC curves of the three markers in differentiating RA patients from healthy controls. (B) Comparison of ROC curves among the three markers. ROC, receiver operating characteristic curve; RBC, red blood cells; Hb: hemoglobin; PLT: platelet.

Fig. 2 Performance of blood RBC, Hb, and PLT in discriminating active RA patients from inactive RA patients. Comparison of ROC curves between RBC (A), Hb (B) or PLT (C) and other parameters including ESR, CRP, RF and CCP in differentiating RA patients from healthy controls. ROC, receiver operating characteristic curve; RBC, red blood cells; Hb: hemoglobin; PLT: platelet; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; CCP, anti-cyclic citrullinated peptide antibody.

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