Mean Platelet Volume and Neutrophil Lymphocyte Ratio as Related to Inflammation Markers and Anti-CCP in Rheumatoid Arthritis

Mittleres Thrombozytenvolumen und Neutrophilen-Lymphozyten-Ratio in Bezug zu Entzündungsmarkern und Anti-CCP bei rheumatoider Arthritis

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Key words
- rheumatoid arthritis
- mean platelet volume
- neutrophil-to-lymphocyte ratio
- inflammation

Schlüsselwörter
- Rheumatoide Arthritis
- Mittlere Plättchenvolumen
- Neutrophilen-to-Lymphozyten-Verhältnis
- Entzündung

Abstract

Background: Various thrombocyte markers and white blood cell levels and their subtypes have recently been investigated in association with inflammation. The purpose of this study was to determine the correlation of mean platelet volume (MPV) and neutrophil/lymphocyte ratio (NLR) with disease activation and clinical parameters in rheumatoid arthritis (RA) patients.

Methods: 84 RA patients and 60 healthy controls were included. Platelet, MPV, white cell, neutrophil and lymphocyte levels in full blood counts were investigated, and NLR was calculated. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), disease activation score (DAS 28) and a health assessment questionnaire (HAQ) were used in the evaluation of RA.

Results: In the present study a total of 144 patients was enrolled, 84 with RA and 60 healthy individuals. 75.2% (n=108) were women and 24.8% (n=36) were men. The patients with RA had lower MPV than control individuals (MPV; 8.52±1.15 fL and 8.92±0.87 fL, respectively) and CRP (r: −0.234, p=0.005). RA patients' mean NLR was significantly higher than that of the control group (2.74±1.74 and 1.80±0.78, respectively; p<0.001). Furthermore, anti-CCP positive patients had higher NLR than anti-CCP negative patients (NLR; 2.51±1.92 and 1.95±1.22, p=0.019 respectively). NLR was positively correlated with ESR (r=0.190, p=0.023), CRP (r=0.230, p=0.035) and anti-CCP (r=0.300, p=0.005).

Conclusion: In conclusion, MPV and NLR together with acute phase reactants can be a useful index for showing inflammation in RA patients.

Zusammenfassung

Einleitung: Verschiedene thrombozytäre Marker und Grenzwerte von Leukozyten und ihren Subtypen sind in der jüngeren Vergangenheit bezüglich ihres Zusammenhangs mit Entzündung untersucht worden. Ziel dieser Untersuchung war die Korrelation des mittleren Thrombozytenvolumens (MPV) und der Neutrophilen/Lymphozyten Ratio (NLR) mit der Krankheitsaktivität und klinischen Parametern bei Patienten mit rheumatoider Arthritis (RA).

Methoden: 84 RA Patienten und 60 gesunde Kontrollen wurden eingeschlossen. Aus dem Blutbild wurden Thrombozyten, MPV, Leukozyten-, Neutrophilen- und Lymphozytenwerte untersucht und die NLR errechnet. Die Erythrozytensedimentationsrate (ESR), C-reaktives Protein, Rheumafaktor (RF), anti-zyklisches citrullinieres Peptid (anti-CCP), der Krankheitsaktivität-Score DAS 28 und ein Gesundheitsbewertungsbogen (HAQ) wurden für die Bewertung der RA herangezogen.

Ergebnis: In die vorliegende Studie wurden 144 Patienten eingeschlossen, 84 mit RA und 60 gesunde Kontrollen. 75,2% (n=108) waren Frauen und 24,8% (n=36) waren Männer. Patienten mit RA hatten ein niedrigeres MPV als die Kontrollen (MPV; 8,52±1,15 fL bzw. 8,92±0,87 fL) und CRP (r: −0,234, p=0,005). Die mittlere NLR von RA Patienten war signifikant höher als die der Kontrollgruppe (2,74±1,74 bzw. 1,80±0,78; p<0,001). Außerdem hatten anti-CCP positive Patienten höhere NLRs als anti-CCP negative Patienten (NLR; 2,51±1,92 bzw. 1,95±1,22 p=0,019). Die NLR korrelierte positiv mit der ESR (r=0,190, p=0,023), dem CRP (r=0,230, p=0,035) und dem anti-CCP (r=0,300, p=0,005).

Schlussfolgerung: MPV und NLR können gemeinsam mit Akutphase-Proteinen ein hilfreicher Index für die Entzündungsaktivität bei RA Patienten sein.

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Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory and multisystemic disease involving the joints and characterized by deformities. It has a global prevalence of 0.5-1 % [1]. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) of laboratory parameters are used in assessment of disease activity. Particularly CRP is closely correlated with the degree of disease activation [2]. Recent studies have claimed that mean platelet volume (MPV) and neutrophil-lymphocyte (NLR) ratio are associated with inflammatory disease. However, previous reports demonstrating a relationship between MPV and RA are conflicting.

Mean platelet volume: Recent advances in clinical laboratory techniques have opened a new horizon in understanding the role of platelets in thrombosis, inflammation and angiogenesis [3–5]. Experimental and clinical studies have shown that platelet specific agents are an important element of the inflammatory response in inflammatory diseases such as RA and systemic lupus erythematosus (SLE) [6–8]. Of these, MPV shows the level of production of bone marrow platelets and is used as a marker of the severity of inflammation and platelet activation [9].

Many authors have argued that NLR can be considered to be a new inflammation marker. Changes occur in the circulating levels of white blood cells as a response to systemic inflammation. The best known of these is the relative lymphopenia accompanying neutrophilia [10]. In studies in recent years, NLR was thought to be a potential marker for showing inflammation and for prognosis in both rheumatic and non-rheumatic diseases [11–13]. There is no consensus from studies performed with MPV in RA patients, and according to our knowledge, there are no studies concerning NLR in patients with RA. The aim of our study was to determine the level of MPV and NLR in RA patients as routinely measured in complete blood counts. In addition, we aimed to evaluate the relationship of MPV and NLR with laboratory and clinical parameters.

Methods

Sample selection

84 patients with RA and 60 control subjects were enrolled in the study. All patients gave a consent form and this study was approved by the local ethical committee. Patients were asked about age, gender, duration of disease, cigarette and alcohol use status, presence of accompanying chronic diseases, morning stiffness in the joints and duration thereof, family history of rheumatic disease and drugs currently being used by the patient. Laboratory and clinical parameters, such as ESR, CRP, Health Assessment Questionnaire (HAQ) score and Disease Activity Score (DAS) were used in assessing disease activation. Platelet, white cell, MPV, neutrophils and lymphocytes were determined using an automated blood cell counter by a Beckman Coulter LH 780 Hematology Analyzer (Beckman Coulter Ireland Inc Mervue, Galway, Ireland). The NLR was noted as a ratio between the neutrophil and lymphocyte counts. Patients with acute or chronic infections, diabetes, hypertension, acute or chronic kidney failure, chronic hepatic disease, a history of allergic diseases or any malign disease that might affect MPV and NLR were excluded.

Measurement-assessment tools employed

Disease Activity Score (DAS 28): This score was used in evaluation disease severity in RA patients as a result of inspection of 28 joints. Total score is calculated by this formula:

DAS28 = (0.56 × number of tender joints X ½) + (0.28 × number of swollen joints X ½) + (0.7 × ESH) + (0.014 × global assessment by patient [VAS-mm]) [14].

Health Assessment Questionnaire (HAQ): HAQ is assessed with 20 questions. The questionnaire asks into dressing, arising, eating, walking, and hygiene, understanding and daily tasks. Every answer is scored between 0 (good) and 3 (bad). The HAQ score reflects functional status and has been demonstrated to be correlated with disease activity markers [15].

Statistical analysis

Analysis of data was performed with SPSS 15.0. Normal distribution of data was examined using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive variables were expressed as mean, standard deviation, median, minimum, maximum, frequency and percentage values. Variables normally distributed between groups were analyzed using the independent groups t test and non-normally distributed variables using the Mann-Whitney U test. The independent groups t test was also used to compare patient and control group mean MPVs. The non-parametric Mann-Whitney U test was used for the comparison of NLRs and platelet values. Correlation between variables in the case group was analyzed using Spearman's correlation test. $P < 0.05$ was regarded as statistically significant.

Results

In the present study we enrolled a total of 144 patients, with 84 RA and 60 healthy individuals. 75.2 % (n = 108) were women and 24.8 % (n = 36) were men. The mean age was 54.5 ± 11.49 and 51.2 ± 8.60 years, respectively, in the control and RA groups. Age and gender rates did not differ significantly between the control and RA groups (respectively, p = 0.15 and p = 0.69). Mean disease duration of RA patients was 7.47 ± 6.44 years (median = 5, min-max = 0.08–30). 69 % of patients had a DAS-28 score > 3.2. History of drug use of patients: 91.7 % of patients were taking Disease Modifying Anti-Rheumatic Drugs (DMARD). Only methotrexate (10–15 mg/week) frequency is 23.8 % (n: 20), methotrexate (10–15 mg/week) + sulfasalazine (2000 mg/day) are 29.8 % (n: 25), methotrexate (10–15 mg/week) + hydroxychloroquine (200–400 mg/day) are 21.4 % (n: 18), methotrexate (10–15 mg/week) + sulfasalazine (2000 mg/day) + hydroxychloroquine (200–400 mg/day) are 16.7 % (n: 14) and methotrexate (10–15 mg/week) + anti-tumor necrosis factor-alpha are 8.3 % (n: 7).

We analysed full blood counts of the 2 groups. Platelet count of patient (Plt; $277.74 \pm 89.92 \times 10^3/\mu L$) and control groups (Plt; $247.30 \pm 41.55 \times 10^3/\mu L$) were comparable (p = 0.08). The patients with RA had a lower MPV than the control individuals (MPV; 8.52 ± 1.15 fl and 8.92 ± 0.87 fl, respectively). There was a significant difference between the 2 groups (p = 0.020) (**Fig. 1**). The baseline demographic and biochemical parameters of the 2 groups are presented in **Table 1**. We performed a correlation analysis of the relationship between MPV and clinical parame-

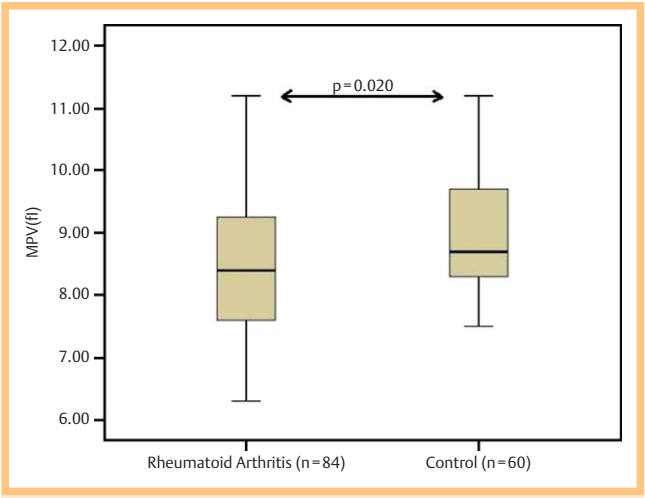

Fig. 1 MPV values in the rheumatoid arthritis and control groups.

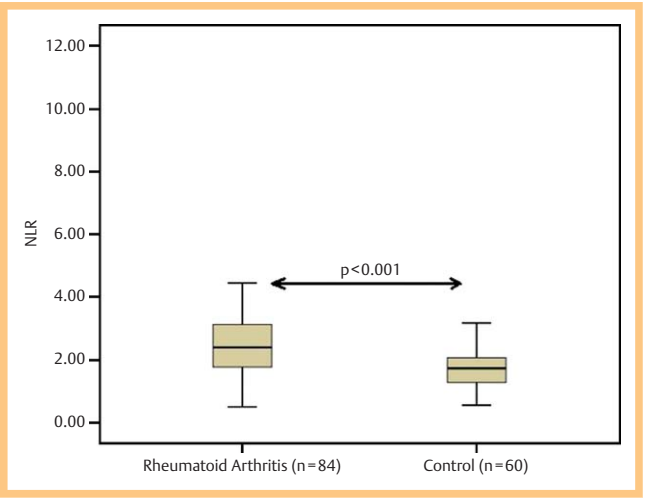

Fig. 2 NLR values in the rheumatoid arthritis and control groups.

Table 1 Comparison of RA patient and control group clinical and laboratory data.

Variables	Patient (n: 84)	Control (n: 60)	p
age (years)	54.5±11.49	51.2±8.60	0.157**
female (%)	64 (76.2%)	44 (73.3%)	*0.696*
male (%)	20 (23.8%)	16 (26.7%)	
ESR (mm/h)	32.4±20.41	10.66±7.76	**<0.001***
CRP (mg/dl)	1.5±2.03	0.27±0.24	**<0.001***
RF (IU/ml)	140.12±299.75	–	–
Anti-CCP (IU/ml)	98.15±92.02	–	–
anti-CCP (positive/ negative)	54 (%64.3)/30 (%35.7)		
DAS 28	3.83±1.21	–	–
HAQ	0.94±0.90	–	–
WBC (×10^3/μL)	7.58±2.46	7.45±1.46	0.715*
neutrophil (×10^3/μL)	4.86±2.05	3.91±0.89	**0.004***
lymphocyte (×10^3/μL)	2.13±1.32	2.39±0.65	**<0.001***
platelet (×10^3/μL)	277.74±89.92	247.30±41.55	0.082*
MPV (fl)	8.52±1.15	8.92±0.87	**0.020****
NLR	2.74±1.74	1.80±0.78	**<0.001***

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Anti-CCP: anti-cyclic citrullinated peptide; RF: rheumatoid factor; DAS-28: disease activity score; HAQ: health assessment questionnaire; WBC: white blood cell; MPV: mean platelet volume; NLR: neutrophil/lymphocyte ratio
*Mann-Whitney U test, **Independent Samples T test

ters. Significant negative correlations were observed between MPV and CRP (r=−0.234, p=0.005). On the other hand, DAS-28, disease duration and ESR were not correlated with MPV (p>0.05).

From the full blood counts of the 2 groups we also found that the white blood cell count of patient (WBC; 7.58±2.46×10^3/μL) and control groups (WBC: 7.45±1.46) were comparable (p=0.7). The patients with RA had higher neutrophil and lower lymphocyte counts than the control individuals [neutrophil (4.86±2.05 vs. 3.91±0.89, respectively p=0.004) and lymphocyte (2.13±1.32 vs. 2.39±0.65, respectively, p<0.001]. The patients with RA had higher NLR than the control individuals (mean NLR, 2.74±1.74 and 1.80±0.78, respectively, p<0.001) (**Fig. 2**). There was a significant difference between the 2 groups (p<0.001). Furthermore, anti-CCP positive patients had a higher NLR than the anti-CCP negative patients (NLR; 2.51±1.92 and 1.95±1.22, respectively, p=0.019). We performed a correlation analysis of the relationship between NLR and clinical parameters. Significant positive correlations were observed between NLR and ESR (r=0.190, p=0.023), CRP (r=0.230, p=0.035) and anti-CCP titer (r=0.300, p=0.005). On the other hand, DAS-28 was not correlated with NLR (p>0.05)

Discussion

In the present study, we found lower MPV and higher NLR in patients with RA. Another important finding was that the NLR was higher in anti-CCP positive patients than in anti-CCP negative patients. There was a negative correlation between MPV and CRP. However, there was a positive correlation between NLR and ESH, CRP, and anti-CCP titer.

In various clinical studies in recent years, many diseases characterized by inflammation have been found to have a relationship with platelet activation [16]. MPV is a marker obtained from routine blood count and shows the level of platelet production in bone marrow. The most recent studies have shown that MPV is a marker of thrombocyte function and activation. It can also be used in the determination of disease activity in some diseases [17]. A number of studies showed MPV levels to be connected to rheumatologic diseases. Kisacik et al. demonstrated MPV levels to be significantly decreased in RA patients. Researchers have also determined that MPV is negatively correlated with disease activity [18]. At the same time, recent studies claimed that MPV levels changed with treatment. Gasparyan et al. found MPV values in RA patients were statistically increased after treatment. In contrast to, Yazıcı et al., showed that MPV were significantly higher in untreated patients with RA and decrease significantly upon treatment [19]. We observed a significant negative correlation between MPV and CRP. MPV could be helpful as an inflammation marker and in monitoring anti-inflammatory treatment in RA patients [20]. We found that the MPV values of patients were significantly lower than those of the control group. Therefore, we think that MPV may be related to inflammation in RA patients. In addition, since most of the patients in the present study received DMARDs, it could be possible that the observed decreased MPV was also related to treatment.

Another simple and easy method to use for detection of inflammation is the NLR. Several studies have recognized white blood cell count and subtype count as markers of inflammation in

some rheumatic diseases [10]. In the literature there are also a few studies showing a relationship between NLR and inflammatory rheumatic diseases [12, 13]. Ahsen et al. studied 68 familial mediterranean fever (FMF) patients and discovered a significantly increased NLR compared to healthy subjects. In this study they also observed a significant positive correlation between NLR and CRP [12]. An additional study in patients with FMF showed a significant increase in the NLR. The NLR in patients with amyloidosis was found to be increased and that it was higher during attacks. The results of this study indicated that NLR may be a marker of rising inflammation during attacks and may be a useful marker for predicting the development of amyloidosis [13]. We found that the NLR values of patients were significantly higher than those of the control group. In our study we also observed a significant positive correlation between NLR and ESR, CRP, and anti-CCP titer. We propose NLR as a test for detecting inflammation in RA patients. However, correlation coefficients were found to be lower than expected. To demonstrate more clearly relationship between NLR and anti-CCP. We believe that the foundation of our work future studies with large case.

Our study has shown that anti-CCP positive patients had a significantly higher NLR. Anti-CCP is an important marker in determining the prognosis of rheumatoid arthritis and is associated with the development of disease activity and bone erosion [21]. A possible conclusion from this result might be that high NLR levels in patients with RA is important in predicting prognosis of disease and may lead to the development of more joint damage. These results provide further support for the hypothesis that NLR may be considered to be an indicator of inflammation, disease activity and disease prognosis, being higher in patients with RA.

In conclusion, to the best of our knowledge, this is the first study to evaluate NLR in RA patients. The present study was designed to determine the levels of MPV and NLR in rheumatic diseases. Our study has shown decreased MPV and elevated NLR in RA. One of the more significant findings to emerge from this study is that MPV negatively correlated with CRP, and NLR positive correlated with ESR, CRP and anti-CCP. Another important finding was that anti-CCP positive patients had a higher NLR. Therefore, similar to MPV, NLR is a simple index of inflammation.

Conflict of interest: There are no conflicts of interest.

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