

# Neutrophil to Lymphocyte Ratio in Patients with Age Related Macular Degeneration Patients

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## Abstract

**Aim:** To compare the serum levels of neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) between patients with neovascular age-related macular degeneration (AMD) and healthy controls.

## Material and Methods

In this retrospective study, 56 patients with neovascular AMD and 54 healthy controls were included. Neovascular AMD was diagnosed following an ophthalmic examination, optical coherence tomography (OCT) and fluorescein angiography (FA). Blood neutrophil, lymphocyte, and platelet counts were recorded. The levels of NLR and PLR were calculated in two groups. Statistical analysis was carried out to find any association between the levels of NLR and PLR and neovascular AMD.

## Results

There were 56 patients (26 male [M], 30 females [F]) in the neovascular AMD group and 54 patients (25 M, 29 F) in the control group. No significant differences were found between patients

with neovascular AMD and the control subjects in terms of gender and age ( $P > 0.05$ ). The mean  $NLR \pm SD$  was  $2.4 \pm 0.7$  in the neovascular AMD group and  $1.8 \pm 0.4$  in the control group. NLR was significantly higher in patients with neovascular AMD compared to the control group ( $p < 0.001$ ). There was no significant difference between the two groups for PLR.

## Conclusion

NLR was significantly increased in patients with neovascular AMD compared to the control subjects suggesting a possible association of NLR with neovascular AMD.

**Keywords:** neovascular age-related macular degeneration, inflammation, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio.

## 1. Introduction

AMD is a chronic degenerative disease of the retina and one of the common reasons for blindness in developed countries [1]. Inflammation is an important component of various age-related diseases such as Alzheimer's disease and atherosclerosis [2]. In recent years, the blood neutrophil to lymphocyte ratio (NLR) has been identified as a potentially useful marker of clinical outcome in inflammatory diseases [2]. Several previous studies have shown that the NLR and platelet-to-lymphocyte ratio (PLR) are indicative of systemic inflammation [3,4]. There are many risk factors of AMD, such as aging, smoking, sunlight exposure, and specific gene mutations [5]. Recent studies have revealed that the role of inflammation in the pathogenesis of AMD is crucial [6,7]. Since inflammation has a role in AMD pathogenesis, a high NLR level may be a possible marker of neovascular AMD. In this study, we aimed to compare NLR and PLR values between patients with neovascular AMD and healthy subjects.

## 2. Materials and Methods

In this retrospective study, we included patients diagnosed with neovascular AMD and age-matched controls without any sign of AMD.

Institution review board approved the study. The study was conducted according to the Declaration of Helsinki. Patients underwent complete ophthalmologic examinations including best-corrected visual acuity (BCVA) with using Early Treatment Diabetic Retinopathy Study (ETDRS) charts, intraocular pressure (IOP) measurement, fundus examination, spectral-domain optical coherence tomography (SD-OCT, Heidelberg Spectralis, Heidelberg Engineering, Heidelberg, Germany) and fundus fluorescein angiography scanning.

The inclusion criteria were set as follows: the existence of leakage demonstrative of the choroidal neovascular membrane (CNVM) or macular edema according to fundus fluorescein angiography; the presence of subretinal fluid, cystic maculopathy or central macular thickness (CMT) of at least  $250 \mu\text{m}$ , as detected by OCT.

Patients and controls were excluded from the study if they had active systemic or ocular infection, chronic inflammatory disease or autoimmune disease, chronic ophthalmic or systemic disease (diabetes mellitus, hypertension etc..) ocular trauma or if they had undergone previous eye surgery (other than uneventful cataract surgery). Current smokers were also excluded. Subjects with the best-corrected visual acuity of were included in the control group. Patients with early cataracts whose macula can be

evaluated were not excluded. Venous blood samples were drawn into ethylene diamine tetraacetic acid (EDTA) tubes. The following combined indexes were evaluated:  $NLR = \frac{\text{neutrophils}}{\text{lymphocyte}}$ ,  $PLR = \frac{\text{platelet}}{\text{lymphocyte}}$  ratio.

## 2.1. Biochemical and Hematological Analyses

The hematological measurements were obtained by using an automated blood cell counter (Beckman-Coulter LH 780 Analyzer, Miami, Florida, USA). White blood cell (WBC), neutrophil, lymphocyte, monocyte, hemoglobin, platelet, and NLR levels were recorded. The following reference values were determined: for WBC:  $4-10 \times 10^3/\text{mm}^3$ , neutrophil:  $1.5-7 \times 10^3/\text{mm}^3$ , lymphocyte:  $1-3.7 \times 10^3/\text{mm}^3$ , monocyte:  $0-0.7 \times 10^3/\text{mm}^3$  and platelet:  $160-450 \times 10^3/\text{mm}^3$ .

## 2.2. Statistical Analysis

SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) 18.0 program was used for statistical analysis. Continuous variables were presented as mean $\pm$ SD. Values and categorical variables were expressed as a percentage. When the mean between the groups was compared, the normality of data was tested by the Shapiro-Wilks tests. Data comparison was performed with independent sample t-test.  $p < 0.05$  was accepted as statistically significant. Spearman correlation analysis was performed to evaluate the correlation between NLR values BCVA and CMT.

## 3. Results

There were 56 patients (26 male [M], 30 female [F]) in the neovascular AMD group and 54 patients (25 M, 29 F) in the control group ( $p > 0.05$ ). The mean age was  $71.72 \pm 7.4$  years in neovascular AMD group,  $70.42 \pm 10.2$  years in the control group ( $p = 0.1$ ). The mean CMT of the neovascular AMD patients were  $290.96 \pm 133.98$  in right eyes and  $281.46 \pm 108.90$  in left eyes.

The laboratory findings of the study patients were presented in (Table 1). The mean neutrophil

level was  $5.3 \pm 1.4$  in the neovascular AMD group and  $3.94 \pm 0.9$  in the control group. The mean neutrophil level was significantly higher than that of the control group ( $p = 0.01$ ). The mean lymphocyte level was  $2.4 \pm 0.7$  in the neovascular AMD group and  $2.2 \pm 0.5$  in the control group. The mean platelet level was  $255.6 \pm 65.3$  in the neovascular AMD group and  $242.3 \pm 57.1$  in the control group.

The mean  $NLR \pm SD$  was  $2.4 \pm 0.7$  in the neovascular AMD group and  $1.8 \pm 0.4$  in the control group. The mean NLR was significantly higher in patients with AMD compared to the control subjects ( $p = 0.00$ ). The mean  $PLR \pm SD$  was  $116.3 \pm 42.02$  in the AMD group and  $114.2 \pm 32.2$  in the control group. There was no significant difference between the two groups for PLR ( $p = 0.14$ ). There was no significant correlation between NLR and CMT in patients with AMD ( $r = 74$ ,  $p = 0.5$  for the right eyes;  $r = 112$ ,  $p = 0.6$  for the left eyes).

## 4. Discussion

In our study, we found that NLR was associated with AMD, but did not show a correlation with CMT. PLR was not associated with AMD. Before discussing the compatible and incompatible aspects of our study with previous studies, it would be appropriate to mention the calculation and meaning of NLR. Neutrophil to lymphocyte ratio is simply calculated by division of peripheral neutrophil account to lymphocyte account. NLR is a simple, reliable and low-cost indicator for inflammation. PLR is also another indicator for inflammation and it has been shown that PLR could be a prognostic marker for some kind of tumors [8,9]. Since NLR is calculated by two independent inflammatory markers, the stability of NLR is a better criterion than other leukocyte parameters [10].

Some studies have shown that NLR is a new indicator of inflammatory activity associated with ocular diseases. Li et al reported the diagnostic value of white blood cell count in patients with primary angle-closure glaucoma [11].

Age	AMD(n) Mean±SD	Control (n) Mean±SD	p- values
		71.67±7.2	70.42±10.2
Neutrophil (103/mm <sup>3</sup> )	5.02±1.5	3,96±1.03	0.01
Platelet (103/mm <sup>3</sup> )	255.6±65.3	242.3±57.1	0.58
Lymphocyte (103/mm <sup>3</sup> )	2.34±0.7	2.2±0.5	0.65
NLR	2.3±0.7	1.9±0.5	0
PLR	117.3±44.1	119.6±39.3	0.55

**Table 1:** The laboratory findings of the study patients.

Ozgonul et al indicated that NLR could be a new biomarker for primary open-angle glaucoma [12]. Ilhan et al. demonstrated that NLR was significantly higher in vitreomacular traction syndrome patients than in the control group [13]. These studies demonstrated the diagnostic value of NLR and the critical role of the inflammatory cascade in the pathophysiology of these ocular diseases.

Besides genetic and environmental factors, inflammation also has a role in the pathogenesis of AMD [14,15]. As a result of the inflammatory process, oxidative stress and endothelial dysfunction cause an increase in the incidence and severity of AMD. An experimental study of choroidal neovascularization on mice showed that neutrophils caused the breakdown of blood-retinal-barrier [16]. Notably, in the individuals with mutant complement factor H gene, para-inflammation caused by normal ageing is believed to trigger inflammation at the retinal pigment epithelium-Bruch's membrane complex, leading to the development of AMD and as an acute-phase reactant, hsCRP level is believed to be increased in those individuals [17].

There are comparison studies about NLR in AMD vs healthy control groups in the literature. Ilhan et al reported that NLR was higher in AMD group and there was a positive correlation between NLR and disease severity [18]. Kurtul et al also showed that NLR was higher in neovascular AMD group than in control subjects [19]. Sengul et al showed that neovascular AMD patients were found to have statistically significantly higher NLR and

PLR levels as compared to controls. They also showed a negative correlation between BCVA and both NLR and PLR whereas a positive correlation between CMT and both NLR and PLR [20]. In contrary to these studies, Pinna et al had found no statistically significant relationship between AMD and NLR and PLR in their study including 79 Sardinian male patients. The limitations of the study were reported as only male patients and the certain race was included in the study [21]. In our study, although NLR was statistically higher in neovascular AMD group, we could not find any correlation between NLR and CMT, unlike other studies.

High platelet levels intervene in angiogenesis, tumor growth, and inflammation, which is controlling lymphatic vessel development and produce relative thrombocytosis [22]. Also, it has been known that platelets are critical sources, especially for VEGF, in the wound healing process [23]. So, larger and activated platelet release may have an important role in angiogenesis. In our study, although PLR was higher in the AMD group the difference was not statistically significant between AMD and control groups.

Our study has some limitations such as retrospective design and low numbers of patients. Besides, we are aware that the most important difficulty in using blood parameters in diagnosis and treatment follow-up is that these values are affected by various factors. Using a single blood sample may not predict whether PLR and NLR will show the same association over time.

The use of repeated multiple blood samples will demonstrate the persistence of PLR and NLR over time, and therefore longitudinal studies are warranted to evaluate predictive power.

In conclusion, we found that NLR was significantly higher in patients with AMD than in control subjects. Our findings verify previous studies showing that a possible link exists between AMD and NLR. Longitudinal studies are needed to show whether this relationship between AMD and NLR persist over time.

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