JAMA Cardiology | Original Investigation

Association of Neutrophil-to-Lymphocyte Ratio With Mortality and Cardiovascular Disease in the Jackson Heart Study and Modification by the Duffy Antigen Variant

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IMPORTANCE The neutrophil-to-lymphocyte ratio (NLR) is associated with mortality and cardiovascular disease at the time of incident disease, but it is not known whether this is true in prospective studies. Further, a common genetic variant of African origin associated with a relative neutropenia, the Duffy antigen variant, is a candidate to modify associations between NLR and outcomes.

OBJECTIVE To investigate the association between NLR and mortality and cardiovascular-related outcomes in the Jackson Heart Study (JHS) and validated our findings in the Normative Aging Study (NAS). We also evaluated whether the Duffy antigen variant modifies these associations in the JHS.

DESIGN, SETTING, AND PARTICIPANTS The JHS is a large prospective cohort study designed to examine risk factors and cardiovascular disease among African American individuals residing in Jackson, Mississippi. The NAS is a longitudinal cohort established by the United States Department of Veterans Affairs in 1963. The JHS is a population-based longitudinal study. The NAS is an interdisciplinary longitudinal study located in the Veterans Affairs Outpatient Clinic in Boston, Massachusetts. A total of 5301 participants were recruited for the JHS at baseline. Genotype data on the Duffy antigen variant were available in the JHS. The participants in the NAS were white men only and free of chronic disease at the time of recruitment and were invited for in-person examinations every 3 years since 1986. Data were analyzed between November 2016 and January 2018.

MAIN OUTCOMES AND MEASURES All-cause mortality, coronary heart disease (CHD), stroke, and heart failure (HF). Two NLR cutoff values (≥2.15 for overall and ≥1.77 for African American participants) were used as the exposure measurements.

RESULTS The participants were African American men and women, aged 21 to 93 years, residing in Jackson, Mississippi. For NLR <2.15, the mean age was 54.2 (12.5); for NLR >2.15, the mean age was 56.5 (13.8); for NLR <1.77, the mean age is 54.1 (12.4); and for NLR >1.77, the mean age was 55.8 (13.6). Adjusting for potential confounders, elevated NLR (≥2.15) was significantly associated with an increased risk for all-cause mortality (hazard ratio, 1.40; 95% CI, 1.14-1.70) and CHD (hazard ratio, 1.69; 95% CI, 1.23-2.34) in JHS. Using a lower NLR cutoff (≥1.77) for African American participants did not alter the significant associations. In the NAS, elevated NLR was associated with an increased risk of mortality (hazard ratio, 1.32; 95% CI, 0.99-1.76), with no statistical significance. In both prospective studies, NLR was less of a robust predictor when the time of event was more distant. The Duffy antigen variant was associated with neutrophil count, and NLR (≥1.77) was significantly associated with mortality, CHD, stroke, and HF in the Duffy antigen–negative group.

CONCLUSIONS AND RELEVANCE Neutrophil-to-lymphocyte ratio was prospectively associated with all-cause mortality, CHD, and HF, with closer median time to event diagnoses in the JHS. Furthermore, the Duffy antigen variant locus was associated with a lower baseline NLR and modified the mortality, CHD, stroke, and HF associations.

JAMA Cardiol. doi:10.1001/jamacardio.2018.1042
Published online May 2, 2018.

Supplemental content

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Inflammation is recognized to play a critical role in the pathophysiology of cardiovascular disease (CVD) and cancer.[1] Studies have consistently shown a strong association between changes in biomarkers of inflammation and CVD.[2-4] In the past few decades, an elevated white blood cell count has been suggested to be an independent risk factor and possible biomarker for major CVD.[3,5,6] There has been an emerging focus on components of the differential white blood cell count, and particular cell types (neutrophils, lymphocytes, and monocytes) have been proposed as stronger predictors of CVD risk than the total white blood cell count.[3,7] Evidence suggests a possible role of neutrophil count alone as an independent predictive factor in both acute and chronic CVD.[3,7] Interestingly, as the literature examining the role of inflammation in cancer has matured, consensus has developed that the best predictor of solid tumor survival is the neutrophil-to-lymphocyte ratio (NLR).[8] The NLR reflects the balance between the innate (ie, neutrophils) and adaptive (ie, lymphocytes) immune responses in the body.[3,4] This work has spurred the investigation of NLR as a predictor of CVD and overall mortality, with studies now confirming an elevated NLR to be a significant predictor of adverse CVD outcomes.[3,4,9-15]

Studies using NLR as a predictor for mortality or CVD are overwhelmingly case-control or retrospective cohort studies. The predictive capability of NLR in prospective cohort studies has been poorly studied. Further, most studies of the NLR have been carried out in ethnically homogeneous European populations. This is important because it is well recognized that there exists a common genetic variant of African origin (the Duffy antigen) that causes a relative neutropenia in otherwise healthy carriers of this trait.[16] To our knowledge, there has been no evaluation of the possible effect of this trait on the association of the NLR with mortality or CVD. Hence, the association between NLR as a biomarker of systemic inflammation and mortality and cardiovascular outcomes in African American individuals was evaluated in the Jackson Heart Study (JHS), with confirmation of our findings using data from another prospective cohort of white men, the Normative Aging Study (NAS). Using genetic data from the JHS, we also examined whether effect modification exists for the Duffy antigen variant on the association between NLR and mortality and CVD outcomes.

Key Points

Question Is the neutrophil-to-lymphocyte ratio prospectively associated with mortality or cardiovascular-related outcomes, especially among African American individuals with Duffy antigen variant known to alter neutrophil count?

Findings In this cohort study, neutrophil-to-lymphocyte ratio was prospectively and significantly associated with all-cause mortality, coronary heart disease, and heart failure in the Jackson Heart Study. The associations were also significantly modified by the Duffy antigen negative trait in the Jackson Heart Study.

Meaning Neutrophil-to-lymphocyte ratio is associated with cancer and chronic disease outcome at diagnosis and may also be associated with mortality, coronary heart disease, and heart failure; the Duffy antigen variant may modify the neutrophil-to-lymphocyte ratio association with mortality and cardiovascular-related outcomes.

Methods

The data from JHS were obtained after signing the data and materials distribution agreement under the Brown University institutional agreement and approved by the JHS Publications and Presentations Subcommittee. The data from Normative Aging Study (NAS) were publicly available from dbGAP. All participants provided written informed consent including written informed consent for genetic studies. All JHS protocols were reviewed and approved by the institutional review boards at Jackson State University, Jackson, Mississippi; Tougaloo College, Jackson, Mississippi; and the University of Mississippi Medical Center. Institutional review board approval was obtained separately from each participating institution.

Jackson Heart Study Data Set

The JHS is a prospective cohort study designed to examine risk factors and CVD among African American men and women, aged 21 to 93 years, residing in Jackson, Mississippi.[17,18] A total of 5301 participants were recruited for the JHS at baseline, and data collection methods have been previously described.[17-19] The baseline data collection occurred between 2000 and 2004, and 2 follow-up clinical visits were conducted between 2005 and 2008 and between 2009 and 2012.[17-19] The examination and event data sets as well as the genetic data set on Duffy antigen variant were obtained from the JHS Vanguard Center with official request and approval.[17,18,20] The examination 1 (2000-2004) data include demographics (ie, age and sex) and medical history (ie, smoking status, body mass index [BMI], white blood cell count, history of stroke, history of CHD, diabetes, and blood pressure medication).[17,18,20] Data on white blood cell count and differential white blood cell counts were only collected on examination 1 (specifically the "loca" data set).[17,18,20]

Jackson Heart Study Genotype Data on Duffy Antigen (rs2814778)

Direct genotyping data for single-nucleotide polymorphism of rs2814778 were obtained for each JHS participant with official approval from the JHS publications and presentations subcommittee (data set accession: phs000286.v4.p1).[17,18,20] We designate genotype CC for being Duffy antigen negative and genotype TT/CT indicated for being Duffy antigen positive.[17,18,20] Hardy-Weinberg equilibrium was computed using a χ^2 test (R package HardyWeinberg [R Programming]).

Normative Aging Study Data Set

The NAS is a longitudinal cohort established by the US Department of Veterans Affairs in 1963.[21] The participants in the study were white men only, free of chronic disease at the time of recruitment, and was invited for an in-person examination every 3 to 5 years.[21] The study has gathered information on the medical history (ie, history of CHD, diabetes, and hypertension), lifestyle and demographic factors (ie, age and smoking status), as well as physical examination and laboratory tests

for each visit.[21] Laboratory test results of neutrophil and lymphocyte counts and information on overall mortality were obtained from the database of genotypes and phenotypes (data set accession: pht004429.v1.p1).

NLR Measurement in JHS and NAS

Neutrophil-to-lymphocyte ratio was calculated for each participant by dividing the reported total absolute neutrophil counts by the total absolute lymphocyte counts. Two different NLR values were used as cutoff points for low and high NLR in the JHS: (1) a value of 2.15, which is the mean NLR of participants in the National Health and Nutrition Examination Survey of aggregated cross-sectional data collected from 2007 to 2010; and (2) a value of 1.77, which is a 2016 pretreatment median NLR determined in black patients with breast cancer.[4,22] The NLR cutoff of 1.77 is comparable with non-Hispanic black participants in the 2007 to 2010 National Health and Nutrition Examination Survey, which had a mean NLR value of 1.76 (95% CI, 1.71-1.81), and it is close in value to the median NLR determined in the JHS cohort (1.54).[4]

Outcomes of Interest in JHS and NAS

Incident data on events, such as all-cause mortality (primary outcome), and cardiovascular-related outcomes, such as heart failure (HF), stroke, and coronary heart disease (CHD), reported from JHS first examination visit (September 2000 to March 2004) were the outcomes of interest in this examination.[17,18] Event date and year were noted for each of these 4 events of interest from the JHS data dictionary.[17,18] In NAS, death was reported longitudinally and was the primary outcome of interest.

Standard Risk Factors of CVD

We evaluated the following risk factors associated with CVD in JHS: age, sex, BMI, current smoking status (yes or no), diabetes status (yes or no), blood pressure medication status, history of CHD, and history of stroke.[17,18] Similarly, the following risk factors associated with CVD were evaluated in NAS: age, smoking, diabetes status, hypertension status, and history of CHD.[21]

Statistical Analyses

All statistical analyses were performed using R statistical programming language, version 3.3.1 (R Programming). Statistical significance was determined to be P less than .05, and the P value was 2-sided. We initially used the method of Rimando et al[22] to identify an optimal cutoff point for NLR point by comparing the hazard ratios and P values for varying cutoff point selections, selected to span the range of NLR in JHS.[22] The cutoff points resulting in the largest hazard ratio (HR) and lowest P value for each primary outcome of interest in JHS were selected as the optimal cutoff points of NLR (eTables 1-4 in the Supplement). This empirical method of determining optimal NLR cutoff point works best if, for a given cutoff point, the number of events for each group is large; yet at higher NLR cutoff points (ie, >5), the number of participants with NLR greater than 5 is relatively small even if larger cutoff points appear optimal. We determined that cutoff points greater than 5 were too far outside the range of our data. Thus, we took an agnostic approach and used the standard NLR cutoff points of 2.15 and 1.77 (as mentioned in previous sections) for our analyses in JHS and standard cutoff point of 2.15 for analyses in NAS. We also validated that these NLR cutoff points were reasonable using a model selection criteria method based on a comparison of the Akaike information criterion for varying cutoff point selections and found the cutoff points (2.15 and 1.77) to be close to those determined by Akaike information criterion: 2.3 and 2.8, in the JHS and NAS, respectively (eFigures 1 and 2 in the Supplement).

The distributions of categorical variables (ie, sex and smoking) were reported as frequencies and percentages, and the distributions of continuous variables were reported as mean (SD). On outcomes for which longitudinal data were available in JHS (all-cause mortality, HF, stroke, and CHD), survival analyses on events were performed using survival package in R, and HRs with 95% confidence intervals were reported from the fitted Cox proportional hazard models. Age was treated as the underlying time scale in all fitted Cox proportional hazards models.[23] Statistical interactions between NLR and genotype for Duffy antigen (NLR × genotype) were formally evaluated by including main effects for these 2 variables as well as their 2-way interaction in the multivariate Cox models fit to each of the primary outcomes in the JHS. Subsequently, multivariate Cox models were conducted adjusting for sex, BMI, smoking, history of stroke, history of CHD, diabetes, and blood pressure medication. Because age was used as the underlying time scale in our models, age at blood draw was not used as a covariate in our models. Cox models for each outcome were run using continuous NLR as the predictor and adjusting for the same covariates that were adjusted for in the main models. Hazard ratios per unit increase in NLR were reported. Models were then rerun using a natural spline of log NLR, with 3 *df* as the predictor. An analysis of variance was used to compare the models with a linear association between NLR and outcome with the models with spline of NLR, and P values from the analysis of variance were reported. A significant P value indicates that a nonlinear association may be more appropriate.

The proportional hazard assumption was checked for all models and predictors using formal statistical tests[24] and graphic plots of the scaled Schoenfeld residuals against transformed time. Diabetes appeared to violate the assumption of proportional hazards and was accordingly used as a stratification factor in our models. Because the NLR and Duffy antigen genotype interaction was significant ($P < .05$), multivariate Cox models were fit separately by genotype TT/CT (Duffy antigen positive) and CC (Duffy antigen negative) to estimate the association between NLR and mortality among Duffy antigen–negative and Duffy antigen–positive patients. For validation of analyses using NAS, the NLR cutoff of 2.15 was used, and multivariate Cox models were adjusted for smoking, history of CHD, diabetes, and hypertension. P values for difference between groups were calculated using t tests for continuous variables and χ^2 tests for categorical variables.

Table 1. Baseline Characteristics of Jackson Heart Study Participants Stratified by NLR

Characteristic	No. (%) NLR <2.15 (n = 3548)	No. (%) NLR ≥2.15 (n = 1084)	P Value[a]	No. (%) NLR <1.77 (n = 2901)	No. (%) NLR ≥1.77 (n = 1731)	P Value[a]
Age, mean (SD)	54.2 (12.5)	56.5 (13.8)	<.001	54.1 (12.4)	55.8 (13.6)	<.001
Sex						
Male	1270 (35.8)	427 (39.4)	.03	1013 (34.9)	584 (39.5)	.002
Female	2278 (64.2)	657 (60.6)		1888 (65.1)	1047 (60.5)	
Smoking						
Ever	1113 (31.4)	337 (31.1)	.91	912 (31.4)	538 (31.1)	.82
Never	2429 (68.5)	744 (68.6)		1983 (68.4)	1190 (68.7)	
BMI, mean (SD)	31.9 (7.1)	31.8 (8.2)	.61	31.9 (7.0)	31.7 (8.0)	.39
Blood pressure medication	1624 (45.8)	550 (50.7)	.002	1326 (45.7)	848 (49.0)	.02
History of CHD	238 (6.7)	95 (8.8)	.03	195 (6.7)	138 (8.0)	.12
History of stroke	133 (3.7)	59 (5.4)	.02	119 (4.1)	73 (4.2)	.91
Diabetes	581 (16.4)	204 (18.8)	.06	462 (15.9)	323 (18.7)	.02

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CHD, coronary heart disease; NLR neutrophil-to-lymphocyte ratio.

[a] Using *t* test for continuous variables and χ^2 test for categorical variables.

Results

Baseline Characteristics of JHS and NAS Participants

Table 1 delineates the demographic characteristics in JHS. The univariate comparisons in Table 1 are stratified by an NLR value of 2.15, the mean NLR from the National Health and Nutrition Examination Survey data, and 1.77, the mean NLR estimated from an independent study of African American control participants participating in a study of survival from breast cancer.[4,22] Sex, blood pressure medication, and diabetes were statistically significant covariates determined from univariate analyses testing the association between the variables and the outcomes. In addition, smoking, BMI, history of stroke, and history of CHD were also adjusted for in the multivariate regression models because these factors have been associated with elevated NLR as well as CVD.[4,25,26] The demographics of the confirmatory cohort (NAS) are shown in eTable 5 in the Supplement, stratified by mean NLR (2.15). Sex, smoking, diabetes, history of CHD, and history of CHD did not result in statistical significance as determined from the univariate analyses; however, because these are important covariates, associated with both NLR and CVD, they were accordingly adjusted for in the NAS analyses.

Association Between NLR and Mortality and CVD-Related Outcomes in the JHS and NAS

Table 2 presents the results from Cox regression models examining all-cause mortality, CHD, stroke, and HF in JHS. Adjusting for potential confounders (ie, sex, smoking, BMI, history of stroke, history of CHD, diabetes, and blood pressure medication), NLR of at least 2.15 was associated with a statistically significant increased risk of all-cause mortality (HR, 1.40; 95% CI, 1.14-1.70; $P < .001$) and CHD (HR, 1.69; 95% CI, 1.23-2.34; $P < .001$) compared with NLR less than 2.15 in the JHS (Table 2). Similarly, the hazard of all-cause mortality and CHD was significantly elevated in those with NLR of at least 1.77 compared with NLR less than 1.77 (Table 2). Additionally, we tested the associations with continuous NLR and the outcomes in JHS, and NLR was again significantly associated with all-cause mortality and CHD and was not significantly associated with HF (eTable 6 in the Supplement). In the NAS, elevated NLR was associated with overall mortality (HR, 1.32; 95% CI, 0.99-1.76) with no statistical significance ($P = .06$) after controlling for age, smoking, history of CHD, diabetes, and hypertension (Table 2).

Prospective Utility of NLR as a Biomarker for Mortality and CVD-Related Outcomes

To more clearly define the prospective predictive power of the NLR, we assessed whether time from the diagnosis to death or CVD-related outcomes modified the strength of the association between NLR and these events. Participants were grouped based on the median time to each event, and Cox regression models were fit separately to those whose event occurred before and after the median. In JHS, NLR was significantly associated with all-cause mortality, CHD, and HF in patients in whom the event occurred more proximally to the time of blood draw (**Table 3**). For all-cause mortality assessed with NLR cutoff of 2.15, the HR calculated among patients experiencing the event prior to the median time to mortality was 1.71 (95% CI, 1.29-2.27) compared with 1.23 (95% CI, 0.93-1.62) in those experiencing the event after the median time to mortality. For CHD and HF assessed with NLR cutoff of 2.15, the HRs calculated among participants experiencing the event prior to the median time to CHD and HF events were 2.13 (95% CI, 1.23-3.68) and 1.46 (95% CI, 1.03-2.08), respectively (Table 3). The associations with NLR cutoff of 1.77 and all-cause mortality, CHD, and HF were also statistically significant (Table 3). For stroke, NLR cutoff points did not have significant associations for closer median time of event (Table 3). In the NAS, the HR for under the median time to death was 1.30 (95% CI, 0.87-1.93), while the HR for those diagnosed at a time beyond the median was 1.41 (95% CI, 0.93-2.13), and both did not reach statistical significance (eTable 7 in the Supplement).

Table 2. Hazard Ratios Reported for Each Event of Interest in JHS and NAS

Outcome	HR (95% CI)[a]	*P* Value
JHS		
All-cause mortality		
NLR <2.15	1 [Reference]	<.001
NLR ≥2.15	1.40 (1.14-1.70)	
NLR <1.77	1 [Reference]	.007
NLR ≥1.77	1.30 (1.07-1.56)	
CHD		
NLR <2.15	1 [Reference]	.001
NLR ≥2.15	1.69 (1.23-2.34)	
NLR <1.77	1 [Reference]	.008
NLR ≥1.77	1.51 (1.11-2.05)	
Stroke		
NLR <2.15	1 [Reference]	.27
NLR ≥2.15	1.24 (0.85-1.80)	
NLR <1.77	1 [Reference]	.08
NLR ≥1.77	1.36 (0.97-1.92)	
HF		
NLR <2.15	1 [Reference]	.18
NLR ≥2.15	1.19 (0.92-1.54)	
NLR <1.77	1 [Reference]	.08
NLR ≥1.77	1.24 (0.98-1.57)	
NAS		
Death		
NLR <2.15	1 [Reference]	.06
NLR ≥2.15	1.32 (0.99-1.76)	

Abbreviations: CHD, coronary heart disease; HF, heart failure; JHS, Jackson Heart Study; NAS, National Aging Study; NLR, neutrophil-to-lymphocyte ratio.

[a] Adjusted for sex, smoking, body mass index, history of stroke, history of CHD, diabetes (stratified), and blood pressure medication.

[b] Adjusted for smoking, history of CHD, diabetes, and hypertension.

Table 3. NLR Association With Median Time-to-Event of Mortality and Cardiovascular-Related Outcomes in JHS

Outcome	HR (95% CI)[a]	*P* Value
All-cause mortality		
NLR <2.15 before August 8, 2008	1 [Reference]	<.001
NLR ≥2.15 before August 8, 2008	1.71 (1.29-2.27)	
NLR <2.15 after August 8, 2008	1 [Reference]	.15
NLR ≥2.15 after August 8, 2008	1.23 (0.93-1.62)	
NLR <1.77 before August 8, 2008	1 [Reference]	<.001
NLR ≥1.77 before August 8, 2008	1.64 (1.25-2.15)	
NLR <1.77 after August 8, 2008	1 [Reference]	.44
NLR ≥1.77 after August 8, 2008	1.11 (0.85-1.44)	
CHD		
NLR <2.15 before	1 [Reference]	.007
NLR ≥2.15 before January 7, 2006	2.13 (1.23-3.68)	
NLR <2.15 after January 7, 2006	1 [Reference]	.02
NLR ≥2.15 after January 7, 2006	1.57 (1.06-2.32)	
NLR <1.77 before January 7, 2006	1 [Reference]	.03
NLR ≥1.77 before January 7, 2006	1.78 (1.05-3.04)	
NLR <1.77 after January 7, 2006	1 [Reference]	.07
NLR ≥1.77 after January 7, 2006	1.42 (0.98-2.05)	
Stroke		
NLR <2.15 before August 20, 2005	1 [Reference]	.05
NLR ≥2.15 before August 20, 2005	1.84 (1.00-3.41)	
NLR <2.15 after August 20, 2005	1 [Reference]	.99
NLR ≥2.15 after August 20, 2005	1.00 (0.62-1.61)	
NLR <1.77 before August 20, 2005	1 [Reference]	.07
NLR ≥1.77 before August 20, 2005	1.73 (0.96-3.12)	
NLR <1.77 after August 20, 2005	1 [Reference]	.37
NLR ≥1.77 after August 20, 2005	1.21 (0.79-1.85)	
HF		
NLR <2.15 before September 5, 2008	1 [Reference]	.03
NLR ≥2.15 before September 5, 2008	1.46 (1.03-2.08)	
NLR <2.15 after September 5, 2008	1 [Reference]	.90
NLR ≥2.15 after September 5, 2008	0.97 (0.66-1.43)	
NLR <1.77 before September 5, 2008	1 [Reference]	.04
NLR ≥1.77 before September 5, 2008	1.43 (1.03-1.99)	
NLR <1.77 after September 5, 2008	1 [Reference]	.50
NLR ≥1.77 after September 5, 2008	1.12 (0.80-1.57)	

Abbreviations: CHD, coronary heart disease; HF, heart failure; JHS, Jackson Heart Study; NLR, neutrophil-to-lymphocyte ratio.

[a] Adjusted for sex, smoking, body mass index, history of stroke, history of CHD, diabetes (stratified), and blood pressure medication.

Effect Modification by Duffy Antigen Genotype

There were more patients who were Duffy antigen negative as indicated by genotype CC (n = 3190; 69.8%) and 1378 patients who were Duffy antigen positive as determined by genotypes TT (n = 165; 3.6%) and CT (n = 1213; 26.6%) (eTable 8 in the Supplement). The trait was not in Hardy-Weinberg equilibrium, which was not unexpected because there is likely significant population stratification within the cohort, and the study participants include familial groups. Participants who were Duffy antigen negative (CC or benign neutropenia) had lower mean neutrophil counts (51.1 per mL^3) compared with participants who were Duffy antigen positive (TT/CT) with counts of 60.55 (eTable 8 in the Supplement).

Table 4 shows the Cox regression models stratified by the Duffy antigen variant. In the Duffy antigen–negative (CC) group, there was a significantly higher point estimate (adjusted HR, 1.84; 95% CI, 1.40-2.41 and adjusted HR, 1.82; 95% CI, 1.43-2.31) for all-cause mortality for those with an NLR of at least 2.15 (also shown in eFigure 3 in the Supplement) and at least 1.77, respectively. In this same group, there was a significant association (adjusted HR, 1.65; 95% CI, 1.04-2.60 and adjusted HR, 1.53; 95% CI, 1.02-2.31) of CHD for those with an NLR of at least 2.15 and at least 1.77, respectively (Table 4). There were also 72% and 47% significantly higher HRs (HR, 1.72; 95% CI, 1.08-2.77 and HR, 1.47; 95% CI, 1.08-1.99) for stroke and HF, respectively, for those with high NLR of at least 1.77 (Table 4). There were no significant associations seen with stroke and HF when the high NLR cutoff was set at at least 2.15 (Table 4). Conversely, no significant associations were seen between NLR of at least 1.77 and stroke, all-cause mortality, CHD, and HF in the Duffy antigen–positive (TT/CT) group (Table 4). In the Duffy antigen–positive group, a significant association was only found with CHD and stroke for high NLR of at least 2.15 (Table 4).

Table 4. NLR Association With Each Time-to-Event by Effect Modification by Duffy Antigen Status in JHS

Outcome	HR (95% CI)[a]	*P* Value
TT/CT (n = 1378)		
All-cause mortality		
NLR <2.15	1 [Reference]	.19
NLR ≥2.15	1.28 (0.88-1.87)	
NLR <1.77	1 [Reference]	.19
NLR ≥1.77	0.77 (0.52-1.14)	
CHD		
NLR <2.15	1 [Reference]	.03
NLR ≥2.15	1.92 (1.07-3.45)	
NLR <1.77	1 [Reference]	.08
NLR ≥1.77	1.90 (0.94-3.84)	
Stroke		
NLR <2.15	1 [Reference]	.05
NLR ≥2.15	1.91 (1.01-3.63)	
NLR <1.77	1 [Reference]	.79
NLR ≥1.77	1.10 (0.55-2.19)	
HF		
NLR <2.15	1 [Reference]	.87
NLR ≥2.15	0.96 (0.59-1.56)	
NLR <1.77	1 [Reference]	.83
NLR ≥1.77	1.06 (0.62-1.81)	
CC (n = 3190)		
All-cause mortality		
NLR <2.15	1 [Reference]	<.001
NLR ≥2.15	1.84 (1.40-2.41)	
NLR <1.77	1 [Reference]	<.001
NLR ≥1.77	1.82 (1.43-2.31)	
CHD		
NLR <2.15	1 [Reference]	.03
NLR ≥2.15	1.65 (1.04-2.60)	
NLR <1.77	1 [Reference]	.04
NLR ≥1.77	1.53 (1.02-2.31)	
Stroke		
NLR <2.15	1 [Reference]	.76
NLR ≥2.15	1.10 (0.60-2.01)	
NLR <1.77	1 [Reference]	.02
NLR ≥1.77	1.72 (1.08-2.77)	
HF		
NLR <2.15	1 [Reference]	.10
NLR ≥2.15	1.35 (0.94-1.94)	
NLR <1.77	1 [Reference]	.02
NLR ≥1.77	1.47 (1.08-1.99)	

Abbreviations: CHD, coronary heart disease; HF, heart failure; JHS, Jackson Heart Study; NLR, neutrophil-to-lymphocyte ratio.

[a] Adjusted for sex, smoking, body mass index, history of stroke, history of CHD, diabetes, and blood pressure medication.

To more closely examine the biological basis for the modification of the NLR by genotype in the JHS, NLR was divided into tertiles, and we assessed the mean lymphocyte and neutrophil count by genotype (eTable 9 in the Supplement). Interestingly, both the mean lymphocyte and neutrophil count varied by genotype in each NLR group. Hence, changes in both cell types drive the alterations in NLR observed in homozygous variant carriers. *P* values for trend were calculated using linear models with continuous genotype values (0 for CC, 1 for CT, and 2 for TT). For each tertile and for both lymphocytes and neutrophils, the trend was statistically significant.

Discussion

Neutrophil-to-lymphocyte ratio has been proposed as a reliable systemic inflammatory marker, and higher NLR has been associated with worse prognosis in patients with various forms of CVD.[27-31] For example, NLR is associated with CHD mortality,[32] left atrial thrombus,[33] and myocardial perfusion.[34] Neutrophil-to-lymphocyte ratio has been found to predict CVD mortality better than the Framingham Risk Score model in an asymptomatic general population cohort in the United States.[35] Neutrophil-to-lymphocyte ratio may also be useful when combined with other clinical mortality risk prediction scores or inflammatory markers.[31]

Importantly, there are no universal standardized cutoff values of NLR that determine a health outcome as "normal" or "adverse." Studies often use varying NLR cutoff points and do not consider well-known population differences (ie, Asian and African) in the normal range of neutrophil and lymphocyte counts.[4] Thus, when applying NLR ratios as prognostic markers for health outcomes, it may be appropriate to consider underlying genetic variation, which can affect estimates of what is considered "normal."

Here, we demonstrate that NLR is significantly associated with all-cause mortality in prospective cohorts (JHS and NAS). Our analyses with JHS data show significant associations between NLR (cutoffs of ≥2.15 and ≥1.77) with all-cause mortality and CHD, and the closer median time from the diagnosis of events (all-cause mortality, CHD, and HF) were significantly associated with both NLR points in the JHS. This suggests that the NLR varies over time, consistent with notion that pathogenic inflammatory processes give rise to the elevated NLR, and this measurable biomarker reaches a maximum around the time of an event (ie, death).

Admixture mapping has identified ethnic neutropenia[35-37] to arise as via a single-nucleotide polymorphism (rs2814778), also known as the Duffy antigen variant, on chromosome 1q22.[16,36,37] This gene is a 7-transmembrane receptor that binds inflammatory CXC and CC chemokines involved in neutrophil recruitment.[16] In JHS, African American individuals who were Duffy antigen negative had a lower baseline NLR compared with those who were Duffy antigen positive. Thus the lower determined NLR cutoff of at least 1.77 showed significant associations with all outcomes: all-cause mortality, CHD, stroke, and HF among those with benign neutropenia. Using the higher NLR cutoff of at least 2.15 revealed significant associations only with all-cause mortality and CHD among those with benign neutropenia. Hence, the cutoff determination for this type of analysis will affect the sensitivity and specificity of the biomarker, suggesting the need to consider racial/ethnic origin in applying this clinically.

The association of NLR with CVD, overall mortality, and cancer survival has generally been thought to be driven by chronic inflammation. However, work published within the last several years suggests a more specific mechanism; it is now known that numerous chronic disease states (including cancer,[38] hypertension,[32] and heart disease[39]) stimulate the release of granulocytic myeloid-derived suppressor cells (gMDSCs) from the bone marrow. These cells are immunoregulatory and are well known to suppress the lymphocyte response.[40] As a result, it is reasonable to speculate that the NLR is a measure of the phenotypic activity of gMDSCs that are released as a result of chronic disease insult. The increase in the neutrophils is observed because gMDSCs elaborated from the bone marrow can increase to some 10% of the peripheral blood leukocytes. Their regulatory action simultaneously suppresses the numbers of lymphocytes observed in the peripheral blood, such that a high NLR reflects the phenotype of the gMDSC. Interestingly, this is consistent with our observations (eTable 9 in the Supplement) and could suggest a mechanism to explain the interaction we observed; the elaboration of gMDSCs from the bone marrow may not differ by genotype. If this were true, one might expect a larger suppressive effect of the gMDSCs because they would be in relative excess (relative to granulocytes) in the Duffy antigen–positive group.

Limitations

The limitations of this study include the fact that we had data from only 1 complete blood cell count. Patients with ST-elevation myocardial infarction have peripheral leukocyte counts that increase within hours after the onset of chest pain, and because neutrophils are known to have shorter lifespan with a rapid turnover, serial neutrophil counts may be of considerably more benefit than a single measurement at the time of admission.[31] In addition, a 2017 study has shown that baseline NLR and the difference in NLR (from baseline) were significant independent prognostic factors for patients with advanced pancreatic cancer.[41] Furthermore, NLR can differ drastically depending on timing with respect to underlying health (ie, blood samples collected at home or on a regular clinical visit vs collected during hospitalization).[4] It has been suggested that the ideal timing to measure and use NLR would be proximal to an event, although prospective data are lacking for this assertion.[3,4] Hence, future work is needed to further validate our results, studying NLR over time in other prospective cohort studies investigating CVD as well as cancer.

Conclusions

The closer median time from the diagnosis of event modified the strength of the association of NLR with all-cause mortality, CHD, and HF in the prospective cohort study, JHS. This suggests that the NLR increases over time in individuals with CVD and reaches a maximum value around the time of an event. We have also observed that the Duffy antigen variant interacts with NLR (≥1.77) in predicting all-cause mortality, CHD, stroke, and HF in people with the Duffy antigen negative genotype. Simple calculation of NLR from a complete blood cell count by clinicians treating patients with CVD may add some information to the overall assessment of future risk of adverse outcomes, keeping in mind that this could be modified by the Duffy antigen variant.

ARTICLE INFORMATION

Accepted for Publication: March 14, 2018.

Published Online: May 2, 2018. doi:10.1001/jamacardio.2018.1042

Author Contributions: Dr Kelsey had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Wu, Kelsey.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Kim, Koestler, Kelsey.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Kim, Eliot, Kelsey.
Administrative, technical, or material support: Kim, Kelsey.
Supervision: Koestler, Wu, Kelsey.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: The Jackson Heart Study is supported and conducted in collaboration with Jackson State University (HHSN268201300049C and HHSN268201300050C), Tougaloo College (HHSN268201300048C), and the University of Mississippi Medical Center (HHSN268201300046C and HHSN268201300047C) contracts from the National Heart, Lung, and Blood Institute and the National Institute for Minority Health and Health Disparities. This work was supported by National Institutes of Health grant R01363267.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Disclaimer: The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the US Department of Health and Human Services.

Additional Contributions: We thank the staffs and participants of the Jackson Heart Study. No compensation was received from a funding sponsor for such contributions.

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