Predictive Roles of Neutrophil-to-Lymphocyte Ratio and C-Reactive Protein in Patients with Calcific Aortic Valve Disease

Jian Song,1,* MM, Qiang Zheng,1,* MM, Xiaochun Ma,1 MD, Qian Zhang,1 MD, Zhenqiang Xu,1 MM, Chengwei Zou,1 MD and Zhengjun Wang,1 MD

Summary
The neutrophil-to-lymphocyte ratio (NLR) and C-reactive protein (CRP) are emerging indirect blood markers to roughly reflect the inflammation level in our body while some pathological changes occurring in aortic valve tissue. Few recent studies demonstrated that NLR is related to calcific aortic valve disease (CAVD). However, the extent of the relationship between them and the impact of CRP on CAVD are not clear. This study aimed to investigate the diagnostic influence and surgical predictive effect of NLR and CRP on CAVD.

A total of 278 consecutive patients with CAVD (123 patients with bicuspid aortic valve and others with tricuspid aortic valve) and 108 healthy individuals who were included in the control group were enrolled in the study. The NLR was calculated from the complete blood count, and the CRP was measured from peripheral blood samples. Echocardiography was used to evaluate the severity of aortic stenosis. Intraoperation/postoperation indicators were collected in 166 patients from the total consecutive patients who underwent aortic valve replacement (AVR) alone.

Significantly higher NLR was measured in both the BAV group (1.96 ± 0.78 versus 0.97 ± 0.15, \( P < 0.001 \)) and the TAV group (2.51 ± 2.03 versus 0.97 ± 0.15, \( P < 0.001 \)) compared with the control group. Moreover, the NLR level was significantly higher (\( P < 0.001 \)) and the CRP level was significantly lower (\( P = 0.007 \)) of the TAV group than that of the BAV group; a significant positive correlation between the NLR and the maximum gradient of aortic valve was detected. Furthermore, there was a moderate correlation between the NLR and the postoperative mechanical ventilation time.

Our results indicated that the NLR and CRP were novel and useful predictive factors in patients with CAVD, and these two potential factors have guiding significance for the prediction of different pathological typing (BAV or TAV). Higher NLR level will not extend the cardiopulmonary bypass time (CPB); however, it will prolong the operation time and the postoperative mechanical ventilation time.

Key words: Aortic stenosis, Inflammation

Calcific aortic valve disease (CAVD) accounts for about 15,000 patient deaths annually in the United States, and intervention occurs only when there is severe stenosis.1 CAVD is a chronic progressive disease, including early aortic valve sclerosis (ASC) and late CAVS;2 the former is present in approximately 20%-30% of individuals aged over 65 years and in 48% of patients over 85 years, while stenosis significantly affects 2%-3% of those aged over 65 years and up to 8% of those over 85 years.3,4 Once symptomatic severe CAVS has developed, prognosis without intervention is dismal, and some patients can have sudden death. With the rapid aging of society and longer life expectancy, the incidence of CAVD is increasing worldwide.

CAVD has previously been considered to be a passive and degenerative process of aging, but current data indicated that it is an active and highly regulated automatic pathophysiological process and histologically similar to coronary atherosclerosis, which is a chronic inflammatory process.5,6 The excitation of inflammatory cells and the strengthening of inflammatory reaction can lead to the process and rupture of atheroma, and then the occurrence of clinical events.7 The neutrophil-to-lymphocyte ratio (NLR) and CRP detection, two fast and simple methods for assessing inflammatory status, have been recently investigated as new predictors for cardiovascular risk.8,13 A lot of researches have shown that obviously elevated NLR and high CRP level are the independent risk factors of cardiac death in patients with coronary heart disease and...
Data are AAD, ascending aortic diameter, and CA VD. The relationship between typical inflammatory markers provide some reference for clinical treatment by studying valve group (TA V, AS and AI and 74 patients for simple AI. Meanwhile, several clinical indicators, such as aortic cross-clamp time, cardiopulmonary bypass time, and mechanical ventilation time, were collected from 166 patients who underwent AVR only. The preoperative lung function of all cases in the experimental group was measured, and it showed that the percentage of ventilatory reserve was more than 70%, and there was no significant difference between the TAV and BAV groups (Tables I, II). Patients with a history of rheumatic disease, mitral/tricuspid valve disease, endocarditis, atrial fibrillation, malignant tumor in the whole body system, and hematologic diseases including leukocytosis, acute or chronic infection, or inflammatory condition were excluded from the study. The protocol was approved by the Institutional Committee on Clinical Investigation of Shandong Provincial Hospital affiliated to Shandong University, and informed consent was obtained from the subjects.

Methods

Study population: We performed a retrospective review of 278 consecutive patients with calcific aortic valve disease who underwent AVR with or without aortic surgery at our center between January 2015 and November 2017. They were in line with the diagnosis criteria proposed by the American College of Cardiology (ACC) and the American Heart Association (AHA). One hundred and eight healthy people in our physical examination center were randomly selected into this study to be included in the control group during the corresponding period. With respect to the specific phenotype from the echocardiography results, we divided the patient group into bicuspid aortic valve group (BA V, n = 123) and tricuspid aortic valve group (TAV, n = 155), with or without ascending aortic dilatation, and further divided them into subgroups according to aortic stenosis or aortic insufficiency (the former defined as AS-predominant group). In the BAV group, besides the AS-predominant cases, 34 patients underwent AVR for AS and AI and 20 patients for simple AI. Thirty-six cases in the TAV group underwent AVR for AS and AI and 74 patients for simple AI. Meanwhile, several clinical indicators, such as aortic cross-clamp time, cardiopulmonary bypass time, and mechanical ventilation time, were collected from 166 patients who underwent AVR only. The preoperative lung function of all cases in the experimental group was measured, and it showed that the percentage of ventilatory reserve was more than 70%.

Table I. Baseline Characteristics and Laboratory Parameters of the Study Groups (n = 386)

|                          | Control (n = 108) | BAV (n = 123) | TAV (n = 155) | P-value1 | P-value2 | P-value3 | P-value4 |
|--------------------------|------------------|--------------|--------------|----------|----------|----------|----------|
| Age, years               | 56.8 ± 9.1       | 56.3 ± 10.7  | 58.8 ± 9.6   | 0.088    | 0.710    | 0.096    | 0.045    |
| Male, n (%)              | 63 (58.3%)       | 77 (62.6%)   | 111 (71.6%)  | 0.067    | 0.508    | 0.025    | 0.111    |
| Hypertension, n (%)      | 21 (19.4%)       | 32 (26%)     | 69 (44.5%)   | < 0.001  | 0.236    | < 0.001  | 0.002    |
| Diabetes mellitus, n (%) | 8 (7.4%)         | 20 (16.3%)   | 20 (12.9%)   | 0.123    | 0.040    | 0.155    | 0.428    |
| Smoking, n (%)           | 19 (17.6%)       | 45 (37.1%)   | 71 (45.8%)   | < 0.001  | 0.001    | < 0.001  | 0.122    |
| WBC, ×109/L              | 5.58 ± 1.11      | 6.23 ± 1.53  | 6.02 ± 1.41  | 0.002    | < 0.001  | < 0.001  | 0.237    |
| Neutrophil, ×109/L       | 2.53 ± 0.65      | 3.51 ± 1.22  | 3.80 ± 1.10  | < 0.001  | < 0.001  | < 0.001  | 0.042    |
| Platelet, ×109/L         | 243.66 ± 46.14   | 194.67 ± 53.97 | 199.53 ± 55.50 | < 0.001  | < 0.001  | < 0.001  | 0.465    |
| Lymphocyte, ×109/L       | 2.63 ± 0.65      | 1.89 ± 0.52  | 1.70 ± 0.48  | < 0.001  | < 0.001  | < 0.001  | 0.002    |
| NLR                      | 96.95 ± 25.45    | 108.38 ± 35.28 | 126.51 ± 48.82 | < 0.001  | < 0.001  | < 0.001  | 0.005    |
| PLR                      | 6.02 ± 1.41      | 2.38 ± 4.71  | 2.94 ± 9.55  | < 0.001  | < 0.001  | < 0.001  | 0.466    |
| CRP, mg/L                | 5.53 ± 1.04      | 4.51 ± 0.78  | 4.44 ± 0.94  | 0.046    | 0.620    | 0.830    | 0.936    |
| LVEDD, cm                | 423.29 ± 117.92  | 346.30 ± 55.78 | 350.00 ± 55.80 | < 0.001  | < 0.001  | < 0.001  | 0.001    |
| LV EF, %                 | 76.65 ± 37.06    | 55.78 ± 41.06 | 55.40 ± 41.06 | < 0.001  | < 0.001  | < 0.001  | 0.001    |
| Percentage of ventilatory reservation, % | 86.64 ± 1.73 | 86.62 ± 1.69 | 86.62 ± 1.69 | < 0.001  | < 0.001  | < 0.001  | 0.001    |

Data are given as mean ± SD, n (%); †P-value between all groups; †P-value between control and BAV group; *P-value between control and TAV group; ‡P-value between BAV group and TAV group. *value is measured by the axial image of the CT scan. WBC indicates white blood cell; CRP, C-reactive protein; LDL, low-density lipoprotein; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; and AAD, ascending aortic diameter.

long-term mortality in patients with myocardial infarction, respectively. Therefore, the purpose of this study is to provide some reference for clinical treatment by studying the relationship between typical inflammatory markers (NLR and CRP) and CAVD.

Statistical analysis: Data were collected and analyzed using the SPSS Statistics (Version 19.0, IBM, USA). Con-
Continuous variables were presented as mean ± standard deviation, and categorical variables are expressed as frequencies and percentages. One-way ANOVA analysis was used to compare the continuous numeric parameters of each group, whereas chi-square test was used to compare categorical variables. Pearson correlation analysis was performed to examine the relationship between NLR, aortic valve maximum pressure gradient, CPB time, and postoperative ventilation time. Logistic regression modeling was used to investigate the association between the incidence of CAVD and the different susceptibility factors. In logistic regression analysis, if B (β) > 0, Exp (B) > 1, and Sig. < 0.05, then a certain set of data can be considered a risk factor. P-value < 0.05 was considered significant for all analyses.

**Results**

Baseline characteristics and laboratory parameters of the study groups are shown in Tables I, II. Surgical data of random patients is included in Table III. The healthy control, the BAV group, and the TAV group were similar with respect to age (56.8 ± 9.1 years versus 56.3 ± 10.7 years versus 58.8 ± 9.5 years; \( P = 0.088 \)), gender (male: 58.3% versus 62.6% versus 71.6%; \( P = 0.067 \)), prevalence of diabetes mellitus, and LDL-C levels. Both the BAV group and the TAV group had significantly higher NLR and higher WBC and neutrophil counts but significantly lower platelet and lymphocyte counts compared with the control group. Moreover, a significantly higher rate of hypertension was found in the TAV group compared with the control group, and there was no difference between the BAV group and the control group, which suggests that hypertension perhaps plays an important role in tricuspid aortic stenosis. Thirdly, in the patient group, there was a significantly higher number of smokers compared with the control group, which illustrates that bad living habits perhaps play an important role in aortic lesion.

Furthermore, NLR of the TAV group was apparently higher than that of the BAV group (2.51 ± 2.03 versus 1.96 ± 0.78, \( P = 0.005 \)) (Figure 1), and there is an obvious difference between the BAV group and the TAV group in aortic cross-clamp time (74.97 ± 21.78 versus 64.54 ± 19.85, \( P = 0.002 \)) and CPB time (100.10 ± 25.25 versus 88.39 ± 25.00, \( P = 0.003 \)), but not in mechanical ventilation time (\( P = 0.333 \)) (Table III). As for the two subgroups, the CRP level of the BAV AS-predominant group was significantly higher than that of the TAV AS-predominant group (2.30 ± 2.18 versus 1.3 ± 1.17, \( P = 0.007 \)) (Figure 2). Pearson correlation analysis indicated

| Table II. Basic and Clinical Characteristics of the Two Subgroups |
|---------------------------------------------------------------|
| **BAV AS-predominant** | **TAV AS-predominant** | **P-value** |
|------------------------|------------------------|-------------|
| Age, years             | 57.6 ± 9.0             | 61.53 ± 9.0 | 0.023       |
| Male, n (%)            | 37 (53.6%)             | 31 (68.9%)  | 0.104       |
| Hypertension, n (%)    | 11 (15.9%)             | 17 (37.8%)  | 0.008       |
| Diabetes mellitus, n (%)| 9 (13.0%)             | 6 (13.3%)  | 0.964       |
| Smoking, n (%)         | 25 (36.2%)             | 21 (46.7%)  | 0.267       |
| WBC, 10⁹/L             | 6.08 ± 1.45            | 6.28 ± 1.36 | 0.455       |
| Neutrophil, 10⁹/L      | 3.60 ± 1.25            | 4.06 ± 1.04 | 0.042       |
| Platelet, 10⁹/L        | 202.17 ± 57.67         | 195.49 ± 54.80 | 0.559     |
| Lymphocyte, 10⁹/L      | 1.94 ± 0.44            | 1.86 ± 0.53 | 0.336       |
| NLR                    | 1.93 ± 0.75            | 2.37 ± 0.95 | 0.007       |
| CRP                    | 107.52 ± 34.52         | 105.80 ± 41.67 | 0.811     |
| LDLC, mmol/L           | 2.30 ± 2.18            | 1.3 ± 1.17  | 0.007       |
| LVEDD, cm              | 4.96 ± 0.75            | 5.13 ± 0.50  | 0.200      |
| AAD, cm²               | 4.42 ± 0.65            | 4.06 ± 0.65  | 0.004      |
| LVEF, %                | 57.78 ± 6.10           | 57.29 ± 5.17  | 0.656      |
| Maximum forward velocity, cm/second | 475.87 ± 69.79 | 495.36 ± 50.38 | 0.109      |
| Maximum gradient, mmHg | 107.72 ± 20.41         | 105.73 ± 19.85 | 0.608     |
| Percentage of ventilatory reservation, % | 86.20 ± 3.11 | 85.50 ± 2.38 | 0.203      |

Data are given as mean ± SD, n (%). *value is measured by the axial image of the CT scan. WBC indicates white blood cell; CRP, C-reactive protein; LDL, low-density lipoprotein; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; and AAD, ascending aortic diameter.

| Table III. Surgical Data Collected from 166 Patients who Underwent AVR Alone |
|---------------------------------------------------------------|
| **BAV (n = 71)** | **TAV (n = 95)** | **P-value** |
|------------------|------------------|-------------|
| NLR              | 1.91 ± 0.72      | 2.33 ± 1.11 | 0.007       |
| Aortic cross-clamp time, minutes | 74.97 ± 21.78 | 64.54 ± 19.85 | 0.002      |
| CPB time, minutes | 100.10 ± 25.25  | 88.39 ± 25.00 | 0.003      |
| Mechanical ventilation time, minutes | 761.85 ± 413.23 | 692.73 ± 481.30 | 0.333      |

Data are given as mean ± SD, n (%).
that the NLR of the BAV AS-predominant group was strongly and positively correlated with its maximum gradient \( r = 0.708, P < 0.001 \) (Figure 3A), and that of the TAV AS-predominant group was positively correlated with its maximum gradient \( r = 0.507, P < 0.001 \) (Figure 3B). Moreover, although there was no correlation between CPB time and NLR \( r = 0.040, P = 0.607 \) (Figure 4A), the postoperative ventilation time was moderately correlated with the NLR level \( r = 0.394, P < 0.001 \) (Figure 4B).

To determine the risk factors for CAVD, multivariate logistic regression analysis was performed, wherein hypertension \( (\beta) = 0.860, \text{Exp}(B) = 2.364, \text{Sig.} = 0.002 \), smoking history \( (\beta) = 1.256, \text{Exp}(B) = 3.510, \text{Sig.} = 0.000 \), and elevated NLR \( (\beta) = 9.823, \text{Exp}(B) = 18456.685, \text{Sig.} = 0.000 \) and PLR \( (\beta) = 0.017, \text{Exp}(B) = 1.017, \text{Sig.} = 0.000 \) levels were found to be significantly and independently associated with the presence of CAVD (Table IV).

**Discussions**

The purpose of this study is to investigate any relationship between elevated NLR, CRP values, and calcific aortic valve disease, and it further provides guidance for surgical treatment such as anti-inflammatory measures through cardiopulmonary bypass time (CPB), aortic cross-clamp time, postoperative respiratory management, and the use of antibiotics. On this basis, our team speculates that it may be more difficult for certain CAVD patients with higher NLR level to escape from CPB and that they need more ventilatory support. However, in this study, we found that there was indeed no correlation between them. To the best of our knowledge, our study is the largest series reported in the previous literature and the first to report a correlation between elevated CRP and CAVD. In our study, we found that NLR and CRP are novel reliable predictors of CAVD, and these two potential factors have guiding significance for the prediction of different pathological typing and the postoperative management of respiration.

Furthermore, our study showed that elevated NLR was associated positively with the severity of aortic stenosis in patients with either BAV or TAV. As previously mentioned, at the beginning of calcific aortic stenosis, aortic valve sclerosis has a similar process and mechanism with coronary/carotid artery atherosclerosis. As we had hypothesized, NLR value was significantly higher in patients with CAVD, and this value also differs significantly
in the BAV group and the TAV group. Besides, we found that NLR, PLR, smoking history, and history of hypertension were also independently associated with the presence of CAVD.

Valve interstitial cells (VICs) are an important part of aortic valve issue, as their primary function is to maintain valve homeostasis. But under the condition of mechanical stress damage caused by radical hemodynamics or some congenital factors such as bicuspid valve deformity, VICs are also prone to damage. After the initial procedure, under the effect of inflammatory cells/immune cells (especially macrophages) infiltration, lipid retention/modification, endothelial to mesenchymal transition, and so on, valve interstitial cells are stimulated and then transformed from quiescent VICs to activated VICs. This kind of transformation can trigger calcification and sclerosis process. Another notable viewpoint has been emphasized in various studies in recent years, i.e., the inflammation reaction which runs through the whole sclerosis process. This is also reflected in the following aspects: 1) The presence of chronic inflammation infiltrates was connected to osteochondrogenic metaplasia and neovascularization; 2) the density or counts of leukocytes correlated with the expression of tumor necrosis factor-α and the hemodynamic progression rate; 3) inflammation is accompanied by the expression of metalloproteinases (MMPs), which participate in the remodeling of tissues; and 4) inflammatory cells produce different cytokines that might promote mineralization of the aortic valve. In short, detecting inflammation reaction roughly equals to judge the progress of CAVD.

The NLR is a marker for inflammation and oxidative stress in the body, and NLR increase is a predictor of atherosclerotic lesion progression, aortic valve disease, and mitral annular calcification, according to some studies in the recent years. Whether in the process of atherosclerosis or in the progress of calcification of the aortic valve, neutrophils can also damage endothelial cells by releasing multiple inflammatory mediators, affecting the cells’ vascular permeability and self-regulating ability. Neutrophils reflect the stage of subclinical inflammation which can cause the damage to endothelial cells and VICs and platelet aggregation by increasing the release of Prooxidant and coagulation factors. Therefore, the NLR value is the result of two important and opposite immune approaches. NLR is not affected by the acute inflammation of the whole body; unlike the absolute value of simple neutrophils, it is a more stable indicator of systemic inflammation and a prognostic marker in patients with cancer, coronary artery disease, heat failure, TIA, and calcification aortic valve disease. The NLR is more stable than the pure white blood cell count, which is not easily disturbed by the other factors in the blood samples, and has a more predictive value. In 2013, Bilen, et al. found that BAV is associated with elevated mean platelet volume (MPV). MPV is a morphological parameter of platelets for showing newly produced platelets. Increase of MPV may occur because of the synthesis of prothrombotic and pro-inflammatory agents. Bilen’s research revealed the characteristic hematological predictors of the bicuspid aortic valve. Just recently, Yayla, et al. suggested that many hematological indicators associated with inflammation and
thrombocyte, such as NLR and PLR, were also significantly connected with aortic stenosis.\(^{30}\) However, they could not comprehensively compare the relationship between the BAV and the TAV groups in these inflammatory markers. In light of our research, as we expected, there was a significant difference in the level of NLR between the bicuspid aortic valve group and the tricuspid aortic valve group. It can be suggested that NLR may have an important role in the process of two main pathological subtypes of CAVD.

Although there was no statistical difference in CRP between the TAV and BAV groups, CRP level of BAV AS-predominant group was obviously higher than that of the TAV AS-predominant group. The most likely reason is a less-efficient distribution and concentration of mechanical forces within the two-cusp valve so that AS develops almost invariably than in patients with tricuspid valve.\(^{31}\) BAV cases were more prone to lipid deposition and inflammation cell aggregation; our data can provide favorable validation.

Despite the statistical significance, NLR and CPB time showed the opposite trend between the BAV and TAV groups, but it is hard to make meaningful explanations. The reasons may go as follows: later onset and slower process of TAV patients may lead to a delay in medical treatment; therefore, the surgeon needs more time and effort to exclude calcification issue. However, another difference demands our attention is that the postoperative ventilation time will increase disproportionately to the NLR throughout the whole consecutive samples underwent alone AVR. Hence, doctors need to pay more attention to postoperative airway management and to properly prolong the time of antibiotic intake to prevent infection. However, neither can we offer insight into the mechanism that NLR increase causes CAVD nor answer the question of whether prophylactic treatment with anti-inflammatory agents can block or slow down the development of CAVD; these are the topics for future investigation.

There are some limitations and uncertainties in this study. First of all, this is a retrospective study which ignores the criteria for disease progression and the initial age of disease occurrence. Secondly, we could not evaluate the association between NLR and clinical conditions. The criteria proposed by ACC and AHA were used for qualitative diagnosis rather than specific classification. Last but not the least, we did not take other cytokines or inflammatory markers such as fibrinogen, interleukin, tumor necrosis factor, and platelet-activating factor into account. To clarify our hypothesis, multicenter, large-scale, randomized, and prospective studies are needed.

**Conclusion**

NLR was a significantly independent predictor of CAVD. Moreover, inflammatory markers such as CRP were also closely correlated with the disease occurrence. To our relief, the value of NLR and the level of CRP can be calculated through blood laboratory examination, which is simple and convenient. Especially for NLR, an indicator of the antagonism between pro-inflammatory and anti-inflammatory may be useful in daily clinical practice; it is meaningless to predict the aortic cross-clamp time and CPB time through NLR alone, but it takes longer to conduct mechanical ventilation for patients with higher NLR level.

**Future perspective:** NLR can be simply calculated from the CBC, and this parameter may be used as an indicator to determine patients who are at higher risk in terms of inflammatory and atherosclerotic burden. In high-risk population of patients with CAVD, more close follow-up visits should be arranged. And this process may play an important role in the early detection of pathological typing.

NLR can also be used as an index to evaluate postoperative respiratory function and to provide anesthetist reference for airway management during the operation.

**Disclosures**

**Conflicts of interest:** The authors have no conflicts of interest to disclose.

**References**

1. Clark CR, Bowler MA, Snider JC, Merryman WD. Targeting cadherin-11 Prevents Notch1-mediated calcific aortic valve disease. Circulation 2017; 135: 2448-50.
2. Yutzey KE, Demer LL, Body SC, et al. Calcific aortic valve disease: a consensus summary from the Alliance of Investigators on Calcific Aortic Valve Disease. Arterioscler Thromb Vasc Biol 2014; 34: 2387-93.
3. Lindroos M, Kupari M, Heikklä J, Tilvis R. Prevalence of aortic valve abnormalities in the elderly: an echocardiographic study of a random population sample. J Am Coll Cardiol 1993; 21: 1220-5.
4. Stewart BF, Siscovick D, Lind BK, et al. Clinical factors associated with calcific aortic valve disease. J Am Coll Cardiol 1997; 29: 630-4.
5. Chen JH, Simmons CA. Cell-matrix interactions in the pathobiology of calcific aortic valve disease: critical roles for matricellular, matricrine, and matrix mechanics cues. Circ Res 2011; 108: 1510-24.
6. Dweck MR, Boon NA, Newby DE. Calcific aortic stenosis: a disease of the valve and the myocardium. J Am Coll Cardiol 2012; 60: 1854-63.
7. Danesh J, Collins R, Appleby P, Petro R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease. JAMA 1998; 279: 1477-82.
8. Azab B, Zaher M, Weiserbs KF, et al. Usefulness of neutrophil to lymphocyte ratio in predicting short- and long-term mortality after non-ST-elevation myocardial infarction. Am J Cardiol 2010; 106: 470-6.
9. Tanndr A, Erkan AF, Alhan A, Töre HF. Arterial stiffness and central arterial wave reflection are associated with serum uric acid, total bilirubin, and neutrophil-to-lymphocyte ratio in patients with coronary artery disease. Anatol J Cardiol 2015; 15: 396-403.
10. Li X, Shen J, Lu Z, Chen M, Fang X, Wang G. High neutrophil-to-lymphocyte ratio is associated with increased carotid artery intima-media thickness in type 2 diabetes. J Diabetes Invest 2017; 8: 101-7.
11. Celikkilek A, Ismailogullari S, Zararsiz G. Neutrophil to lymphocyte ratio predicts poor prognosis in ischemic cerebrovascular disease. J Clin Lab Anal 2014; 28: 27-31.
12. Turkmen K, Tonbul HZ, Erdur FM, et al. Soluble TWEAK independently predicts atherosclerosis in renal transplant patients.
BMC Nephrol 2013; 14: 144.

13. Soeki T, Sata M. Inflammatory biomarkers and atherosclerosis. Int Heart J 2016; 57: 134-9.

14. Isaac V, Wu CY, Huang CT, Baune BT, Tseng CL, McLachlan CS. Elevated neutrophil to lymphocyte ratio predicts mortality in medical inpatients with multiple chronic conditions. Medicine 2016; 95: e3832.

15. Argan O, Ural D, Kozdag G, et al. Associations between neutrophil gelatinase associated lipocalin, neutrophil-to-lymphocyte ratio, atrial fibrillation and renal dysfunction in chronic heart failure. Med Sci Monit 2016; 22: 4765-72.

16. Sasaki Y, Ikeda Y, Iwabayashi M, Akasaki Y, Ohishi M. The impact of autophagy on cardiovascular senescence and diseases. Int Heart J 2017; 58: 666-73.

17. Ann SH, Jung JI, Jung HO, Youn HJ. Aortic valve calcium score is associated with coronary calcified plaque burden. Int Heart J 2013; 54: 355-61.

18. Leopold JA. Cellular mechanisms of aortic valve calcification. Circ Cardiovasc Interv 2012; 5: 605-14.

19. Mathieu P, Boulanger MC. Basic mechanisms of calcific aortic valve disease. Can J Cardiol 2014; 30: 982-93.

20. Yu Z, Seya K, Daitoku K, Fukuda I, Furukawa K. Tumor necrosis factor-alpha accelerates the calcification of human aortic valve interstitial cells obtained from patients with calcific aortic valve stenosis via the BMP2-Dlx5 pathway. J Pharmacol Exp Ther 2011; 337: 16-23.

21. Moreno PR, Astudillo L, Elmariah S, et al. Increased macrophage infiltration and neovascularization in congenital bicuspid aortic valve stenosis. J Thorac Cardiovasc Surg 2011; 142: 895-901.

22. Charest A, Pépin A, Shetty R, et al. Distribution of SPARC during neovascularisation of degenerative aortic stenosis. Heart 2006; 92: 1844-9.

23. Akerström F, Barderas MG, Rodríguez-Padial L. Aortic stenosis: a general overview of clinical, pathophysiological and therapeutic aspects. Expert Rev Cardiovasc Ther 2013; 11: 239-50.

24. Varol E, Aksoy F, Özaydın M, Erdogan D, Dogan A. Association between neutrophil-lymphocyte ratio and mitral annular calcification. Blood Coagul Fibrinolysis 2014; 25: 557-60.

25. Küçükseymen S, Çağrıcı G, Güven R, Arslan Ş. Is neutrophil to lymphocyte ratio really a useful marker for all grades of degenerative aortic stenosis? Turk Kardiyol Dern Ars 2017; 45: 506-13.

26. Corriere T, Di Marca S, Cataudella E, et al. Neutrophil-to-Lymphocyte Ratio is a strong predictor of atherosclerotic carotid plaques in older adults. Nutr Metab Cardiovasc Dis 2018; 28: 23-7.

27. Kurtul S, Sarli B, Baktır AO, et al. Neutrophil to lymphocyte ratio predicts SYNTAX score in patients with non-ST segment elevation myocardial infarction. Int Heart J 2015; 56: 18-21.

28. Olsson M, Thyberg J, Nilsson J. Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves. Arterioscler Thromb Vasc Biol 1999; 19: 1218-22.

29. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol 2005; 91: 181-4.

30. Tamhane UU, Aneja S, Montgomery D, Rogers EK, Eagle KA, Gurm HS. Association between admission neutrophil to lymphocyte ratio and outcomes in patients with acute coronary syndrome. Am J Cardiol 2008; 102: 653-7.

31. Yan W, Liu C, Li R, Mu Y, Jia Q, He K. Usefulness of the neutrophil-to-lymphocyte ratio in predicting adverse events in elderly patients with chronic heart failure. Int Heart J 2016; 57: 615-21.

32. Bilen E, Tanboga IH, Kurt M, et al. Mean platelet volume is increased in patients with bicuspid aortic valve. Clin Appl Thromb Hemost 2012; 18: 351-5.

33. Bath PM, Butterworth RJ. Platelet size: measurement, physiological and vascular disease. Blood Coagul Fibrinolysis 1996; 7: 157-61.

34. Yayla Ç, Açığöz SK, Yayla KG, et al. The association between platelet-to-lymphocyte ratio and inflammatory markers with the severity of aortic stenosis. Biomark Med 2016; 10: 367-73.

35. Pachulska RT, Chan KL. Progression of aortic valve dysfunction in 51 adult patients with congenital bicuspid aortic valve: assessment and follow up by Doppler echocardiography. Br Heart J 1993; 69: 237-40.