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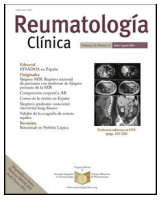

Original Article

Immature platelet fraction in rheumatoid arthritis with interstitial lung disease

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ABSTRACT

Background: Platelets have an effect on the hemostatic defense of the lung. Immature platelet fractions (iPF) reflects the number of young platelets containing ribonucleic acid in the circulation and real-time production. Information about their roles in rheumatic diseases is limited and there are no studies on iPF in RA with interstitial lung disease (ILD). Our aim is to investigate the association between the iPF level and occurrence of ILD in RA and the correlation of iPF with disease activity in general or only in RA with ILD.
Methods: The study included 50 RA patients without ILD, 33 RA patients with ILD, and 30 healthy controls. Demographic data, Disease Activity Score 28 (DAS28), autoantibodies, and iPF were evaluated. ILD was diagnosed by using high-resolution computed tomography with clinical findings and chest X-ray. The samples were analyzed for complete blood count with platelet indices included, on Mindray BC-6800 hematology analyzer, Hamburg, Germany.
Results: iPF levels were higher in RA patients with ILD compared to healthy controls and RA patients without ILD. A weakly positive correlation between DAS28 with iPF was found in all RA patients. iPF levels were found as 2.85 to detect ILD with 66.7% sensitivity and 65% specificity.
Conclusions: Our results showed that the iPF was detected higher in RA with ILD compared to RA without ILD. iPF, a routine cheap and easy test during hemogram, can provide important information in terms of disease activity and lung involvement in RA.

Fracción plaqueta inmadura en artritis reumatoide con enfermedad pulmonar intersticial

Palabras clave:
Artritis reumatoide
Enfermedad pulmonar intersticial
Fracción de plaquetas inmaduras

RESUMEN

Antecedentes: Las plaquetas tienen un efecto sobre la defensa hemostática del pulmón. Las fracciones de plaquetas inmaduras (FPi) reflejan el número de plaquetas jóvenes que contienen ácido ribonucleico en la circulación y la producción en tiempo real. La información sobre su papel en las enfermedades reumáticas es limitada y no existen estudios sobre la FPi en la AR con enfermedad pulmonar intersticial (EPI). Nuestro objetivo es investigar la asociación entre el nivel de FPi y la aparición de EPI en la AR y la correlación de la FPi con la actividad de la enfermedad en general o solo en la AR con EPI.
Métodos: El estudio incluyó a 50 pacientes con AR sin EPI, 33 pacientes con AR con EPI y 30 controles sanos. Se evaluaron los datos demográficos, la puntuación de actividad de la enfermedad 28 (DAS28), los autoanticuerpos y la FPi. La EPI se diagnosticó mediante tomografía computarizada de alta resolución, con hallazgos clínicos y radiografía de tórax. Las muestras fueron analizadas para un recuento sanguíneo completo con índices de plaquetas incluidos, en el analizador de hematología Mindray BC-6800, Hamburgo, Alemania.

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Resultados: Los niveles de FPi fueron más altos en los pacientes con AR con EPI en comparación con los controles sanos y los pacientes con AR sin EPI. Se encontró una correlación débilmente positiva entre DAS28 y FPi en todos los pacientes con AR. Se encontró que los niveles de FPi eran de 2,85 para detectar ILD con una sensibilidad del 66,7% y una especificidad del 65%.
Conclusiones: Nuestros resultados mostraron que la FPi se detectó más en AR con EPI en comparación con AR sin EPI. La FPi, una prueba de rutina barata y fácil durante el hemograma, puede proporcionar información importante en términos de la actividad de la enfermedad y la afectación pulmonar en la AR.

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune systemic disease that may lead to many organ involvements and result in disability, morbidity, and mortality.[1] Functional limitation, positive rheumatoid factor (RF) and/or anti-cyclic citrullinated peptides antibodies (anti-CCP), presence of erosions, high disease activity, and extraarticular disease such as lung involvement are important factors associated with poor prognosis.[2] The clinical findings of pulmonary involvement in RA vary between asymptomatic/subclinical inflammation to pulmonary fibrosis. The median survival was reported as 2.6 years after the diagnosis of interstitial lung disease (ILD) in RA patients.[3] However, RA with ILD may not be diagnosed due to the absence of significant symptoms in the early stages.[4] Therefore, it is important to clarify the pathogenesis of ILD, early diagnosis, and treatment.

The human leukocyte antigen-DRB1 alleles and environmental factors such as cigarettes have been found to be associated with ILD in RA.[5] In addition, the immune cells, antigen-specific T cells, and inflammatory cytokines play an important role in the pathogenesis of RA with ILD.[5] Macrophages are involved in the control of T cells and immune complexes and neutrophils contribute to synovitis with prostaglandin, protease, and reactive oxygen products.[6] It is thought that immunocomplex storage and complement activation are also effective in extra-articular involvement.[6] Platelets play an important role in the inflammation, autoimmunity, and also in the pathogenesis of rheumatoid synovitis and associated cardiovascular events in RA.[7–10] Nevertheless, the studies have generally focused on mean platelet volume (MPV) as a potential biomarker in autoimmune rheumatic disorders.[9]

Reticulated platelets are immature, younger platelets with a half-life of less than a day, and contain a small amount of ribonucleic acid for protein synthesis.[11,12] The immature platelet fraction (iPF) reflects the number of reticulated platelets.[11,12] The benefits of immature platelets measured by automated hematology analyzers in many clinical conditions have been reported.[12] Studies on reticulated platelets have been reported in patients with the acute coronary syndrome, uremia, and thrombocytopenia.[7,13,14] However, the information about the role of iPF in rheumatic diseases is insufficient. The role of iPF is an interesting subject to be investigated in inflammatory rheumatic diseases. In particular, according to our knowledge, there is no study on iPF in RA patients with pulmonary involvement.

In the study, the aim was to investigate: (a) the iPF levels in RA patients with or without ILD; (b) correlation of iPF with disease activity in general or only in RA with ILD.

Materials and methods

This was a descriptive, cross-sectional study performed in RA patients and healthy controls. All patients fulfilled the criteria of the 2010 American College of Rheumatology classification criteria for RA.[15] Patients who were under 18 years old, autoimmune diseases except for RA, hematologic diseases, patients with thrombocytopenia, using antiaggregant, malignancies, and pulmonary infections such as upper respiratory tract infection, tuberculosis, etc. were excluded from the study. The study protocol was approved by the Faculty of Medicine Ethics Committee and designed consistent with the Declaration of Helsinki (approval number 2020/10775).

Age, gender, clinical characteristics such as disease duration, smoking status, medication, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF, anti-CCP, and platelet counts were collected from medical records, retrospectively. In addition, the following parameters about platelets were recorded: iPF, plateletcrit, MPV, and platelet distribution width (PDW). The samples were analyzed for complete blood count with platelet indices included, on Mindray BC-6800 hematology analyzer, Hamburg, Germany. Disease activity was assessed with Disease Activity Score 28-ESR (DAS28) including visual analog scale, ESR, the number of tender and swollen joints.[16] Chest X-ray was performed on RA patients with suspected ILD with clinical findings (cough, wheezing, bilateral pulmonary crackles, breathlessness, etc). Then, high-resolution computed tomography (HRCT) was performed for suspected ILD patients with chest X-ray. Patients with RA were included in the study as ILD group, if they had the reticular opacities, traction bronchiectasis, ground-glass opacities to honeycomb pattern on HRCT.[17,18] ILD was diagnosed by using HRCT. Infections, idiopathic, lymphoid diseases, granulomatosis diseases (sarcoidosis, etc.), diffuse alveolar hemorrhage, vasculitis, exposure-related factors such as drugs/inhalational, and other rheumatic diseases except for RA were excluded for the ILD group.

The statistical analyses were carried out by Statistical Package for Social Sciences (SPSS) for Windows (SPSS version 20.0, IBM, USA). The data were expressed as mean ± standard deviation, median [25th–75th percentile], frequency (n), and percentage (%) according to the distribution of normality analyzed by the Kolmogorov–Smirnov test. Differences between groups were made by either independent-samples t-test, Mann–Whitney-U test, and Kruskal–Wallis test according to the normality distribution. The chi-squared test was used for the analysis of categorical data and independence between variables. Correlation between parameters was assessed by means of Spearman's correlation analysis. The value of r 0–0.19 was accepted as very weak, 0.2–0.39 as weak, 0.4–0.59 as moderate, 0.6–0.79 as strong, and 0.80–1.0 as a very strong correlation. Receiver-operating characteristic (ROC) curve analysis was performed to distinguish RA patients with ILD from RA patients without ILD. The area under the curve (AUC), the cut-off value, sensitivity, and specificity were also analyzed. A p-value of less than 0.05 was considered statistically significant.

Results

Eighty-three RA patients and 30 healthy subjects who had no evidence of any rheumatic disease were enrolled in the study. No significant differences were found for age, gender, the smoking history between patients with RA and healthy controls. Disease duration and smoking history rates were similar in both RA

Table 1
Demographic characteristics and laboratory values of the rheumatoid arthritis patients with and without interstitial lung disease.

	Rheumatoid arthritis without ILD ($n=50$)	Rheumatoid arthritis with ILD ($n=33$)	p-value
Age[*] (years)	57.6 ± 10.7	62.1 ± 8.9	NS
Gender (male/female)	10/40	12/21	NS
Disease duration, months	31.1 ± 12.1	34.9 ± 7.3	NS
Smoking history, n (%)	4 (12)	7 (14)	NS
Steroid use, n (%)	46 (92)	30 (90.9)	NS
DMARDs, n (%)			
Hydroxychloroquine	38 (76)	26 (78.8)	NS
Sulfasalazine	8 (16)	4 (12.1)	NS
Methotrexate	45 (90)	6 (18.2)	<0.001
Leflunomide	7 (14)	25 (75.8)	<0.001
RF titers[**] (IU/mL)	63.0 [7–60.6]	211.2 [7–303.2]	0.003
Anti-CCP titers[**] (U/mL)	44.0 [0.5–68.6]	83.0 [1.6–170.3]	0.01
ESR[*] (mm/h)	33.5 ± 15.9	46.7 ± 20.1	0.002
CRP[**] (mg/L)	3.2 (2.0–10.7)	21.9 [8.9–36.3]	<0.001

ILD: interstitial lung disease, DMARDs: disease-modifying anti-rheumatic drugs, RF: rheumatoid factor.
Anti-CCP: anti-cyclic citrullinated peptide, ESR: erythrocyte sedimentation rate.
[*] Mean ± standard deviation.
[**] Median [25th–75th percentile].

groups. Leflunomide use was higher in RA patients with ILD, and methotrexate use was higher in RA patients without ILD ($p<0.001$). There were no significant differences in the use of steroid, hydroxychloroquine, and sulfasalazine in both groups. The demographic and laboratory features of RA patients with or without ILD are shown in Table 1.

The data of the platelet parameters obtained from peripheral blood were analyzed in all patients with RA included in the study. The MPV, plateletcrit, PDW, and platelet levels were higher in RA patients with ILD compared to RA patients without ILD ($p>0.05$). iPF was significantly higher in RA patients with ILD compared to RA patients without ILD ($p=0.02$) and healthy controls ($p=0.01$). The distribution of data about peripheral blood platelet parameters in patients with rheumatoid arthritis is shown in Table 2.

In RA patients with ILD, iPF was moderately correlated with MPV ($r=0.589$, $p<0.001$). And, DAS28-ESR was weakly correlated with iPF ($r=0.396$, $p<0.001$) in all RA patients with or without ILD. In addition, a significant correlation was found between the DAS28-ESR and iPF in both groups ($r=0.508$, $p=0.03$ with ILD; $r=0.332$, $p=0.01$ without ILD). There were no significant differences for iPF between genders and we found no correlation between iPF and age. ROC analysis was used as a general performance measure to differentiate between the two RA groups. The AUC is 0.65 (CI for 95%: 0.53–0.77). The iPF percentage was 2.85 to detect ILD of RA with 66.7% sensitivity and 65% specificity.

Discussion

The topic of the article is actually due to insufficient data regarding the platelet indices in RA with organ involvement. This study was conducted to assess RA patients with a level of iPF and its relationship to the presence of ILD. In this study, the iPF levels were higher in RA patients with ILD compared to healthy controls and RA patients without ILD. Also, the relationship of iPF levels with disease activity was evaluated. A weakly positive correlation between DAS28-ESR with iPF was found in all RA patients. We conclude that patients with high disease activity have higher iPF levels in RA. In addition, iPF levels were found as 2.85 to detect ILD with 66.7% sensitivity and 65% specificity.

It has been reported that platelets receive less attention than they deserve regarding their role and relationship in RA pathogenesis.[19] Platelet-related markers such as platelet factor 4, soluble P-selectin, CD40 ligand/CD40 complex, and particularly MPV, have been evaluated in inflammatory rheumatic diseases.[20] Many studies reported the reticulated platelet/iPF as a useful marker for bone marrow megakaryopoiesis activity and the cause of thrombocytopenia.[12] But, the information about the role of iPF in autoimmune rheumatic diseases is insufficient and an interesting subject worth investigating. Considering that lung involvement in RA is a poor prognostic factor, it is important to determine ILD at an early stage.[18] Our hypothesis is that iPF can be a helpful marker in this regard since the pulmonary regions are an important region in platelet production and platelet biogenesis.[21] Therefore, we evaluated iPF in RA patients with and without ILD, in our study.

RP values differ depending on the techniques used for measurement.[13] iPF levels are usually low in thrombocytopenic patients with suppressed bone marrow activity.[9] In cases with platelet destruction such as immune thrombocytopenic purpura and disseminated intravascular coagulation, iPF levels are high.[9] We found higher iPF, MPV, PDW, and platelet levels in RA patients with ILD compared to RA patients without ILD. There were no significant differences were for MPV, PDW, and platelet levels between groups. Although the data on the subject are limited; Koh et al. concluded that reticulated platelets could not be used in the differential diagnosis of thrombocytopenia due to small differences between groups.[22] On the contrary; there is also a study reporting that reticulated platelet is a useful non-invasive test in differentiating between thrombocytopenia with low and high thrombopoietic activity.[13] We found a significant difference for iPF in RA patients between with ILD and without ILD. Also, it was found that iPF was significantly higher in RA patients compared to healthy controls.

The correlations between iPF and parameters of other platelet series vary among studies.[23,24] In a relatively small group investigating the correlation between reticulated platelet and MPV, no significant correlation was found.[23] In another study including a larger patient group, a significantly negative correlation was reported between reticulated platelet and MPV.[24] In addition, a significant correlation has been reported between reticulated platelet level and the age of those with normal platelet counts. We found a moderate correlation between iPF and MPV. But, there was no correlation between iPF and age. In terms of disease activity, high iPF levels were reported in non-complicated sepsis, and a correlation was found with disease severity scores.[25] In RA, the DAS28, indicating disease activity increases as part of active inflammation. In a study, platelet-derived microparticles levels correlated with DAS28 in RA patients.[26] In our study, a significant correlation was found between the DAS28-ESR and iPF in all RA patients with or without ILD.

Table 2
Distribution of data about peripheral blood platelet parameters in patients with rheumatoid arthritis.

	Interstitial lung disease (+) $n = 33$	Interstitial lung disease (−) $n = 50$	p-value
Platelet* ($\times 10^9$/L)	305.0 ± 99.8	296.0 ± 76.4	NS
Plateletcrit** (%)	0.3 [0.2–0.4]	0.3 [0.2–0.4]	NS
Mean platelet volume* (fL)	10.4 ± 1.6	9.9 ± 1.5	NS
Platelet distribution width* (%)	16.0 ± 0.3	15.9 ± 0.3	NS
IPF* (%)	3.8 ± 1.8	2.9 ± 1.2	0.02

IPF: immature platelet fraction, NS: not significant.
* Mean ± standard deviation.
** Median [25th–75th percentile].

It would be useful to determine the iPF cut-off value to indicate the presence of ILD in RA. To our knowledge, no cut-off value has been determined for inflammatory rheumatic diseases. A cut-off value of 11% was diagnostic for thrombocytopenia with increased thrombopoietic activity with a sensitivity of 93% and a specificity of 85%.[13] We found the iPF cut-off value of 2.85 (with 65% specificity and 66.7% sensitivity) to detect ILD in RA patients. However, the sensitivity and specificity would not enough for early detection of ILD involvement in RA. The limitations of the study were the retrospective design, the small sample size, and the lack of follow-up of long-term results and treatment outcomes. Also, we did not examine the parameters with flow cytometry.

In conclusion, the present study has demonstrated the relationship between the levels of iPF and ILD among RA patients. Our results showed that the MPV, mature platelet, and iPF were detected higher in RA with ILD compared to RA without ILD. And, the DAS28-ESR was positively correlated with iPF in RA patients with ILD. The study covers a clinically relevant aspect which is the possibility of early detection of ILD in patients with RA, something for which, at this time, there are no adequate tools available, with a very simple determination for its application in daily clinical practice. iPF, a routine cheap and easy test during hemogram, can provide important information in terms of disease activity and organ involvement in RA patients.

Ethical statement

The study protocol was approved by the Faculty of Medicine Ethics Committee and designed consistent with the Declaration of Helsinki (approval number 2020/10775).

Conflict of interest

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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