Original Article

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Internal Medicine Section

Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratio in Adult-Onset Still Disease, their Relationship with Baseline Disease Activity and Subsequent Disease Course: A Retrospective Cohort Study

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ABSTRACT

Introduction: Adult-onset Still Disease (AoSD) is a rare systemic polygenic non-familial autoinflammatory disease of unknown aetiology. The long-term course of the disease can be categorised in three different definitions including self-limited course, intermittent course or chronic course. Recently, Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) were investigated in various rheumatic diseases as an informative markers in evaluating severity of inflammation and disease activity.

Aim: To explore association between baseline NLR, PLR, disease activity score and subsequent disease course in patients with AoSD.

Materials and Methods: This retrospective cohort study enrolled 61 patients with AoSD and 61 age-matched patients with Fibromyalgia Syndrome (FMS). Pouchot score was specifically used in AoSD patients to assess disease activity based on symptoms, physical examination findings and laboratory results from April 2020 to July 2020. Patients with AoSD were subgrouped into three groups: self-limited; intermittent; and chronic course. The association of NLR and PLR with disease activity score was analysed between groups by using independent samples t-test, Mann-Whitney U test and Kruskal Wallis Variance Analysis. Differences between categorical variables were analysed using Chi-square test. A $p \leq 0.05$ was considered statistically significant.

Results: The mean follow-up of the 61 patients with AoSD was 74 months (range, 14-169). Eighteen patients (29.5%) had a self-limited disease course, nine patients (14.8%) an intermittent disease course and 34 (55.7%) a chronic disease course. AoSD patients had significantly higher serum NLR, PLR and lower Mean Platelet Volume (MPV) values than FMS patients {6.68 (1.67-19.7), 1.83 (1.1-4) p=0.0001; 187 (82.9-549), 114 (72-246) p=0.0001; 8.3 (6.4-11.3), 9.3 (7.7-11.7) p=0.0001, respectively}. NLR, PLR and Pouchot score were similar among AoSD subgroups, which were grouped according to disease pattern. The majority of patients in the self-limited and chronic course groups had higher baseline Pouchot score without statistical significance. The NLR and C-Reactive Protein (CRP) were significantly higher in AoSD patients with active disease than inactive disease {7.02 (1.8-19.7), 4.17 (1.67-14.8) p=0.06; 13 (1.9-29.5), 9 (1.6-20.9) p=0.046, respectively)}.

Conclusion: High NLR and elevated CRP levels are related to active disease in AoSD patients. Although NLR, PLR and Pouchot score were similar among subgroups, patients with a chronic course or self-limited course had higher NLR values and more active disease at diagnosis compared with patients with an intermittent course.

Keywords: Autoinflammation, Biomarkers, C-reactive protein, Pouchot score

INTRODUCTION

The AoSD is a rare systemic polygenic non-familial autoinflammatory disease of unknown aetiology that was first defined by Eric Bywaters in 1970 [1]. The long-term course of the disease can be categorised in three different definitions as: (i) self-limited course, defined as a single episode followed by sustained remission throughout the whole follow-up period; (ii) intermittent course, with recurrent systemic flares with remission between flares; (iii) chronic course, at least one episode of persistent symptoms lasting longer than one year [2]. Currently, it is known that the chronic disease course generally develops more disability and has a worse prognosis than other patterns. Lee SW et al., showed that the adjusted level of ferritin had a significant predictive value for progression to chronic disease in AoSD patients [3].

NLR and PLR are an informative markers in evaluating severity of inflammation and disease activity in various rheumatic diseases [4]. These can be easily calculated through complete blood count, defined as the proportion of absolute neutrophil count to lymphocytes and the proportion of absolute platelet count to lymphocytes, respectively. Recently, the Systemic Immune Inflammation Index (SII) combined with ferritin has been suggested as the most effective assessment score for AoSD (SII: platelet count×neutrophil/lymphocyte) count at diagnosis [5]. In addition, in another study, although elevated serum proinflammatory cytokines had limited use to discriminate patients with active AoSD from those with bacterial sepsis, Modified Pouchot activity score had a higher predictive value to confirm the diagnosis of AoSD. A modified Pouchot score ≥4 shows a sensitivity of 92% and a specificity of 93% for distinguishing active and non-active AoSD [6].

There is no well-defined biomarker for monitoring prognosis and disease activity in AoSD patients. Multisystemic involvement and various patterns of disease course in these patients are quite heterogeneous. The ability to predict disease course and early management of AoSD may contribute to the prevention of permanent damage in chronic disease. In this study the authors investigated the predictive role of baseline NLR, PLR and Pouchot score for distinguishing AoSD patients according to their clinical disease course.

MATERIALS AND METHODS

This retrospective cohort study enrolled 61 patients with AoSD and 61 age and sex-matched patients with FMS. All of the AoSD patients were diagnosed initially in the Rheumatology Outpatient clinic at Pamukkale University School of Medicine from 2010 to 2018 based on Yamaguchi diagnostic criteria [7]. These criteria require the presence of in total five features, at least two of which must be major for the diagnosis of AoSD after excluding other mimicker diseases such as malignancy, infectious and inflammatory diseases. Arthralgia, fever, typical rash and leukocytosis are defined as major criteria whereas sore throat, presence of lymphadenopathy and/or splenomegaly, abnormal liver function tests and absence of rheumatoid factor and Anti-Nuclear Antibody (ANA) are minor criteria.

The data of all AoSD and FMS patients who were on regular follow-up between January 2010 and December 2018, in the tertiary health centre was collected in this study. The analysis of the collected data was done between April and July 2020. This study was performed after local Ethics Committee approval of the Pamukkale University Faculty of Medicine (date/no: 03/2020-06) and in accordance with the Helsinki declaration. Informed consent form was obtained from each patient.

Inclusion criteria: AoSD diagnosis fulfilling the Yamaguchi criteria [7] or FMS diagnosis according to the current classification criteria [8] and having regular follow-up for at least one year for both patient groups.

Exclusion criteria: It includes other inflammatory, infectious or malignant disease, using anti-inflammatory drugs at diagnosis, onset of the disease before 18-year-old and insufficient medical record for both patient groups.

NLR and PLR were calculated based on baseline laboratory values at the diagnosis before initiation of corticosteroid treatment to prevent the effects of glucocorticoids on blood leukocyte count and distribution. Pouchot score was specifically used in AoSD patients to assess disease activity based on symptoms, physical examination findings and laboratory results. Total score varied between 0 and 12, with each of the clinical manifestations being assigned as 1 point: typical rash, sore throat, fever, leukocytosis ≥15,000, hepatomegaly, splenomegaly, lymphadenopathy, myalgia, abdominal pain, pneumonia, pleuritis and pericarditis [9].

Patients were categorised in three different clinical courses as self-limited, intermittent and chronic courses at follow-up [2]. If there was a single attack, it was defined as a self-limited course. If there was more than one attack with relapses, this was evaluated as intermittent course. Persistent active disease and joint symptoms longer than one year were accepted as chronic course. In this context, remission was accepted as no disease activity according to the global assessment of the physician and laboratory parameters [10].

Primary outcome was the association between NLR, PLR, Pouchot score and the three different disease courses in AoSD patients. Secondary outcome was the relationship between Pouchot score and inflammatory markers in AoSD patients.

STATISTICAL ANALYSIS

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) 25.0 software. Kolmogorov Smirnov and Shapiro Wilk tests were used for determination of normal distribution. Continuous variables were defined as the mean±standard deviation and median (minimum-maximum values), and categorical variables were defined by number and percentages. For independent group comparisons, we used independent samples t-test when parametric test assumptions were met and Mann-Whitney U test and Kruskal Wallis Variance Analysis were used when parametric test assumptions were not met. Differences between categorical variables were analysed using Chi-square test. A p-value ≤0.05 was considered statistically significant.

RESULTS

The majority of patients were female in both groups (57.4% (n=35) of patients with AoSD vs 72.1% (n=44) of patients with FMS) [Table/Fig-1]. The median age was 39 years in AoSD patients and 43 years in FMS patients (p=0.666) [Table/Fig-1].

Variables	AoSD (n=61)	FMS (n=61)	p-value
Female (n, %)	35 (57.4%)	44 (72.1%)	0.088 (χ^2=2.909)*
Age (in years)	39 (19 - 73)	43 (19 - 65)	0.666 (z=-0.433)**
NLR	6.68 (1.67-19.7)	1.83 (1.1-4)	0.0001 (z=-8.803)**
PLR	187 (82.9-549)	114 (72-246)	0.0001 (z=-6.701)**
MPV (cubic µm)	8.3 (6.4-11.3)	9.3 (7.7-11.7)	0.0001 (z=-5.009)**
ESR (mm/hour)	83 (3-150)	11 (2-39)	0.0001 (z=-8.421)**
CRP (mg/L)	12 (1.6-29.5)	0.9 (0.14-8.5)	0.0001 (z=-8.409)**

[Table/Fig-1]: Comparison of patient characteristics in the whole study group including AoSD and FMS patients.
*: Chi-square test was used, **: All variables were analysed with Mann-Whitney U test due to non-parametric data and noted as median (minimum-maximum).
NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; MPV: Mean platelet volume; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; AoSD: Adult-onset still disease; FMS: Fibromyalgia syndrome

The mean follow-up of the 61 patients with AoSD was 74 months (range, 14-169). Eighteen patients (29.5%) had a self-limited disease course, nine patients (14.8%) an intermittent disease course and 34 (55.7%) a chronic disease course. The baseline Pouchot score was ≥4 in 72.1% (n=44) of AoSD patients [Table/Fig-2].

Variables	n (%)
Gender	
Female	35 (57.4)
Male	26 (42.6)
Disease courses	
Self-limited	18 (29.5)
Intermittent	9 (14.8)
Chronic	34 (55.7)
Pouchot score	
<4 (inactive)	17 (27.9)
≥4 (active)	44 (72.1)

[Table/Fig-2]: Characteristic features of patients with AoSD.

AoSD patients had significantly higher serum NLR, PLR and lower mean platelet volume (MPV) values than FMS patients {6.68 (1.67-19.7), 1.83 (1.1-4) p=0.0001; 187 (82.9-549), 114 (72-246) p=0.0001; 8.3 (6.4-11.3), 9.3 (7.7-11.7) p=0.0001, respectively} [Table/Fig-1]. ESR and CRP were also at higher titers than in FMS [Table/Fig-1]. Baseline NLR, PLR and Pouchot score were similar between three subgroups according to the disease course [Table/Fig-3]. There was no statistical difference in terms of age and gender in these three subgroups (p>0.05 vs p>0.05, respectively). The majority of patients in the self-limited and chronic course groups had higher baseline Pouchot score. The NLR and CRP were significantly higher in AoSD patients with active disease than inactive disease {7.02 (1.8-19.7), 4.17 (1.67-14.8) p=0.06; 13 (1.9-29.5), 9 (1.6-20.9) p=0.046, respectively} [Table/Fig-4,5]. PLR and ESR did not show a statistically significant difference between active and inactive AoSD patients [Table/Fig-5].

DISCUSSION

This study was designed to reveal any association between disease activity, baseline inflammatory markers and subsquent disease course in order to find a novel measure for prediction of disease course. The majority of patients in the self-limited and chronic course groups had higher baseline Pouchot score. The baseline NLR and CRP were higher in active than inactive AoSD patients with a statistical significance. Pouchot J et al., also showed that

Variables	Disease course	Mean±SD	Med (min-max)	p-value
NLR	Self-limited	7.4±3.36	6.75 (1.8-14.6)	0.271 (H=2.614)*
	Intermittent	5.95±4.78	4.17 (1.67-14.7)	
	Chronic	7.97±4.68	6.93 (2.18-19.7)	
PLR	Self-limited	195.22±63.4	194 (82.9-324)	0.848 (H=0.329)*
	Intermittent	202.9±105.6	161 (94.1-424)	
	Chronic	213.58±101.36	187 (91.6-549)	
Pouchot score	Self-limited	4.39±1.33	4 (2-7)	0.578 (H=1.079)*
	Intermittent	4±1.8	5 (1-6)	
	Chronic	4.68±1.3	5 (2-7)	

[Table/Fig-3]: Mean values of NLR, PLR and Pouchot scores in three subgroups (AoSD) according to the disease course.*: Kruskal Wallis Variance Analysis was used.
NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio

Pouchot score	Disease course				p-value
	Self-limited n (%)	Intermittent n (%)	Chronic n (%)	Total	
<4	5 (27.8)	4 (44.4)	8 (23.5)	17 (27.9)	0.484 (χ^2=1.453)*
≥4	13 (72.2)	5 (55.6)	26 (76.5)	44 (72.1)	

[Table/Fig-4]: Patient distribution by the disease activity scores and the clinical courses.
*: Chi-square test was used

Variables	Pouchot score in AoSD patients	Med (min-max)	p-value
NLR	<4	4.17 (1.67-14.8)	0.06 (z=-1.882)**
	≥4	7.02 (1.8-19.7)	
PLR	<4	199 (91.6-390)	0.557 (z=-0.587)**
	≥4	178.5 (82.9-549)	
ESR	<4	67±39.89	0.126 (t=-1.551)*
	≥4	83.34±35.72	
CRP	<4	9 (1.6-20.9)	0.046 (z=-1.995)**
	≥4	13 (1.9-29.5)	

[Table/Fig-5]: Comparison of variables between AoSD patients with active and inactive disease.
*: Independent samples t-test was used and the variable was shown as (mean±SD), **: All variables were noted as median (minimum-maximum) and were analysed with Mann-Whitney U test due to non-parametric data.
ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein

the most common course is a chronic pattern (36%), followed by self-limited (34%) and intermittent (24%) patterns among 62 AoSD patients with 70 months mean follow-up time [9].

In a large multicentre cohort including 356 patients with 22 months median follow-up, male gender, delayed diagnosis, arthritis involving wrist/elbow joints and failure to achieve remission with initial treatment were poor prognostic factors for developing chronic disease [11]. In addition, 76.5% of patients (n=26) who developed a chronic course had baseline active disease based on Pouchot score in this study. Also, the mean NLR and PLR were higher in the vast majority of the chronic course group, though not statistically signficant. These statistical inconsistencies may be due to a very limited patient size in each subgroup, especially in the intermittent group.

NLR and PLR have been investigated in many rheumatic diseases as predictors of disease activity. Pan L et al., observed a positive correlation between these inflammatory ratios and Kerr score in patients with Takayasu disease [12]. Two studies including AoSD patients had conflicting results. First, Seo JY et al., investigated NLR as a diagnostic marker and its predictive value for relapse of AoSD. A cut-off value of 3.08 showed the greatest sensitivity (91.7%), specificity (68.4%) and AUC value (0.967) as a diagnostic tool for AoSD [13]. In the second study, Zhang M et al., noted that although PLR was not different between groups, patients with sepsis showed significantly increased NLR compared to AoSD patients [14]. In this cohort, the NLR was significantly higher in active AoSD patients compared with inactive disease in a support of previous literature.

CRP has been defined as an useful marker for monitoring disease activity in AoSD patients due to the main role of Interleukin-6 (IL-6) in the pathogenesis of the disease [15]. In addition, elevated level of IL-18 was detected in active AoSD patients and correlated with disease activity [16]. IL-18 is a member of the IL-1 family and is also well-known to be involved in haematopoietic progenitor cell growth. The other discriminating factor of active and non-active. AoSD patients was elevated CRP titers in this study. IL-18 and IL-6 could lead to this inflammatory response, although the levels could not be analysed due to the retrospective nature of the study.

Platelet size is another useful predictive biomarker of inflammatory processes that may provide important information on the course and prognosis of a variety of pro-inflammatory diseases [17]. Liu JP et al., showed MPV to be at lower titers in AoSD patients than those with sepsis (mean MPV was 9.8±1.2 fL) [18]. Zhang M et al., also showed that a lower value of MPV may be a quantitative assessment tool for the diagnosis of AoSD and other Fever of Unknown Origin (FUO) [19]. Similar to previous reports, in this study the mean MPV values were lower in AoSD patients than FMS patients as a result of inflammation.

Limitation(s)

Due to the low incidence rate and being a single centre experience, the sample size was quite limited. Simultaneously, analysis of pro-inflammatory markers that may show disease activity such as IL-18 could not obtained due to retrospective nature of study.

CONCLUSION(S)

The majority of patients in the self-limited and chronic course groups had higher baseline Pouchot score. The baseline NLR and CRP were higher in active AoSD patients compared with inactive AoSD patients. These findings suggest that NLR may be a useful, easy and less costly index for active patients. Further well-designed prospective, randomised-controlled studies in larger homogeneous populations are required to define risk factors for accepted disease courses.

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