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Prognostic Utility of Neutrophil-to-Lymphocyte Ratio on Adverse Clinical Outcomes in Patients with Severe Calcific Aortic Stenosis

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Abbreviations: AS, aortic stenosis; NLR, neutrophil-to-lymphocyte ratio; EuroSCORE-I, European System for Cardiac Operative Risk Evaluation score; NT-proBNP, N-terminal pro-BNP; hs-CRP, high

Abstract

Background

Inflammation is an important factor in the pathogenesis of calcific aortic stenosis (AS). We aimed to evaluate the association between an inflammatory marker, neutrophil-to-lymphocyte ratio (NLR) and major adverse cardiovascular events (MACE) in patients with severe calcific AS.

Methods

A total of 336 patients with isolated severe calcific AS newly diagnosed between 2010 and 2015 were enrolled in this study. Using Cox proportional hazards (PH) regression models, we investigated the prognostic value of NLR adjusted for baseline covariates including logistic European System for Cardiac Operative Risk Evaluation score (EuroSCORE-I) and undergoing aortic valve replacement (AVR). We also evaluated the clinical relevance of NLR risk groups (divided into low, intermediate, high risk) as categorized by NLR cutoff values. MACE was defined as a composite of all-cause mortality, cardiac death and non-fatal myocardial infarction during the follow-up period.

Results

The inflammatory marker NLR was an independent prognostic factor most significantly associated with MACE [hazard ratio (HR), 1.06; 95% confidence interval (CI), 1.04–1.09; p-value <0.001]. The goodness-of-fit and discriminability of the model including EuroSCORE-I and AVR (loglikelihood difference, 15.49; p-value <0.001; c-index difference, 0.035; p-value = 0.03) were significantly improved when NLR was incorporated into the model. The estimated Kaplan-Meier survival rates at 5 years for the NLR risk groups were 84.6% for the

sensitivity C-reactive protein; MACE, major adverse cardiovascular event.

low risk group (NLR $\leq$ 2), 67.7% for the intermediate risk group (2 < NLR $\leq$ 9), and 42.6% for the high risk group (NLR > 9), respectively.

Conclusion

The findings of the present study demonstrate the potential utility of NLR in risk stratification of patients with severe calcific AS.

Introduction

Calcific aortic stenosis (AS) represents a major public health burden associated with progressively increasing morbidity and mortality [1, 2]. This is especially so with a rapidly aging global society and the relatively high prevalence of AS being observed among the elderly. A novel approach enabling the risk stratification of AS patients would undoubtedly furnish an invaluable tool to greatly facilitate clinical decisions. Recent endeavors towards researching the complex abnormalities in risk stratification of AS patients have identified several potential biomarkers. Overall however, there remains a lack of biomarkers to complement the prognostic use of simple risk scores, although levels of natriuretic peptides [3] and high-sensitivity troponin may have a predictive role in AS.

There is an association between inflammation and remodeling of calcific aortic valve disease where inflammation and calcification are believed to play key roles in the disease [4]. Although high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation, may have a role in identifying patients in the early stages of calcific AS [5], there are conflicting study results with respect to the association between hs-CRP level and prognosis of calcific AS [6, 7]. Currently, neutrophil-to-lymphocyte ratio (NLR), a simple and inexpensive method for assessing inflammation, is being investigated as a new predictor of cardiovascular risk as an important inflammatory marker [8, 9]. Recent meta-analysis indicates that NLR is a predictor of all-cause mortality and cardiovascular events [10]. Furthermore, NLR has been identified as an independent factor associated with coronary calcium score [11]. The objective of this study was to investigate the prognostic value of NLR as an independent predictor for adverse clinical outcomes in patients with severe calcific AS and additionally evaluate the clinical relevance of NLR in stratifying AS patients into heterogeneous risk groups.

Methods

Study population

A total of 336 symptomatic or asymptomatic patients newly diagnosed with isolated severe calcific AS between January 2010 and January 2015 were retrospectively enrolled in this study from two university hospitals in South Korea. The definition of severe calcified AS was based on the American Society of Echocardiography (ASE) guidelines (peak velocity $\geq$4 m/s or mean pressure gradient (PG) > 40 mm Hg in the presence of normal left ventricular (LV) function or calculated aortic valve area (AVA) <1.0 cm^2) [12]. In cases where LV systolic dysfunction co-existed with mean PG 30–40 mmHg and AVA of 1.0 cm^2 (low-flow low-gradient AS), dobutamine stress echocardiography was used to discriminate severe calcific AS causing LV systolic dysfunction (n = 25). Patients with systemic diseases and those on treatment with agents affecting white blood cell count including patients with hematological disorders, malignancies, chemotherapy treatment, evidence of concomitant inflammatory disease, acute infection, chronic inflammatory conditions, history of corticosteroid therapy in the preceding 3

months, prior valve replacement surgery, secondary hypertension or end-stage renal disease on dialysis were excluded from this study. In addition, patients with valvular disease in valves other than aortic valve (AV) such as bicuspid AV, rheumatic AS or severe regurgitation of valves were excluded. Clinical risk factors (age, sex, diabetes mellitus, hypertension, hyperlipidemia, smoking status) and New York Heart Association (NYHA) functional class were analyzed by retrospective chart review. European System for Cardiac Operative Risk Evaluation score (EuroSCORE-I) was calculated in each patient via available online tools (http://www.euroscore.org) in order to reflect coexisting clinical risk [13]. This study complies with the Declaration of Helsinki and was approved by the Ulsan University Hospital Institutional Review Board with written informed consent obtained from all participants.

Laboratory analysis

Complete blood counts, which included total white blood cells, neutrophils, lymphocytes, and platelets as well as echocardiography were obtained at the time of admission as part of routine clinical work-up. Liver enzymes, glucose, creatinine, lipid profiles, N-terminal proBNP (NT-proBNP), and hs-CRP were also measured in all patients. NT-proBNP was measured using immunoradiometric assays with a commercial kit for BNP (COBAS 6000 E601 module, Roche Diagnostics HITACH HIGH TECH CORP., Indianapolis, IN, USA), and hs-CRP was measured using fully automated turbid immunometry (Advia 1800, Siemens). NLR was calculated as the ratio of neutrophil count to lymphocyte count.

Echocardiography

Echocardiographic assessments were performed using a Sonos 5500 system (Philips Medical Systems, Bothell, WA) with standardized imaging techniques. A comprehensive echocardiographic examination including M-mode echocardiography, two-dimensional echocardiography, and conventional and color Doppler ultrasonography were performed according to European Society of Echocardiography and ASE criteria [12]. The peak velocity across the valve was measured with continuous-wave Doppler from the window with the strongest velocity signal. AVA was calculated using the continuity equation, and ejection fraction (EF) was calculated using the biplane Simpson method. Measurements of thicknesses of interventricular septum and posterior wall, diameter of the LV cavity, and LV mass index (LVMI) were calculated from the ratio between LV mass. Body mass index (BMI) was also calculated.

Clinical outcomes and definitions

The endpoint of the study was major adverse cardiovascular events (MACE) in the follow-up period defined as a composite of all-cause mortality, cardiac death and non-fatal MI. Intraoperative deaths as well as deaths during the follow-up periods were included in the analysis. Deaths were classified as cardiac or non-cardiac following review of medical records, including autopsy records and death certificates, which were available for all cases. Nonfatal MI was defined using the definitions of the European Society of Cardiology, American College of Cardiology, American Heart Association, and World Heart Federation [14].

Statistical analysis

Differences in baseline characteristics between patient groups (with MACE and without MACE) were tested using t-statistics for continuous variables. In the case of categorical variables, we applied the chi-square test if expected frequencies equal at least 5; otherwise, the Fisher exact test was utilized.

Cox proportional hazards (PH) regression models were employed to investigate the prognostic value of NLR adjusted by the baseline covariates including EuroSCORE-I, AVR, and NLR. The independent prognostic factors significantly associated with MACE were selected in stepwise fashion avoiding over-parameterization. The underlying proportional hazards assumptions of the Cox PH models were verified by Schoenfeld residual tests. The goodness-of-fit and discriminability of Cox PH models were assessed by likelihood ratio tests and Harrell's c-index, respectively [15]. The confidence intervals of the C-index were constructed by the nonparametric bootstrap method [16].

Upon the verification of the prognostic value of NLR as a continuous variable, we further investigated the clinical relevance of the categorized NLR that assign patients into three risk groups (low, intermediate, high). The cutoff values for the NLR risk groups were carefully determined to minimize information loss in terms of loglikelihood difference as discretizing the continuous variable of NLR [17].

The Kaplan-Meier method was applied to estimate survival curves for categorized NLR risk groups without imposing any parametric assumption. In hypothesis testing, a significance level of 0.05 was chosen. All statistical analyses were performed using R (http://www.r-project.org).

Results

Characteristics of the study population

Among the 336 patients enrolled in the study population, 166 (49.4%) were male and the mean age of the patients was 70.1 ± 12.0 years. Fig 1 showed the detail information for the presentation of the cohort.

The median follow-up time was estimated to be 33 months using the reverse Kaplan-Meier method [18]. The clinical characteristics of the study population according to the status of MACE are summarized in Table 1.

Compared to the patient group with no MACE occurrence (MACE (-)), the patient group with MACE (MACE (+)) consisted of older patients, had lower BMI, higher heart rate and higher EuroSCORE-I, and underwent more frequent percutaneous coronary interventions and less frequent AVR (all p-values <0.05). In Table 2, the laboratory characteristics of the study population are compared between the two patient groups. Compared to MACE (-), MACE (+) showed reduced lymphocyte count and hematocrit, higher platelet density width and serum creatinine, and correspondingly lower estimated glomerular filtration rate (eGFR) (all p-values <0.05). S1 Fig showed the empirical joint and marginal distributions of lymphocyte and neutrophil depicted by a scatter plot and histograms.

As depicted in Fig 2, significantly higher NLR, NT-proBNP, and hs-CRP values were also observed in MACE (+).

According to the echocardiographic characteristics of the study population listed in Table 3, MACE (+) had reduced AVA and LVEF, increased trans-valvular PG and left atrial diameter, and higher ratio of peak earlier mitral filling velocity to mitral annulus velocity (E/Ea) compared to MACE (-) (all p-values <0.05).

Prognostic value of NLR in patients with severe calcific AS

According to the Cox PH regression analysis, high EuroSCORE-I and a history of AVR were significantly associated with MACE. Also, the inflammatory variable, NLR, was the independent prognostic factor most significantly associated with MACE (hazard ratio (HR), 1.06; 95% confidence interval (CI), 1.04–1.09; p-value < 0.001; Table 4). S2 Fig showed the empirical distributions of the continuous type of variables present in Table 4 utilizing box-plots

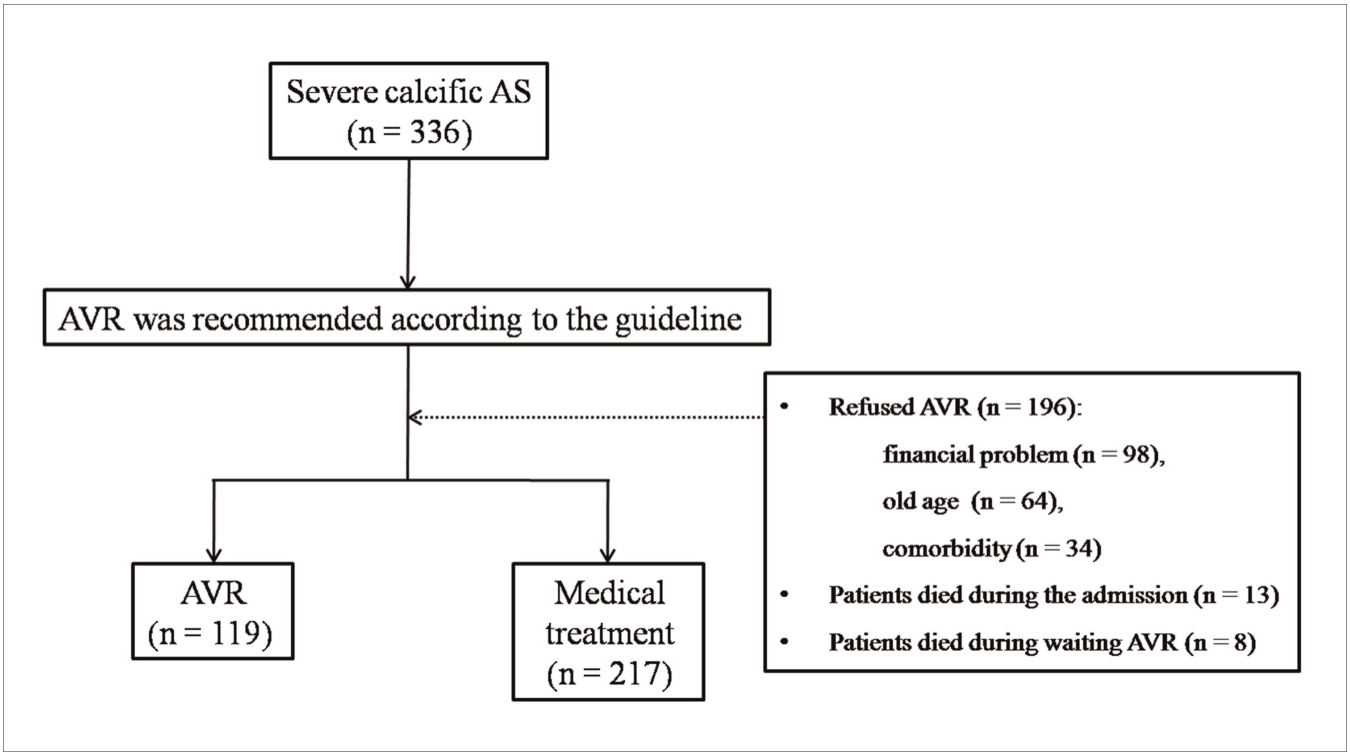

Fig 1. Clinical layout of severe calcific aortic stenosis (AS) cohort. Although aortic valve replacement (AVR) was recommended according to the guideline, only 119 patients underwent AVR because of various situations.

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The likelihood and C-index estimates of Cox PH regression models are summarized in Table 5. The inflammatory variable, NLR, has the highest likelihood value and C-index among three variables including AVR, EuroSCORE-I, and NLR. The incremental effect of NLR on MACE was also demonstrated by comparing three different Cox PH regression models: Model 1 includes AVR; Model 2 includes AVR and EuroSCORE-I; Model 3 includes AVR, EuroSCORE-I, and NLR, respectively. According to the comparison between Model 2 and Model 3, NLR significantly improved the goodness-of-fit and discriminability of the Model 2 including AVR and EuroSCORE-I (loglikelihood difference, 15.49; p-value < 0.001; c-index difference, 0.035; p-value = 0.03).

The 95% CI and p-value of the difference of the C-indexes were calculated using the empirical distribution of the indexes based on 2,000 bootstrap re-samples. When we extended the definition of MACE including a composite of all-cause mortality, cardiac death, non-fatal MI and heart failure admission, NLR still remains as the independent prognostic factor most significantly associated with MACE after properly adjusting the effect of AVR (S1 Table). S3 Fig showed the empirical distributions of NLR according to sub-patient groups classified by the status of AVR (with or without AVR) and by the status of MACE (with or without MACE).

Optimized cutoff values of NLR

The upper and lower cutoff values of NLR were optimized to minimize the information loss in categorizing NLR and were estimated to be 2 and 9, respectively. Among a total of 336 patients, 112 (33.3%), 186 (55.4%), and 38 (11.3%) patients were assigned into three risk groups as follow: the low risk group if NLR ≤ 2, the intermediate risk group if $2 <$NLR ≤ 9, and the high risk group if NLR >9. As shown in Table 6, the categorized NLR was also a significant independent

Table 1. Baseline Clinical Characteristics According to Major Cardiovascular Event.

	MACE (+) (n = 82)	MACE (-) (n = 254)	*p*-value
Age, years	72.3 ± 10.4	69.4 ± 12.4	0.014
BMI, kg/m^2	22.7 ± 3.10	23.6 ± 3.10	0.034
Male, n (%)	38 (46.3%)	128 (50.4%)	0.609
Systolic BP, mmHg	126.5 ± 24.1	126.9 ± 20.1	0.909
Diastolic BP, mmHg	72.0 ± 15.4	71.4 ± 12.6	0.806
Current smoker, n (%)	14 (17.1%)	35 (13.8%)	0.579
Hypertension, n (%)	36 (43.9%)	119 (46.9%)	0.735
Diabetes mellitus, n (%)	17 (20.7%)	59 (23.2%)	0.750
Dyslipidemia, n (%)	17 (20.7%)	74 (29.1%)	0.178
Previous CVA, n (%)	9 (11.0%)	28 (11.0%)	1.000
Significant CAD, n (%)	18 (22.0%)	37 (14.6%)	0.162
Previous PCI	14 (17.1%)	18 (7.1%)	0.014
EuroSCORE-I	8.5 ± 9.4	6.0 ± 5.8	0.024
NYHA functional class, n (%)			0.162
Class I	22 (26.8%)	83 (34.0%)	
Class II	26 (31.7%)	87 (35.7%)	
Class III	21 (25.6%)	54 (22.1%)	
Class IV	13 (15.9%)	20 (8.2%)	
Aortic valve replacement, n (%)	21 (25.6%)	98 (38.6%)	0.045

Values are means ± SDs for continuous variables or frequencies (percentages) for categorical variables.
BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; CVA, cerebrovascular accident; MACE, major adverse cardiovascular event; NYHA, New York Heart Association; PCI, percutaneous coronary intervention

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Table 2. Baseline Laboratory Characteristics According to Major Cardiovascular Event.

	MACE (+) (n = 82)	MACE (-) (n = 254)	*p*-value
White blood cell, x10^9/L	8.8 ± 4.1	7.8 ± 3.5	0.049
Neutrophil, %	69.1 ± 15.0	66.1 ± 37.3	0.293
Lymphocyte,%	20.2 ± 11.6	25.5 ± 11.4	<0.001
Monocyte, %	6.5 ± 2.9	7.3 ± 3.0	0.035
Hemoglobin, g/dL	12.0 ± 4.1	12.2 ± 2.0	0.724
Hematocrit, %	34.7 ± 6.0	36.1 ± 5.7	0.070
Red cell distribution width	14.9 ± 2.2	14.4 ± 2.4	0.062
Platelets, x10^9/L	220.1 ± 88.9	200.5 ± 77.4	0.076
NLR	7.1 ± 10.0	4.1 ± 4.7	0.009
hs-CRP, mg/L	4.4 ± 6.7	2.1 ± 4.0	0.034
Serum creatinine, mg/dL	1.7 ± 2.4	1.3 ± 1.4	0.182
eGFR, ml/min/1.73m^2	62.0 ± 34.2	71.3 ± 29.4	0.040
Total cholesterol, mg/dL	167.1 ± 42.1	169.5 ± 42.3	0.648
LDL cholesterol, mg/dL	94.4 ± 32.8	100.0 ± 39.0	0.286
HDL cholesterol, mg/dL	43.3 ± 14.6	45.0 ± 14.3	0.399
Triglycerides, mg/dl	101.0 ± 89.8	109.9 ± 69.8	0.471
NT-proBNP, ng/mL	7058.0 ± 8026.2	3359.1 ± 6079.8	0.006

Values are means ± SDs for continuous variables or frequencies (percentages) for categorical variables.
NLR, neutrophil-to-lymphocyte ratio; hs-CRP, high sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate according to the Modification of Diet in Renal Disease equation; LDL, low density lipoprotein;HDL, high density lipoprotein; NT-proBNP, n-terminal pro brain natriuretic peptide.

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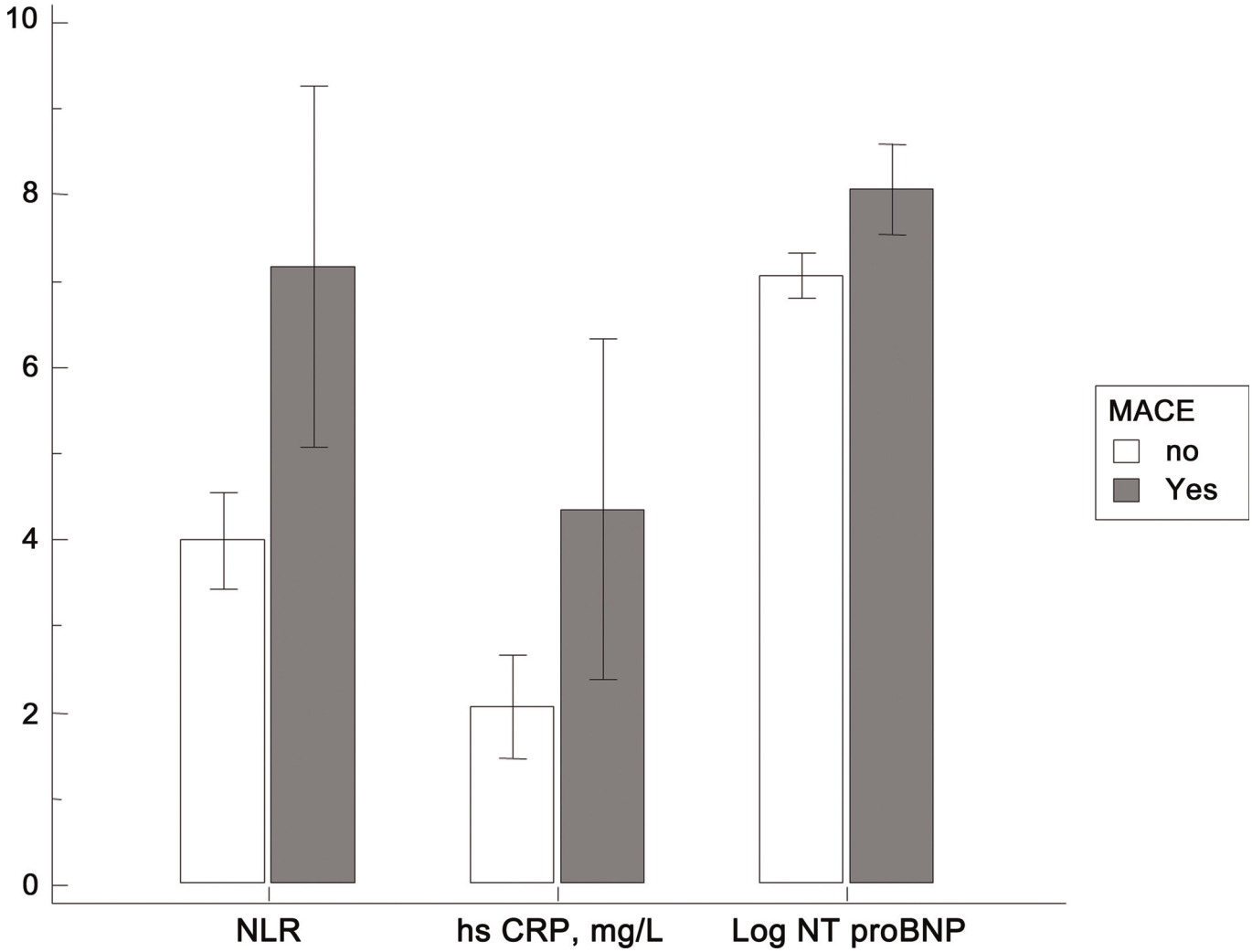

Fig 2. Neutrophil-to lymphocyte ratio (NLR), N-terminal pro-brain natriuretic peptide (NT-proBNP), and high-sensitivity C-reactive protein (hs-CRP) according to major adverse cardiovascular events (MACEs) and New York Heart Association (NYHA) functional class.

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prognostic factor. Compared with the low risk group, the adjusted HR of the intermediate risk group and the high risk group were estimated to be 1.90 (95% CI, 1.07–3.38; p-value = 0.027) and 4.85 (95% CI, 2.38–9.90; p-value <0.001), respectively.

Clinical value of the NLR risk classification

As depicted in Fig 3, there exists a statistically significant separation among the estimated Kaplan-Meier survival curves of the NLR risk groups (p-value <0.001).

The estimated MACE free survival rates at 5 years for the NLR risk groups were 84.6% for the low risk group, 67.7% for the intermediate risk group, and 42.6% for the high risk group, respectively (Table 7).

Discussion

In the present study of 336 consecutive patients diagnosed with severe calcific AS for a given period, we observed the natural history of the study patients and all suitable patients underwent

Table 3. Baseline Echocardiographic Characteristics According to Major Cardiovascular Event (MACE).

	MACE (+) (n = 82)	MACE (-) (n = 254)	*p*-value
AV area, cm^2	0.7 ± 0.2	0.8 ± 0.2	0.018
AV maximal velocity, cm/s	4.5 ± 0.8	4.3 ± 0.8	0.011
AV maximal pressure gradient, mmHg	84.2 ± 29.3	75.7 ± 27.8	0.023
AV mean pressure gradient, mmHg	51.4 ± 19.2	44.1 ± 18.1	0.003
LVEDD, mm	47.7 ± 8.0	46.9 ± 8.0	0.474
LVEDV, ml	93.3 ± 39.3	90.6 ± 42.0	0.597
LVESD, mm	33.4 ± 9.4	30.7 ± 8.6	0.027
LVESV, ml	43.2 ± 29.9	36.0 ± 28.8	0.063
LV ejection fraction, %	57.2 ± 15.0	63.0 ± 12.9	0.002
LV mass index, kg/m^2	160.9 ± 44.9	156.8 ± 58.2	0.540
IVSd, mm	13.5 ± 2.9	14.2 ± 3.6	0.073
PWTd, mm	12.6± 2.2	12.4 ± 2.7	0.622
RWT	0.5± 0.1	0.5± 0.2	0.875
Left atrial diameter, mm	44.7± 8.5	42.5± 8.8	0.052
E/Ea	22.5 ± 10.6	18.9 ± 8.9	0.018

Values are means ± SDs for continuous variables or frequencies (percentages) for categorical variables.
AV, aortic valve; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVESD, left ventricular end-systolic diameter; LVESV, left ventricular end-systolic volume; IVSd, diastolic interventricular septal wall thickness; PWTd, diastolic posterior wall thickness; RWT, relative wall thickness; E, peak early diastolic mitral filling velocity; Ea, mitral annular velocity; EF, ejection fraction.

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AVR. The unique finding of this study was that NLR, a new predictive inflammatory marker, in cardiovascular disease was an important independent predictor of MACE in patients with

Table 4. Cox Proportional Hazards Regression Analysis Regarding Major Cardiovascular Event.

	Simple			Multivariable		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Age, years	1.03	1.01 to 1.05	0.004			
BMI, kg/m^2	0.92	0.86 to 0.99	0.032			
Heart rate, min/sec	1.01	1.00 to 1.02	0.006			
EuroSCORE-I	1.07	1.05 to 1.10	<0.001	1.05	1.03 to 1.08	<0.001
PCI, n (%)	2.49	1.39 to 4.45	0.002			
Aortic valve replacement, n (%)	0.43	0.26 to 0.70	0.001	0.52	0.31 to 0.87	0.012
Lymphocyte,%	0.95	0.93 to 0.97	<0.001			
Hematocrit, %	0.95	0.91 to 0.98	0.004			
Red cell distribution width	1.10	1.02 to 1.17	0.009			
NLR	1.07	1.05 to 1.09	<0.001	1.06	1.04 to 1.09	<0.001
hs-CRP, mg/L	1.09	1.04 to 1.14	<0.001			
eGFR, $ml/min/1.73m^2$	0.99	0.98 to 1.00	0.046			
NT-proBNP, ng/mL	1.00	1.00 to 1.00	<0.001			
LVESD, mm	1.02	1.00 to 1.05	0.043			
LV ejection fraction, %	0.98	0.97 to 1.00	0.013			
E/Ea	1.04	1.01 to 1.04	0.002			

MACE, major adverse cardiovascular event; HR, hazard ratio; CI, confidence interval; BMI, body mass index; PCI, percutaneous coronary intervention; NLR, neutrophil to lymphocyte ratio; hs-CRP, high sensitivity C-reactive protein; eGFR; estimated glomerular filtration rate; NT-proBNP; n-terminal pro brain natriuretic peptide; LVESD, left ventricular end-systolic diameter; LV, left ventricular. E, peak early diastolic mitral filling velocity; Ea, mitral annular velocity.

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Table 5. Comparison of the Goodness-of-fit and discriminability of Cox Proportional Hazards Regression Models.

	Goodness-of-fit			Discriminability			
	Loglik	Diff.	*p*-value*	C-index	Diff.	95% CI+	*p*-value+
Model 1	-397.69			0.58			
Model 2	-389.08			0.66			
Model 3	-381.49			0.70			
Model 1 vs. Model 2		8.61	<0.001		0.079	0.038 to 0.118	0.002
Model 1 vs. Model 3		16.35	<0.001		0.114	0.068 to 0.164	<0.001
Model 2 vs. Model 3		7.74	<0.001		0.035	0.002 to 0.070	0.033

Model 1, AVR; Model 2, AVR + EuroSCORE-I; Model 3, AVR + EuroSCORE-I + NLR; Loglik, loglikelihood; Diff., difference

C-index, Harrell's concordance index; *p*-value* is based on the loglikelihood ratio test

95% confidential interval (CI)+ and *p*-value+ are based on the nonparametric bootstrap method.

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severe calcific AS. In addition, the incorporation of NLR into a model with EuroSCORE-I, a validated clinical risk score for patients with calcific AS [16, 19], and AVR, a procedural factor significantly improved the goodness-of-fit and the discriminability for the long-term clinical outcomes in patients with severe calcific AS. This is the first study, to our knowledge, to explore adverse outcomes in patients with severe calcific AS via the combination of the inflammatory marker and the clinical risk score.

Predicting outcomes in patients recently diagnosed with calcific AS is clinically relevant, and the assessment of hemodynamic obstruction defined by echocardiographic indexes including trans-valvular PG and AVA is suboptimal because of technical difficulties and poor association with symptoms. Apart from natriuretic peptides, biomarkers have not played a significant role in the evaluation or management of patients with calcific AS [3], although their role in clinical management is not clearly defined. Given the association between inflammation and calcific AS including calcification, fibrosis, and lipid storage [1, 20], which indicate the morphological changes seen in atherosclerosis, validation of inflammatory markers in coronary artery disease and calcific AS might be valuable. In this regard, hs-CRP, a useful predictive biomarker of systemic inflammation and coronary atherosclerosis [21], has also been investigated to assess its relationship with the severity, progression rate and prognosis of calcific AS. Although hs-CRP might have a role in identifying patients in the early stages of calcific AS [5], no relationship has been found in a large population-based cohort [22], with conflicting results noted for the association between hs-CRP level and prognosis of calcific AS [6, 7].

Recently, NLR was identified as an important inflammatory marker and is an inexpensive, routine, reproducible and widely available test. Although NLR is known as potential predictor of cardiovascular risk in patients undergoing percutaneous coronary intervention [9, 23, 24], the relationship between NLR and calcific AS has not been sufficiently investigated. However, there Although EuroSCORE-I score is a validated operative risk score for patients with AS [16,

Table 6. Effect of neutrophil to lymphocyte ratio (NLR) Risk Groups on Major Adverse Cardiovascular Event.

	No. of patients (%)	HR*	95% CI	*p*-value
Low (NLR ≤2)	112 (33.3%)	Reference		
Intermediate (2< NLR ≤9)	186 (55.4%)	1.90	1.07 to 3.38	0.027
High (NLR >9)	38 (11.3%)	4.85	2.38 to 9.90	<0.001

HR*, hazard ratios adjusted by covariates including Logistic EuroSCORE I and Aortic valve replacement (AVR) using a Cox proportional hazards regression model.

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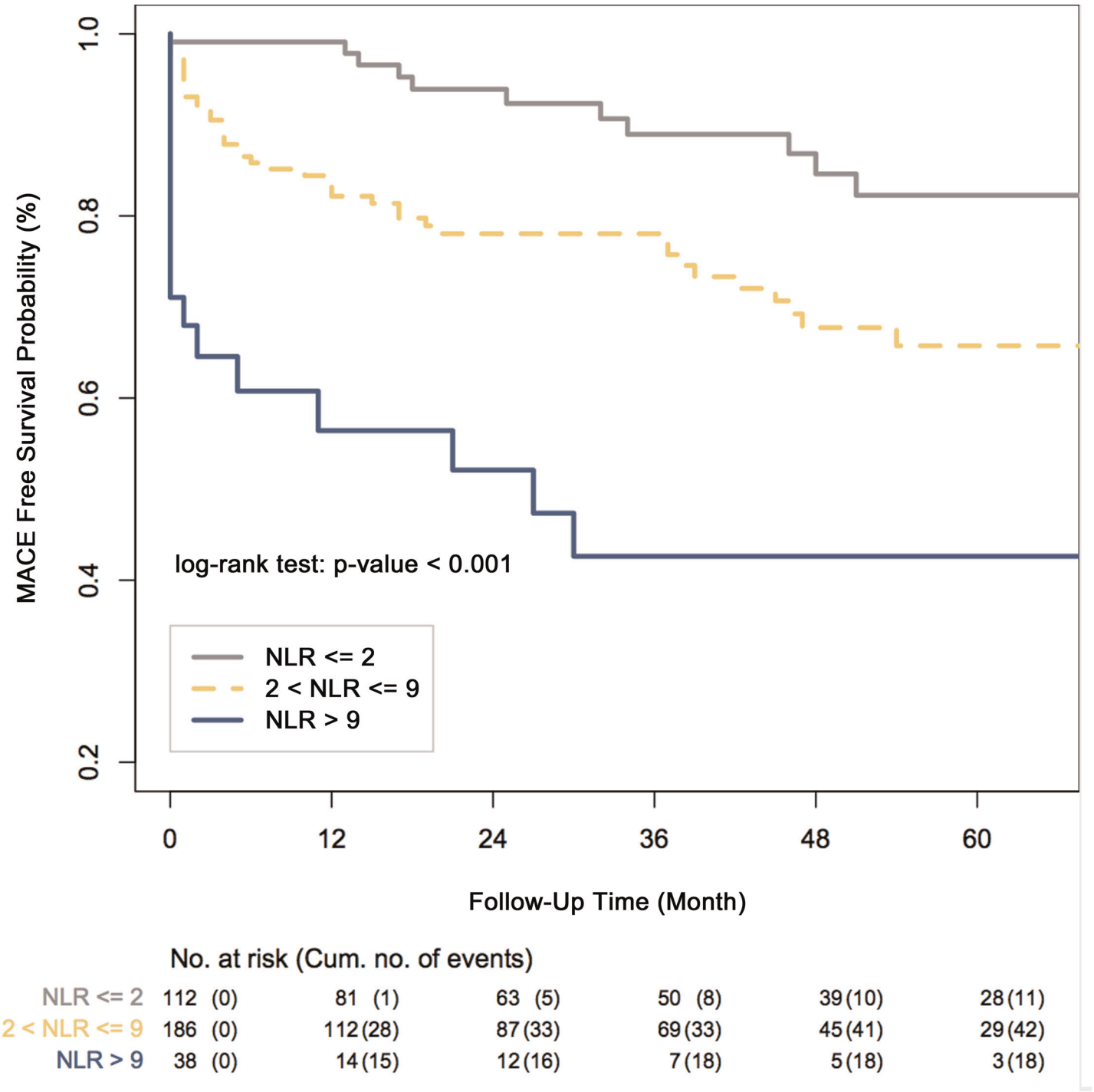

Fig 3. Major Adverse Cardiovascular Event free survival curves of neutrophil to lymphocyte ratio (NLR) risk groups estimated by the Kaplan-Meier method.

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19], it is calculated using age, NYHA functional class, LV systolic function, and renal function, all of which were associated with the long-term likelihood of MACE in patients with severe calcific AS. In the present study, high EuroSCORE-I was significantly associated with MACE. The inflammatory variable, NLR, was the independent prognostic factor most significantly

Table 7. Kaplan Meier Survival Estimates at 5 Years for NLR Risk Groups.

Group	No. of patients	5-year survival rate (%)	95% CI
All patients	336	70.3	64.2 to 77.1
Low (NLR ≤2)	112	84.6	75.9 to 94.3
Intermediate (2< NLR ≤9)	186	67.7	59.4 to 77.3
High (NLR >9)	38	42.6	27.4 to 66.4

NLR, neutrophil to lymphocyte ratio.

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associated with MACE. This corresponds with the result by Avci et al., who showed that an increased NLR is related to the severity of calcific AS and LV systolic dysfunction in patients with severe calcific AS [25]. As expected, the procedural factor AVR was also important for the long-term prognosis, and because 119 patients had AVR during follow-up, we utilized the multivariable Cox hazard regression model for adjusting the heterogeneous effects of AVR. Interestingly, the incorporation of NLR into a model with AVR and EuroSCORE-I significantly improved the discriminability of the model measured by Harrel's concordance index. Our observation of an effective method for stratifying mortality risk beyond the EuroSCORE-I score based on inflammatory marker such as NLR in combination with more accurate clinical risk scores might be useful in identifying subgroups of patients likely to exhibit a dismal prognosis even with valve replacement. We believe that it would be certainly an interesting topic for a future study investigating whether or not it is possible to predict the severity of AS based on the level of NLR. This future study would be plausible if there exist a wide spectrum of patients in the study population in terms of the severity of AS. However, our study population includes patients only with isolated severe calcific AS and thus we may pursue the investigation in the future when we obtain appropriate data sets.

Several limitations should be considered when interpreting the findings of the present study. While the study cohort consisted exclusively of severe calcific AS patients diagnosed from echocardiography, the initial presenting symptoms were heterogeneous. Secondly, despite 70% of the patients in our cohort experiencing heart failure symptoms, only 35% of patients underwent AVR because they were either high risk or refused to undergo surgical AVR. As there are no observational studies comparing AVR and medical observation after diagnosis of calcific AS in Korea, we cannot assume that an accurate proportion of severe calcific AS patients underwent AVR. However, compared to Western countries, our results might reflect the Asian cultural trend of refusing surgery in old age as well as the poor accessibility of transcatheter AVR (TAVR) in Korea due to its high cost. Indeed, as TAVR is not widely performed in Korea, our patients at higher risk were not referred for consideration of TAVR, which limits the generalization of our results. However, considering other studies showing that medical therapy tends to be chosen for older patients with comorbidities [26] and the performance of AVR in 42% of patients with low-gradient severe AS during a mean follow-up of 46 months [27], our result might reflect the real practice in the treatment of severe calcific AS in Korea. Additionally, we used the EuroSCORE-I score as clinical risk factor, which was designed to predict operative mortality and perioperative morbidity and had been validated for patients undergoing heart surgery. As the vast majority of patients included in this series did not undergo AVR, the use of EuroSCORE-I might be controversial. Because the study cohort was enrolled since 2010, we did not use the improved EuroSCORE-II calculator [28] which was updated at 2012. Nevertheless, the EuroSCORE-I score performed reasonably well in predicting longer-term mortality in our study and continues to be the most commonly used risk score

in patients with AS, especially in the absence of a superior alternative [16, 19]. Thus, our findings should be confirmed using serial change in NLR in a future prospective study.

Conclusions

Our results demonstrate the potential utility of NLR as an inflammatory biomarker to improve risk stratification of patients with severe calcific AS. Further studies are needed to evaluate how such biomarker panels influence patient management and treatment decisions.

Supporting Information

S1 Fig. The empirical joint and marginal distributions of lymphocyte and neutrophil depicted by a scatter plot and histograms.
(TIF)

S2 Fig. The empirical distributions of the continuous type of variables present in Table 4 utilizing box-plots.
(TIF)

S3 Fig. The empirical distributions of neutrophil to lymphocyte ratio (NLR) according to sub-patient groups classified by the status of aortic valve replacement (AVR; with or without AVR) and by the status of major cardiovascular event (MACE, with or without MACE).
(TIF)

S1 Table. Multivariable Cox proportional hazards regression analysis regarding major cardiovascular event (MACE*) extended to include heart failure admission (all-cause mortality, non-fatal myocardial infarction and heart failure admission).
(DOCX)

Author Contributions

Conceptualization: KIC.

Data curation: KIC.

Formal analysis: SHC.

Investigation: KIC.

Methodology: ESS.

Resources: GBS.

Supervision: ESS.

Validation: AYH.

Visualization: KIC.

Writing - original draft: KIC.

Writing - review & editing: ESS.

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