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ORIGINAL ARTICLE

Relative Predictive Value of Circulating Immune Markers in US Adults Without Cardiovascular Disease: Implications for Risk Reclassification

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Abstract

Objective: To investigate the relative predictive value of circulating immune cell markers for cardiovascular mortality in ambulatory adults without cardiovascular disease.

Methods: We analyzed data of participants enrolled in the National Health and Nutrition Examination Survey from January 1, 1999, to December 31, 2010, with the total leukocyte count within a normal range (4000-11,000 cells/μL [to convert to cells $\times 10^9$/L, multiply by 0.001]) and without cardiovascular disease. The relative predictive value of circulating immune cell markers measured at enrollment—including total leukocyte count, absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, monocyte-lymphocyte ratio (MLR), neutrophil-lymphocyte ratio, and C-reactive protein—for cardiovascular mortality was evaluated. The marker with the best predictive value was added to the 10-year atherosclerotic cardiovascular disease (ASCVD) risk score to estimate net risk reclassification indices for 10-year cardiovascular mortality.

Results: Among 21,599 participants eligible for this analysis, the median age was 47 years (interquartile range, 34-63 years); 10,651 (49.2%) participants were women, and 10,713 (49.5%) were self-reported non-Hispanic white. During a median follow-up of 9.6 years (interquartile range, 6.8-13.1 years), there were 627 cardiovascular deaths. MLR had the best predictive value for cardiovascular mortality. The addition of elevated MLR ($\geq$0.3) to the 10-year ASCVD risk score improved the classification by 2.7%±1.4% (P=.04). Elevated MLR had better predictive value than C-reactive protein and several components of the 10-year ASCVD risk score.

Conclusion: Among ambulatory US adults without preexisting cardiovascular disease, we found that MLR had the best predictive value for cardiovascular mortality among circulating immune markers. The addition of MLR to the 10-year risk score significantly improved the risk classification of participants.

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Activation of the immune system has been described in cardiovascular diseases, such as atherosclerosis, acute coronary syndrome, and heart failure.[1-4] The associated immune markers may have prognostic significance to detect long-term cardiovascular events. However, the widely used cardiovascular risk prediction models do not include any immune markers.[5,6]

Circulating immune-inflammatory markers such as C-reactive protein (CRP), total leukocyte count, and differential leukocyte count are widely available, and studies

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report their independent association with adverse cardiovascular outcomes.[1-3,7,8] However, their ability to predict the long-term risk of cardiovascular mortality in a population without cardiovascular disease remains unknown. Furthermore, no previous study has compared the relative predictive values of these circulating immune markers. Finally, it remains unclear how the predictive value of circulating immune markers compares with that of traditional cardiovascular risk factors such as age, hypertension, and dyslipidemia.

We investigated the role of widely available circulating immune cell markers to predict cardiovascular mortality in a representative ambulatory US adult population without cardiovascular disease. We hypothesized that circulating immune cell markers in the normal range are differentially associated with cardiovascular risk. We aimed to rank their relative predictive value and to explore how the addition of these circulating immune cell markers to current prediction models modifies cardiovascular risk classification.

METHODS

The study was conducted following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (Supplemental Table 1, available online at http://www.mayoclinicproceedings.org).

Study Design and Participants

The National Health and Nutrition Examination Survey (NHANES) is a serial cross-sectional survey designed to gather data about the health status of the resident civilian noninstitutionalized US population. The details of the interview, laboratory evaluation, and physical examination have been published previously[9] and are given in the Supplemental Methods (available online at http://www.mayoclinicproceedings.org). The data are linked to the National Death Index through December 31, 2011. We used publicly available deidentified data. Therefore, approval from the University of Alabama at Birmingham Institutional Review Board was not required. Data from participants aged 18 years and older from 6 NHANES cycles from 1999 to 2010 were used for the analysis (Figure 1).

We excluded participants with preexisting cardiovascular disease (self-reported angina, heart attack, coronary artery disease, heart failure, stroke, or pacemaker) or total leukocyte count beyond the normal range (<4000 cells/μL or >11,000 cells/μL [to convert to cells $\times 10^9$/L, multiply by 0.001]). Other exclusion criteria are given in the Supplemental Methods.

Study Variables

Several circulating immune markers, such as total and differential leukocyte count and CRP, used in this investigation were available from the blood sample collected at the time of the visit to the mobile examination center during NHANES. Details are given in the Supplemental Methods and Supplemental Table 2 (available online at http://www.mayoclinicproceedings.org). We computed the neutrophil-lymphocyte ratio by dividing the absolute neutrophil count by the absolute lymphocyte count and the monocyte-lymphocyte ratio (MLR) by dividing the absolute monocyte count by the absolute lymphocyte count. Both these ratios are independently associated with worse cardiovascular prognosis.[10,11]

Demographic and Clinical Information

Data on other demographic and clinical characteristics were collected during either the interview or the visit to the mobile examination center. The variable codes and diagnostic criteria to define comorbidities are given in the Supplemental Methods and Supplemental Table 2.

Study Outcome

Cardiovascular mortality during follow-up was the outcome for all survival analyses. Cardiovascular mortality that occurred within 10 years of the visit to the mobile examination center was the outcome for all logistic models. The cause of death was derived from linked death certificate records from the National Death Index.[12] Details are given in the Supplemental Methods and

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Supplemental Table 2. Follow-up time was taken as time from mobile examination center date to the date of death or end of mortality period.

Statistical Analyses

Continuous variables were reported as medians with interquartile ranges (IQRs), and categorical variables were represented as counts with proportions. The Wilcoxon rank sum test and χ^2 test were used to identify the differences in baseline characteristics in continuous and categorical variables, respectively. We estimated the 10-year atherosclerotic cardiovascular disease (ASCVD) and Framingham risk scores for all participants.[5,6] For calculation, participants younger than 40 years or older than 79 years were considered to be 40 years old and 79 years old, respectively. The baseline characteristics of the participants with age younger than 40 years, 40 to 79 years, and older than 79 years are given in Supplemental Table 3 (available online at http://www.mayoclinicproceedings.org).

The 10-year ASCVD and Framingham risk scores varied across the NHANES cycle (Supplemental Table 4, available online at http://www.mayoclinicproceedings.org).

We assessed the relative predictive value of circulating immune markers using the Cox proportional hazards model and the logistic regression model. Details are given in the Supplemental Methods. The optimal cutoff of the circulating immune marker with the best predictive value for 10-year cardiovascular mortality was estimated using the Youden and Liu index.[13,14] We then evaluated the independent association of the marker with the best predictive value at a categorical cutoff ascertained in the previous step. We used both the Cox proportional hazards model and competing risk regression analysis with noncardiovascular mortality as a competing risk for cardiovascular mortality in the adjusted model. The adjusted Cox proportional hazards model included the following covariates: age, sex, race, hypertension, hemoglobin, diabetes mellitus, chronic obstructive pulmonary disease, current smoking, dyslipidemia, estimated glomerular filtration rate, malignant disease, obesity, and NHANES cycle. The variables in multivariate models were included if they were established risk factors for cardiovascular events or associated with inflammation and cardiovascular mortality. We also included the NHANES cycle as a covariate as sampling strategy changed in NHANES over the years, which likely led to variation in 10-year ASCVD risk across the NHANES cycles. The proportionality assumption was verified using the Schoenfeld residuals.[15] We determined whether there was a dose-response relationship of cardiovascular mortality with the marker with the best predictive value. Poisson regression analyses were used to estimate the incident rate (per 100 person-years) of cardiovascular mortality. Missing data for obesity (n=291 [1.4%]) and estimated glomerular filtration rate (n=54 [0.3%]) were imputed using multivariate chained equations with age, sex, and race as predictors.[16] There was no difference in the central tendency, spread, and predictive ability

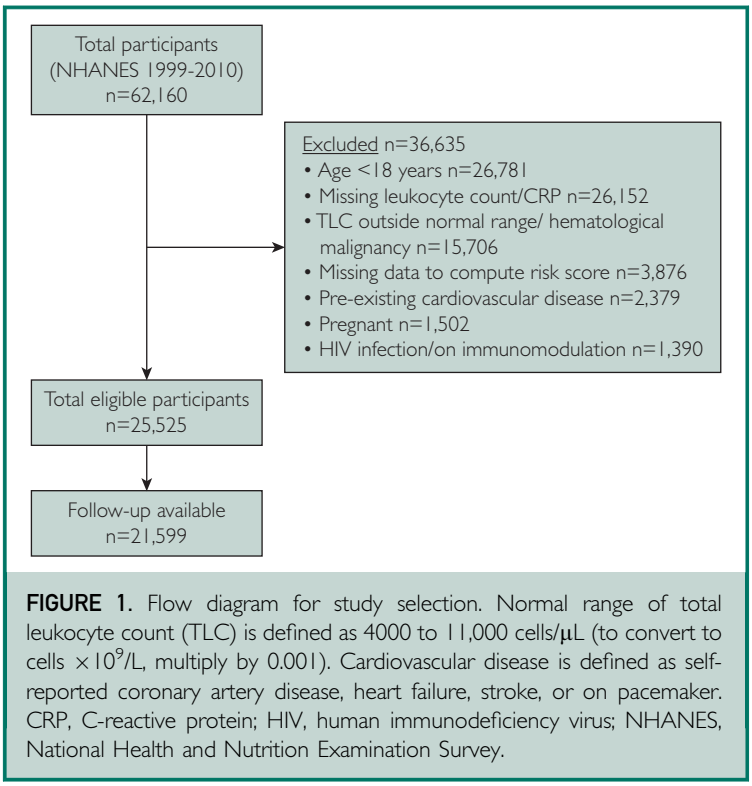

FIGURE 1. Flow diagram for study selection. Normal range of total leukocyte count (TLC) is defined as 4000 to 11,000 cells/μL (to convert to cells $\times 10^9$/L, multiply by 0.001). Cardiovascular disease is defined as self-reported coronary artery disease, heart failure, stroke, or on pacemaker. CRP, C-reactive protein; HIV, human immunodeficiency virus; NHANES, National Health and Nutrition Examination Survey.

in the Cox model between the imputed and the unimputed variables (Supplemental Table 5, available online at http://www.mayoclinicproceedings.org).

We also determined the independent prognostic importance of all circulating immune markers using the Cox proportional hazards model to predict cardiovascular mortality.

Reclassification and Comparison With CRP and Components of ASCVD and Framingham Risk Scores

We then estimated the net reclassification index (NRI) after adding the circulating immune marker with the best predictive value in the same model as the 10-year ASCVD risk score and the Framingham risk score with a prespecified cutoff of 5% (low risk).[17] NRI helps compare the discriminative ability of 2 risk prediction models by logistic regression.[17] The reclassification indices for both scores were also estimated after the addition of CRP, given that it is the most commonly used inflammatory marker to predict cardiovascular outcomes.[18] Furthermore, we ranked the predictive value of the components in the 10-year ASCVD risk score, the Framingham risk score, and the circulating immune marker for 10-year cardiovascular mortality. These factors were ranked by the likelihood and Wald χ^2 statistics for the Cox proportional hazards analyses and by the area under the curve and standardized domination statistic for the logistic regression analyses.[11,12] All statistical analyses were performed in Stata/SE version 15.1 (StataCorp). All *P* values were 2 sided, with *P* value less than .05 considered statistically significant.

RESULTS

Baseline Characteristics

Among 62,160 participants in 6 NHANES cycles spanning 1999 to 2010, there were 21,599 participants (34.7%) included in the analyses (Figure 1). The baseline characteristics of the study population are given in the Table.

The median age of the participants was 47 years (IQR, 34-63 years), with 10,651 (49.2%) women and 10,713 (49.5%) self-identified as non-Hispanic white. The prevalence of diabetes mellitus, hypertension, and dyslipidemia was 13.8%, 52.8%, and 65.0%, respectively (Table). The median leukocyte count was 6900 cells/μL (IQR, 5700-8100 cells/μL). The median absolute neutrophil and monocyte counts were 4000 cells/μL (IQR, 3100-4900 cells/μL) and 500 cells/μL (IQR, 400-600 cells/μL), respectively. The median 10-year ASCVD risk score and Framingham risk score was 5% (IQR, 1.6%-16.7%) and 7.4% (IQR, 2.0%-21.9%), respectively.

The participants in the assembled cohort were observed for a median of 9.6 years (IQR, 6.8-13.1 years). Cause of death was available in 99.8% of participants. There were 627 deaths due to cardiovascular causes at an incident rate of 0.29 per 100 person-years (95% CI, 0.27-0.32). Of these, 509 (81.2%) cardiovascular deaths were within 10 years of follow-up.

Relative Predictive Value of Circulating Immune Markers

The MLR had the best predictive value for 10-year cardiovascular mortality (Figure 2) in both time to event (using likelihood and Wald χ^2 statistics) and logistic regression analyses (area under the curve and standardized domination statistic). The risk of cardiovascular mortality was higher with 1 SD rise of MLR (β=.34) compared with 1 SD rise of CRP (β=.08; Wald χ^2 for comparison of β in survival model = 71.30; P<.001). The optimal cutoff of the MLR to classify patients with the highest accuracy for the risk of 10-year cardiovascular mortality using the Youden index was 0.30 (Supplemental Figure 1, available online at http://www.mayoclinicproceedings.org). This cutoff had a sensitivity and specificity of 55% and 69%. Participants with an elevated MLR (≥0.3) had a higher risk of cardiovascular mortality in the adjusted analysis (hazard ratio, 1.36; 95% CI, 1.15-1.60; P<.001; Figure 3A) and the competing risk analysis with noncardiovascular mortality as a

competing risk (hazard ratio, 1.29; 95% CI, 1.09-1.52; P=.003; Figure 3B) (all P<.05). The effect estimates remained robust in the adjusted model with unimputed data (hazard ratio, 1.37; 95% CI, 1.15-1.63; P<.001). There was a dose-response relationship such that the incidence of cardiovascular mortality increased by 13% (95% CI, 6-21) for every 1 unit increase in the MLR in the adjusted model (Figure 4).

All immune markers except absolute lymphocyte count were independently associated with cardiovascular mortality (Supplemental Table 6, available online at http://www.mayoclinicproceedings.org).

Reclassification and Comparison With CRP and Components of ASCVD and Framingham Risk Scores

Baseline demographic, clinical, and laboratory characteristics of participants by low (<0.3) or elevated (≥0.3) MLR are given in Supplemental Table 7 (available online at http://www.mayoclinicproceedings.org). The participants with elevated MLR were older and included a higher representation of men and non-Hispanic whites and individuals with hypertension, smoking, and malignant disease. Participants in the elevated MLR group had a significantly higher 10-year ASCVD score (8.3% vs 4.2%; P<.001) and Framingham risk score (12.1% vs 6.0%; P<.001).

Addition of elevated MLR to the categorical 10-year ASCVD risk score correctly up-classified 3.2% of participants with cardiovascular mortality (49.3% to 52.2%) and incorrectly up-classified 0.4% of participants with no cardiovascular mortality (8.8% to 9.2%). There was a significant improvement in risk classification (NRI, 2.7±1.4; P=.04; Supplemental Figure 2A, available online at http://www.mayoclinicproceedings.org). Adding elevated CRP to the categorical 10-year ASCVD risk score had no impact on the risk classification (NRI, −0.2±0.0; P=.83; Supplemental Figure 2B). Risk reclassification with addition of other circulating immune markers to the 10-year ASCVD risk score with different

TABLE. Baseline Demographic, Clinical, and Laboratory Parameters in the Assembled NHANES Cohort (1999-2010)[a,b,c]

Factor	Overall (N=21,599)
Demographic parameters	
Age (y)	47.0 (34.0-63.0)
Women	10,651 (49.2)
Race	
Non-Hispanic white	10,713 (49.5)
Non-Hispanic black	3887 (18.0)
Mexican American	4660 (21.5)
Other Hispanic	1544 (7.1)
Other race, including multiracial	845 (3.9)
Anthropometry	
Weight (kg)	77.8 (66.4-91.0)
Height (m)	1.7 (1.6-1.8)
Body mass index (kg/m^2)	27.6 (24.2-31.6)
Comorbidities	
Diabetes mellitus	2977 (13.8)
Dyslipidemia	14,082 (65.0)
Hypertension	11,441 (52.8)
Systolic blood pressure (mm Hg)	122.0 (112.0-135.0)
Diastolic blood pressure (mm Hg)	72.0 (64.0-79.0)
Smoking	10,143 (46.9)
Chronic obstructive pulmonary disease	122 (0.6)
Malignant disease	750 (5.7)
Obesity	7104 (33.3)
Laboratory parameters	
Hemoglobin (g/dL)	14.4 (13.4-15.4)
Platelet count (cells ×10^6/μL)	260.0 (223.0-305.0)
Estimated GFR (mL/min/1.73 m^2)[d]	99.7 (82.1-122.0)
Circulating immune markers	
C-reactive protein (mg/dL)	0.2 (0.1-0.4)
Total leukocyte count (cells ×10^3/μL)	6.9 (5.7-8.1)
Absolute lymphocyte count (cells ×10^3/μL)	2.0 (1.7-2.5)
Absolute neutrophil count (cells ×10^3/μL)	4.0 (3.1-4.9)
Absolute monocyte count (cells ×10^3/μL)	0.5 (0.4-0.6)
Absolute eosinophil count (cells ×10^3/μL)	0.2 (0.1-0.2)
Absolute basophil count (cells ×10^3/μL)	0.0 (0.0-0.1)
Neutrophil-lymphocyte ratio	2.0 (1.5-2.6)
Monocyte-lymphocyte ratio	0.3 (0.2-0.3)
Risk score	
10-year ASCVD risk[e] (%)	5.0 (1.6-16.7)
10-year Framingham risk (%)	7.4 (2.0-22.0)

[a]ASCVD, atherosclerotic cardiovascular disease; GFR, glomerular filtration rate; NHANES, National Health and Nutrition Examination Survey.

[b]To convert hemoglobin values to g/L, multiply by 10; to convert platelet count to cells ×10^9/L, multiply by 0.001; to convert C-reactive protein values to nmol/L, multiply by 95.24; to convert white blood cell counts to cells ×10^9/L, multiply by 0.001.

[c]Data are represented as median (25th-75th percentile) or number (%).

[d]GFR estimated by the Modification of Diet in Renal Disease 4-component study equation.

[e]For calculation of 10-year ASCVD risk, participants aged <40 years (n=7,547 [34.9%]) or >79 years (n=1,315 [6%]) were considered to be 40 and 79 years old, respectively. Neutrophil-lymphocyte and monocyte-lymphocyte ratio derived by dividing absolute neutrophil count and absolute monocyte count by absolute lymphocyte count, respectively.

statistical models is given in Supplemental Table 8 (available online at http://www.mayoclinicproceedings.org).

Similarly, adding elevated MLR to the 10-year Framingham risk score significantly improved the reclassification by correctly up-classifying 4% of participants with cardiovascular mortality (NRI, 4.0±1.2; P=.001). Adding elevated CRP had no impact on the risk classification (NRI, 0.3±0.7; P=.67).

Comparing the Relative Predictive Value of MLR With Components of ASCVD and Framingham Risk Scores

We ranked the predictive value of the 9 components of the 10-year ASCVD risk score and elevated MLR for cardiovascular mortality. Age had the highest predictive value in all models. The MLR ranked fifth and had a higher predictive value than race, smoking status, high-density lipoprotein level, and total cholesterol concentration in both the time to event and logistic regression models (Supplemental Figure 3, available online at http://www.mayoclinicproceedings.org). Similarly, it ranked fifth compared with the components of the Framingham risk score.

DISCUSSION

We found that multiple circulating immune markers were independently associated with cardiovascular mortality during a median follow-up of 9.6 years among ambulatory US adults without preexisting cardiovascular disease and a total leukocyte count within the normal range. Among the available circulating immune markers, the MLR had the best predictive value. The MLR significantly improved the classification power of the 10-year ASCVD risk score and the Framingham risk score to predict cardiovascular mortality and performed better than CRP as a predictor. In addition, it had greater prognostic importance than traditional cardiovascular risk factors such as race, smoking status, high-density lipoprotein level, and total cholesterol concentration.

Immune cells and inflammatory markers have an important pathophysiologic role in cardiovascular diseases. They are influenced by myocardial stressors such as ischemia, volume overload, or pressure overload and by comorbidities commonly associated with cardiovascular diseases, such as dyslipidemia and obesity.[19,20] At a cellular level, there is an increasing appreciation of the interplay between cardiac myocytes and immune cells during physiologic and pathologic states. Extant literature suggests that there is an initial infiltration of blood neutrophils and then proinflammatory monocytes and monocyte-derived macrophages to clear necrotic debris after myocardial injury.[21,22] Thereafter, tissue macrophages transition to a phenotype of inflammation resolution and tissue repair. This complex inflammatory response, initially directed at myocardial repair, may become dysregulated and cause adverse cardiac remodeling and worsen prognosis.[23] These observations lend biologic credence to our findings whereby both neutrophils and monocytes were independent risk factors for increased cardiovascular mortality.

The critical role of monocytes in the pathophysiologic process of cardiovascular diseases such as atherosclerosis, heart failure, and atrial fibrillation is well described.[24,25] Distinct subsets of circulating monocytes with a proinflammatory or reparative phenotype have been identified in humans and mouse models.[21,26] The elevated risk of cardiovascular death with elevated neutrophil and monocyte counts in our study may reflect ongoing myocardial and vascular damage or dysregulated inflammation in ambulatory adults without preexisting cardiovascular disease.

We also found that a low lymphocyte count is not an independent risk factor for cardiovascular mortality, in contrast to previous reports in advanced heart failure.[27] The association of a lower lymphocyte count with cardiovascular mortality was not observed in the fully adjusted model, suggesting that the adverse prognostic role of decreased lymphocytes is likely to be mediated (or modulated) by other covariates. Increased cortisol production during stress shifts the leukocyte production in favor of neutrophils and monocytes over lymphocytes.[28] This might explain why the

neutrophil-lymphocyte ratio and MLR may be a better marker of inflammation than absolute neutrophil or monocyte count.

Elevated CRP is a widely used marker for adverse cardiovascular outcomes, both in the general population and in patients with cardiovascular disease. Furthermore, CRP is the most widely used inflammatory marker in cardiovascular risk prediction models.[7,8] The exact role of CRP in the pathogenesis of cardiovascular diseases is not clear. In atherosclerosis, it has been proposed that some isoforms of CRP drive increased expression of proinflammatory adhesion molecules that mediate the migration of monocytes to atheromatous plaques.[29] Furthermore, activated monocytes in the arterial wall have been reported to be critical for hypertension-induced vascular remodeling in humans.[30] These observations together with a weaker association between CRP and cardiovascular mortality in our study led us to speculate that CRP may not reliably indicate tissue-level immune cell recruitment and dysregulated responses compared with circulating immune cells. Another possibility is that CRP may not be as reliable as MLR as a marker of nonatherosclerotic (such as heart failure and cardiometabolic disease) myocardial insult. Furthermore, the monocytes recruited to the myocardium may be the final pathway after myocardial damage leading to adverse myocardial remodeling. Thus, monocytes have a higher predictive value than lipoproteins that drive atherosclerosis and may associate with the severity of atherosclerosis at the time of measurement in those without established cardiovascular disease.[31,32] Our study finds that MLR may be a better risk prognosticator than CRP in those without prevalent CVD while validating the association of CRP with cardiovascular events. Collectively, these conceivable but hypothesis-generating observations may explain the higher predictive value of the MLR than of CRP and lipoproteins to predict cardiovascular mortality in our study.

The adverse prognostic implications of readily available circulating immune cell markers for adverse cardiovascular outcomes in healthy participants have been previously reported in separate studies.[1,3,7,8,10,11] Despite their independent adverse prognostic role, traditional risk prediction models, such as the 10-year ASCVD risk score, do not include any immune markers.[6] To the best of our knowledge, this is the first time that the relative contribution of total and differential leukocyte counts, neutrophil-lymphocyte

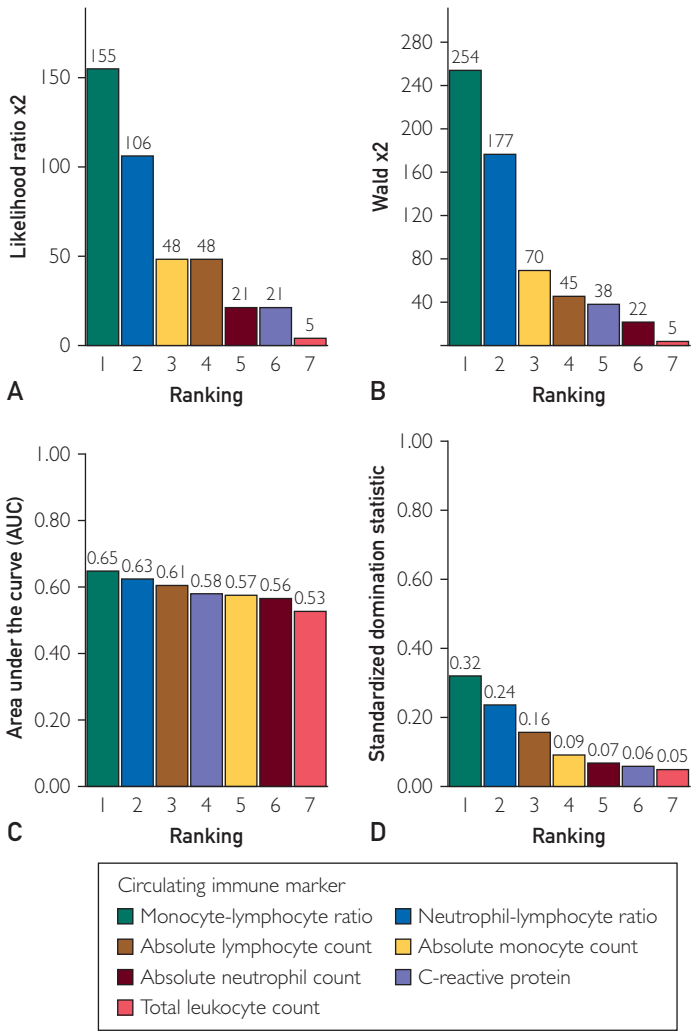

FIGURE 2. The relative predictive value of circulating immune markers to predict CV mortality. The circulating immune markers are arranged in decreasing order of importance. Likelihood ratio χ^2 using Cox Model to predict CV mortality (panel A). Wald χ^2 using Cox Model to predict CV mortality (panel B). The AUC using a logistic model to predict 10-year CV mortality (panel C). Standardized domination statistic using a logistic model to predict the relative importance of immune markers for 10-year CV mortality (panel D).

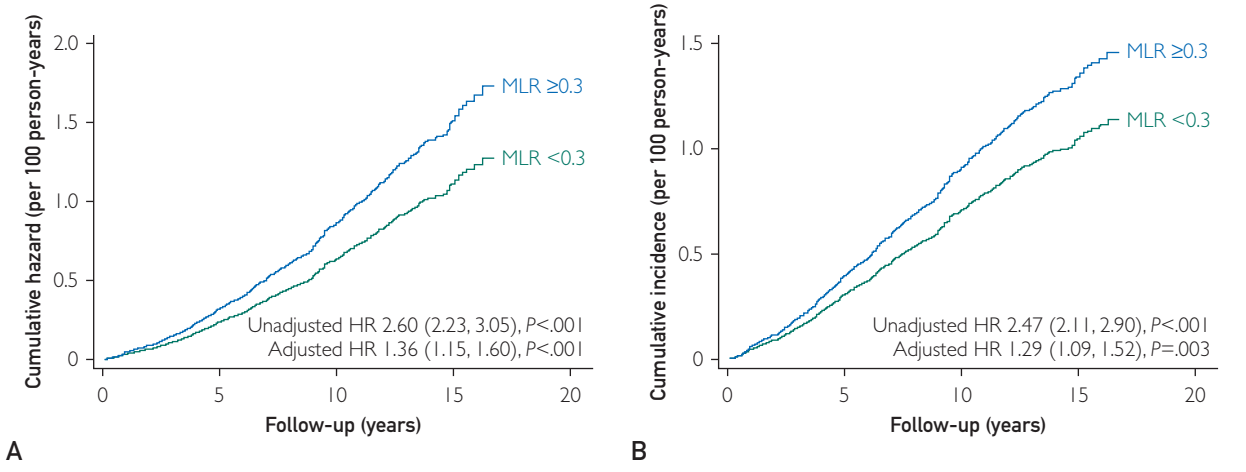

FIGURE 3. Risk of cardiovascular mortality stratified by monocyte-lymphocyte ratio (MLR) in the Cox proportional hazards model (A) and competing risk regression model with noncardiovascular mortality as competing risk (B) adjusted for age, sex, race, hypertension, hemoglobin, diabetes mellitus, chronic obstructive pulmonary disease, current smoking, dyslipidemia, estimated glomerular filtration rate, malignant disease, obesity, and National Health and Nutrition Examination Survey cycle. HR, hazard ratio.

ratio and MLR, and CRP have been compared in a single risk prediction model with an established risk score to predict 10-year cardiovascular mortality. Randomized controlled trials have suggested a beneficial role of immunomodulation in secondary prevention to reduce adverse cardiovascular events.[33,34] Future studies in other prospective cohorts are needed to validate these findings and to investigate whether immunomodulation has a role in the primary prevention of cardiovascular disease in individuals with a cardioinflammatory phenotype. Immune markers, such as MLR, can help identify a subset of patients without cardiovascular disease who may benefit from immunomodulation.

Our study has important limitations. The baseline values of circulating immune markers were used in the prediction model. We could not correlate temporal trends of immune markers with cardiovascular mortality because of a lack of serial measurements in the NHANES. Even though we excluded participants with leukocyte counts outside the normal range, the measures of circulating immune markers could have been confounded by an acute illness at the time of enrollment. We used a self-reported history of heart attack, angina, coronary heart disease, heart failure, stroke, and pacemaker at baseline to exclude patients with preexisting cardiovascular disease that may have resulted in bias. However, previous data show good reliability and validity of self-reported comorbidities in the NHANES data.[35] In the study population, 41% of participants were outside the age range of ASCVD (<40 years or >79 years); the 10-year ASCVD risk score estimation in these participants may have overestimated

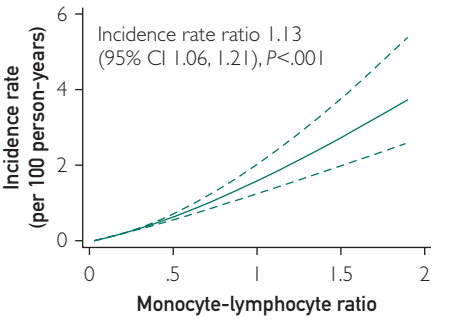

FIGURE 4. Dose-response relationship between monocyte-lymphocyte ratio and cardiovascular mortality. The solid green line represents the incidence rate, and the dashed green lines represent the 95% confidence interval (CI). There was a linear increase in the risk of cardiovascular mortality with increase in the monocyte-lymphocyte ratio.

or underestimated the cardiovascular risk. The 10-year ASCVD risk score predicts future risk of nonfatal myocardial infarction or coronary heart disease death or fatal or nonfatal stroke during 10 years.[6] However, because of the lack of other outcomes in the NHANES, we used cardiovascular mortality as an outcome, and it is plausible that the 10-year ASCVD risk score overestimated the risk. However, the fact that the up-classification of risk of cardiovascular mortality was better than the ASCVD score indicates that it is unlikely that the results observed in our study were simply due to overestimation of risk.

CONCLUSION

Several circulating immune markers are independently associated with cardiovascular mortality in ambulatory US adults. The MLR has the best predictive value for cardiovascular mortality among readily available circulating immune markers. Further study is needed to confirm our findings and to understand the pathobiologic mechanism linking immune cells and cardiovascular risk.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: **ASCVD** = atherosclerotic cardiovascular disease; **CRP** = C-reactive protein; **IQR** = interquartile range; **MLR** = monocyte-lymphocyte ratio; **NHANES** = National Health and Nutrition Examination Survey; **NRI** = net reclassification index

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