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Intermediate-Risk Chronic Stable Angina: Neutrophil-Lymphocyte Ratio and Fibrinogen Levels Improved Predicting Angiographically-Detected Coronary Artery Disease

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Abstract

Background: Coronary heart disease (CHD) is the leading cause of death worldwide. Research indicates that coronary atherosclerosis is the most frequent cause of CHD. Evidence is scarce concerning the clinical efficacy of fibrinogen or neutrophil-lymphocyte ratio (NLR) measurement in risk-stratifying patients with chronic stable angina.
Objectives: To examine the independent and incremental prognostic value of fibrinogen and neutrophil-lymphocyte ratio (NLR) for angiographically-detected coronary artery disease (CAD).
Patients and Methods: In this cross-sectional study, angiography was performed for 183 Iranian patients with chronic stable angina with exercise ECG-determined intermediate risk. Generalized estimated equations were used to obtain the odd ratio (OR) of CAD for a 1-unit increase in log-NLR and a 1-SD increase in plasma fibrinogen. Models were adjusted for established CAD risk factors. Integrated discriminatory improvement index (IDI) and net reclassification improvement index (NRI) were used as measures of predictive ability for CAD, combined with traditional risk factors by NLR and fibrinogen.
Results: The mean age of the participants was 57.5, with 51.9% being male. Only 12% of participants had angiographically-determined patent coronary arteries. The number of participants with one, two, and three-vessel stenosis were 76, 31, 31, respectively, while 45 did not have stenosed vessels. NLR and fibrinogen levels were significantly higher in patients with stenosis in two (2.4 and 512 $mg.dL^{-1}$) or three (2.6 and 517 $mg.dL^{-1}$) coronary arteries, as compared to the group of patients with no significant involvement (2 and 430 $mg.dL^{-1}$) (all $P < 0.01$). Patients with a higher NLR and a higher fibrinogen levels were more likely to have higher grades of CAD. OR log-NLR = 1.36 (95% CI: 1.05 - 1.94) and OR Z-Fibrinogen = 1.61 (95% CI: 1.18 - 2.22). When NLR and fibrinogen were added to the traditional risk factors separately, the NRIs were 0.170 (0.023 - 0.324) and 0.380 (0.214 - 0.543), respectively. The NRI was 0.460 (0.303 - 0.620) when both NLR and fibrinogen added to traditional risk factors simultaneously.
Conclusions: NLR and fibrinogen predicted CAD, independent of traditional CAD risk factors. Both measures (whether separately or together) substantially enhanced the predictive performance of traditional risk factors for identifying patients with CAD.

Keywords: Angiography, Atherosclerosis, Fibrinogen, Lymphocyte, Neutrophil

1. Background

Coronary heart disease (CHD) is the leading cause of death worldwide (1-3). Coronary atherosclerosis is the most frequent cause of CHD (4, 5). Atherosclerosis without thrombosis is manifested by chronic stable angina, which can be complicated by acute thrombosis, which can cause unstable angina, myocardial infarction, and sudden death (5, 6). Inflammatory factors have been implicated in the pathogenesis of acute cardiovascular events (7-9).

Fibrinogen measurements has been suggested to refine the overall risk profiles of individuals and to better design preventive interventions. Patients at risk for fibrinogen-related ischemic complications of atherosclerosis can be easily identified, and treatment strategies have been developed to protect against these complications (10). Researchers have acknowledged that it is time to shift some attention away from lipid-lowering drugs and toward therapies that fight vascular inflammation (11). The white blood cell count, first introduced in 1974 as an independent risk factor for coronary heart disease (12), has been investigated extensively for varying CVD outcomes in both sexes and across populations of different ethnicities (13-16). The most comprehensive assessment of specific leucocyte components in CHD has been performed by Wheeler et al., who found the neutrophil count to be more strongly associated with CHD than other leukocyte components (17). Findings, however, have not been conclusive across other studies (18). It remains to be determined whether the neutrophil dysfunction in coronary artery disease (CAD) is mainly a marker of chronic disease, an atherogenic factor, or a consequence of the drug treatment (19).

Previous studies have largely been conducted among apparently healthy subjects. Evidence is scarce concerning the clinical usefulness of fibrinogen or neutrophil-lymphocyte ratio (NLR) measurement in risk-stratifying patients with chronic stable angina.

2. Objectives

This study therefore sought to examine the independent and incremental prognostic value of angiographically-detected CAD among patients with chronic stable angina.

3. Patients and Methods

3.1. Study Population

Between 15 July 2012 and 15 July 2013, all patients seeking care from the Imam Hospital cardiology clinic, Ahvaz, Iran, were sequentially included in a cross-sectional study and evaluated for chronic stable angina. Patients with the following criteria were recruited for the study: patients with chronic stable angina, confirmed by chest discomfort, and with normal resting electrocardiogram (ECG). Exclusion criteria for this study was based on the self-report of myocardial infarction (MI) or previous revascularization, and any patients whose laboratory findings diagnosed MI, e.g. normal plasma levels of creatinine phosphokinase, muscle type B (CPKMB) and troponin, or also those who were pregnant or lactating. To predict the presence and extent of CAD, all patients whose chest pain was compatible with chronic stable angina were offered a cardiac exercise tolerance tests if they were able to exercise adequately. A standard treadmill exercise ECG test was performed and the Duke treadmill score (DTS) was calculated. We excluded patients who were at low or high risk according to their DTS. The final sample consisted of 183 participants (51 females) with DTS-determined intermediate risk.

Imam hospital is a general, governmental, and referral hospital, located in the center of the Ahvaz city. It has 600 beds and 15 sections.

3.2. Measurements

A trained interviewer collected information using a questionnaire. The information obtained included demographic data, past medical history of CVD, and smoking status. After a 15-minute rest in a sitting position, blood pressure was measured twice, on the right arm, using a standardized mercury sphygmomanometer. The mean of the two measurements was considered the participant's blood pressure.

3.3. Diagnostic Angiography

Diagnostic angiography was performed on all participants. Angiographies were performed using Judkins right and left catheters through the femoral arteries, and with a Siemens Axiom Artis DFC ZEE floor-mounted device. Interpretation of angiographs was performed by two expert cardiologists who were blinded for the results of blood tests (WBC and fibrinogen levels). The quantification analysis of obstructions after calibration of the catheter was performed by moving the curser from the proximal to the distal of each vessel to reveal the length and severity of the obstruction. All three coronary arteries (right coronary artery, [RCA], left coronary artery [LCA] and lateral circumflex [LC]) were evaluated.

3.4. Laboratory Measurement

Hematological assessments were performed by manual counting using a light microscope. Fibrinogen was measured by a chromatographic method using an ACL 7000 coagulometer.

3.5. Exercise Treadmill Testing

Several noninvasive stress testing modalities are currently available, but the exercise ECG is still used as the standard in comparison to other clinical and testing risk markers (8). All patients underwent symptom-limited exercise testing according to the standard Bruce protocol, as was suggested in literature (8). The resting heart rate, blood pressure, and 12-lead ECGs were recorded in the supine and upright positions before exercise. During each minute of exercise, heart rate and blood pressure were measured, and a 12-lead ECG was recorded. Exercise testing was discontinued if exertional hypotension, malignant ventricular arrhythmias, marked ST depression ($\geq$ 3 mm), or limiting chest pain was reported. An abnormal exercise ST response was defined as $\geq$ 1 mm of horizontal or downward sloping ST depression or $\geq$ 1 mm of ST-segment elevation in leads without pathological Q waves (excluding AVR lead).

3.6. Other Medical Problems

Diabetes was ascertained in a participant who either self-reported that he or she used glucose lowering medications or answered yes to the question "Have you ever been told by a doctor that you have diabetes (high blood sugar)?" A participant was considered to be a smoker if he or she answered yes to the question Have you been a smoker during the last year? Hyperlipidemia was also ascertained by patients self-reporting use of lipid lowering medications, or answering yes to the relevant question in questionnaire. The DTS provides accurate diagnostic and prognostic information for the evaluation of symptomatic patients with clinically suspected ischemic heart disease. The Equation 1 for calculating the DTS is as follows:

$$\text{DTS} = \text{Exercise Time} - 5 \times (\text{ST Deviation}) - 4 \times (\text{Exercise Angina}) \quad (1)$$

Where DTS is Duke treadmill score.

Exercise time is in minutes using Bruce protocol, and exercise angina was scored 0, for no angina; 1, for non-limiting angina; and 2; for exercise-limiting angina (stopping exercise due to angina). Participants were classified as low-risk (DTS $\geq$ 5), moderate-risk (-10 $\leq$ DTS $\leq$ 4), and high-risk (DTS $\leq$ -11) (8).

CAD was defined as the presence of $\geq$ 50% stenosis of any of the main coronary arteries (LAD, LCX, and RCA), according to the American college of cardiology and the American heart association lesion classification.

3.7. Statistical Analyses

Data are presented as mean (SD) and frequency (%) for continuously- and categorically-distributed variables, respectively. Deviation from normal distribution was determined by calculating skewness. Before incorporation into regression models, NLR value and fibrinogen levels were naturally log-transformed to attain normality. One-way analysis of variance was used to compare the continuous variables between categories of CAD. The significance of difference between participants with no stenosis and those with different degrees of stenosis was examined using the Dunnett method. A chi-square test of trend was applied for ordinal variables. The expected power of this study is 80.

3.8. Multivariate Analysis

Coronary artery stenosis was modeled by positing a series of generalized estimated equations (GEE), with ordinal distribution as the probability distribution and:

$$\text{Logit}\,(y) = \left(\frac{y}{1-y}\right) \quad (2)$$

as the link function (20). The regression analyses was controlled for confounding variables while examining the contribution of the fibrinogen levels and NLR values to the coronary artery stenosis. Fibrinogen levels were Z-transformed

$$z = \frac{\text{Value}_{\text{actual}} - \text{Mean}}{\text{Standard Deviation}} \quad (3)$$

Analyses were performed at both the individual and vessel levels:

1. At the individual level, the extent of coronary artery disease was scored 0, (absent or minimal atherosclerotic involvement); 1, (single-vessel disease); 2, (two-vessel disease); or 3, (three-vessel and/or main stem disease); according to the number of main vessels with significant stenosis.

2. At the vessel level, stenosis in each of the three cardinal vessels (RCA, LCX and LAD) was categorized into patent (< 50% stenosis), moderate (50 - 70% stenosis), and significant (> 70% stenosis). Using GEE modeling, the correlation between coronary arteries' stenosis observed in a given individual was measured.

To examine clinical usefulness of the NLR value and plasma fibrinogen levels, their association with significant CAD as a binary outcome was investigated. Log-transformed NLR values and log-transformed plasma fibrinogen levels were introduced, both individually and simultaneously, into a Generalized Logit Mixed Model with random intercepts to capture correlation between main coronary arteries in a given participant.

To determine if sex would modify the effect on CAD of fibrinogen or NLR, we introduced the interaction terms male $\times$ (log-transformed fibrinogen levels) and male $\times$ (log-transformed NLR values) into the final main effect models. A likelihood ratio test was used to compare fitness of the nested models with and without these interaction terms. Since no evidence was found that sex might have modified the effects on CAD of fibrinogen or NLR (all P > 0.7), the results presented were obtained from a sex-pooled whole sample, in order to capture full statistical power.

3.9. Assessment of Model Performance

Several criteria were used to compare the overall predictive values of alternative models.

3.10. Goodness of Fit

How effectively a model describes the outcome variable is referred to as its goodness of fit. Various measures of goodness of fit were used for this study. Deviance (D statistic) compares the fit of the saturated model to the fitted model. This will be a small value if the model is adequate. For the purposes of assessing the significance of non-linear terms, the values of D with and without the non-linear terms were compared by computing the deviance difference (G statistic). Akaike information criterion (AIC) was used to account for complexity. Difference in AIC > 10 was considered significant (21).

3.11. Discrimination

Discrimination refers to the ability to distinguish high risk subjects from low risk subjects, and is commonly quantified by a measure of concordance, the C statistic. For binary outcomes, C is identical to the area under the receiver operating characteristic (ROC) curve (AROC). As a general rule, if ROC = 0.5, it suggests no discriminatory power, if $0.70 \leq$ ROC < 0.80, it suggests acceptable discriminatory power, if $0.80 \leq$ ROC < 0.90, it is considered excellent discriminatory power, and if ROC $\geq$ 0.90, it is considered outstanding discriminatory power (22, 23).

3.12. Calibration

Calibration describes how closely predicted probabilities agree numerically with actual outcomes. Calibration was examined using the Hosmer-Lemeshow test (23).

3.13. Added Predictive Ability

Traditionally, risk models have been evaluated by using Harrell's C statistic, but this method is not insensitive enough in comparing models, and is of little direct clinical relevance. New methods have recently been proposed to evaluate and compare predictive risk models. These are based primarily on stratification into clinical categories on the basis of risk, and they attempt to assess the ability of new models to more accurately reclassify individuals into higher or lower risk strata. Absolute and relative integrated discriminatory improvement index (IDI) and cut-point-based and cut-point-free net reclassification improvement index (NRI) were used as measures of predictive ability added to traditional CHD risk factors by NLR and fibrinogen, separately and together. Reclassification improvement is defined as an increase in risk category for patients who develop events and as a decrease for those who do not. Net reclassification improvement considers movement between categories in the wrong direction and applies different weights to events and nonevents (24).

3.14. Nonlinear Associations

Instead of using arbitrary predetermined cut-points, restricted cubic splines functions of the NLR values and fibrinogen levels were used to represent their continuous relationship with CAD so that the relationships were meaningful, in accordance with substantive background knowledge. Because of this, possible nonlinear associations were able to be captured. Restricted cubic splines function allowed the researchers to flexibly model continuous predictor (NLR values and fibrinogen levels) while allowing some control over the excessive instability and tendency of spline functions to generate artefactual and uninterpretable features of a curve. Multivariate restricted cubic splines regression models were started using three knots defined at 25^{th}, 50^{th}, and 75^{th} percentiles. Splines were then selected using backward elimination. The significant level was set at unity for restricted cubic spline functions of the NLR values and fibrinogen levels, forcing them into the multivariate regression models, to allow capture of any nonlinearity in the associations. The likelihood ratio test between models with and without spline functions examines the null hypothesis that the association under the investigation is linear (25).

All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Informed written consent was obtained from all participants, and the ethical committee of Ahvaz Jundishapur University of Medical Sciences approved this study in 2012 (ETH-646). The study protocol conforms to the ethical guidelines of the 2008 declaration of Helsinki. The statistical significance level was set at a two-tailed type I error of 0.05. All statistical analyses were performed using STATA version 12 (STATA, College Station, Texas USA) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

4. Results

The mean age of the participants was 57.5 years, with 51.9% being male. The number of participants with one, two, and three-vessel stenosis were 76, 31, 31, respectively, while 45 did not have stenosed vessels (Table 1). The NLR and fibrinogen levels were significantly higher in patients with stenosis in 2 (2.4 and 512 $mg.dL^{-1}$) or 3 (2.6 and 517 $mg.dL^{-1}$) coronary arteries as compared to the group of patients with no significant involvement (2 and 430 $mg.dL^{-1}$) (all P < 0.01) (Table 1).

As shown in Table 2, patients with a higher NLR and fibrinogen levels were more likely to have higher grades of CAD. The associations were consistent at both individual and vessel levels [at individual levels: OR NLR = 1.36 (95% CI: 1.05 - 1.94) and OR Fibrinogen = 1.61 (95% CI: 1.18 - 2.22); at the vessel level OR NLR = 1.38 (95% CI: 1.09 - 1.74) and OR Fibrinogen = 1.54 (95% CI: 1.17- 2.02)].

Table 3 compares the basic model incorporating traditional CAD risk factors with enhanced models. Three enhanced models were developed by incorporating NLR and fibrinogen into the basic model, separately and in combination. Both NLR and fibrinogen as standalone variables

Table 1. Characterization of the Participants in Relation to the Extent of Coronary Artery Disease[a]

Variables	Total	Coronary Artery Involvement				P Value
		Patent	One-Vessel	Two-Vessel	Three-Vessel	
Number of participants	183	76	31	31	45	-
Age, y (mean ± SD)	57.48 ± 12.57	53.07 ± 12.29)	58.24 ± 11.53	57.90 ± 11.75	63.78 ± 11.77[c]	< 0.001
Gender (male vs. female)	95 (51.9)	25 (34.2)	17 (50.0)	21 (67.7)	32 (71.1)	< 0.001
Smoking (yes vs. no)	61 (33.3)	11 (15.1)	7 (20.6)	16 (51.6)	27 (60.0)	< 0.001
Diabetes mellitus (yes vs. no)	70 (38.3)	16 (21.9)	9 (26.5)	14 (45.2)	31 (68.9)	< 0.001
Hypertension (yes vs. no)	67 (36.6)	17 (23.3)	9 (26.5)	12 (38.7)	29 (64.4)	< 0.001
Hyperlipidemia (yes vs. no)	78 (42.6)	18 (24.7)	18 (52.9)	12 (38.7)	30 (66.7)	< 0.001
Diastolic blood pressure (mmHg)	80.05 (10.72)	80.0 (11.05)	80.59 (8.86)	78.39 (12.41	80.89 (10.41)	0.778
Systolic blood pressure (mmHg)	160.8 (26.6)	158.4 (27.9)	161.5 (24.0	156.1 (28.2)	167.3 (24.9)	0.233
Neutrophil-Lymphocyte ratio	2.31 (0.88)	2.07 (0.46)	2.45 (0.85)	2.43 (0.67)[b]	2.55 (1.35)[b]	0.013
Fibrinogen, mg.dl[-1]	468.4 (117.6)	430.5 (101.1)	442.1 (117.6)	511.5 (129.9)[c]	516.9 (124.8)[c]	< 0.001
Neutrophil count	61.07 (8.87)	58.29 (7.59)	64.50 (8.11)[b]	61.11 (8.59)	62.96 (10.30)[b]	0.002
Lymphocyte count	28.31 (6.88)	29.40 (6.46)	28.56 (7.63)	26.25 (5.18)	27.79 (7.75)	0.178
Ejection fraction	48.18 (8.72)	51.75 (8.44)	46.47 (7.91)	46.17 (7.78)	45.66 (9.31)[b]	0.041

[a]Values are expressed as No. (%) unless otherwise indicated.
[c]P < 0.01 compared to the patent group.
[b]P < 0.05 compared to the patent group.

Table 2. Predictors of Coronary Artery Stenosis in Multiple Proportional Odds Regression Model[a]

Variable	Individuals[b]		Vessels[c]	
	Odds Ratio 95% Confidence Interval	P Value	Odds Ratio (95%) Confidence Interval	P Value
Age, y	1.029 (1.00 - 1.06)	0.036	1.026 (1.00 - 1.02)	0.035
Gender (male vs. female)	2.79 (1.35 - 5.76)	0.006	2.31 (1.23 - 4.33)	0.009
Smoking (yes vs. no)	2.57 (1.22 - 5.45)	0.013	2.16 (1.10 - 4.21)	0.024
Diabetes mellitus (yes vs. no)	2.52 (1.21 - 5.27)	0.014	1.91 (0.99 - 3.69)	0.014
Hypertension (yes vs. no)	1.79 (0.94 - 3.42)	0.079	1.72 (0.99 - 3.00)	0.055
Hyperlipidemia (yes vs. no)	2.15 (1.04 - 4.43)	0.038	1.49 (0.76 - 2.90)	0.240
Log-NLR	1.36 (1.05 - 1.94)	0.034	1.38 (1.09 - 1.74)	0.008
Fibrinogen (per 1-SD change)	1.61 (1.18 - 2.22)	0.003	1.54 (1.17 - 2.02)	0.002

[a]Analyses were performed at both the individual and vessel levels. The regression analyses were controlled for confounding bias due to potential confounders while examining the contribution of the fibrinogen levels and neutrophil-lymphocyte ratio values to the coronary artery stenosis.
[b]At the individual level, the extent of coronary artery disease was categorized into 0, (absent or minimal atherosclerotic involvement); 1, (single-vessel disease); 2, (two-vessel disease); and 3, (three-vessel and/or main stem disease), according to the number of main vessels with significant stenosis.
[c]At the vessel level, coronary artery stenosis (as per vessel) was modeled by positing a series generalized estimated equations (GEE) with ordinal distribution as the probability distribution and logit as the link function to allow for intra-individual correlation among vessels. For the vessel level, stenosis in each of the three most important vessels (RCA, LCX, and LAD) were categorized into patent (< 50 stenosis), moderate (50 - 70stenosis) and significant (> 70 stenosis). Using GEE modeling, we were able to allow for the inter-correlation between coronary arteries in the same individuals.

added to the predictive value achieved by traditional CAD risk factors; the yield, however, was highest when both measures were simultaneously added to the basic model. Hosmer-Lemeshow χ^2 (P value) for the enhanced model with both NLR and fibrinogen was 5.3 (0.868), indicating that model has the best calibration or best agreement between actual observed CAD and estimated probability of having CAD. AIC and BIC for this model were 595 and 638, respectively, supporting the notion that this model best fits the actual data. The Harrell's C discrimination index value of 0.80 indicates that the model's ability to discriminate those with CAD from those without CAD has been excellent.

Predictive ability added to the basic model by models incorporating NLR and/or fibrinogen was generally statistically significant. The magnitude of the added predictive ability was highest when both NLR and fibrinogen were simultaneously added to the basic model.

The nonlinear association between the probability of having CAD and NLR values or plasma fibrinogen levels was examined. Estimated probabilities of CAD based on models incorporating restricted cubic spline functions of NLR values and plasma fibrinogen levels did not fit the data better than the models incorporating linear terms only (fibrinogen: likelihood ratio $\chi^2 = 2.4$, $P = 0.496$; NLR: likelihood ratio $\chi^2 = 4.1$, $P = 0.252$). As shown in Figure 1, the gradient of the curve representing the association between the probability of CAD and fibrinogen increased for fibrinogen levels above 470 $mg.dL^{-1}$ and decreased for fibrinogen levels below 370 $mg.dL^{-1}$. There was no evidence of a threshold within the range of usual NLR values (Figure 2).

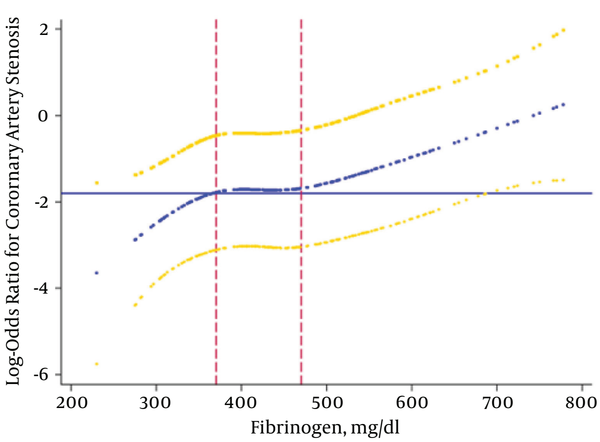

Figure 1. Nonlinear Contribution of the Fibrinogen to the Log-Odds of Coronary Artery Disease

5. Discussion

In a sample of individuals with chronic stable angina at intermediate risk for acute coronary syndrome as measured by exercise electrocardiograph, NLR and fibrinogen predicted varying degrees of angiographically-detected coronary artery stenosis, independent of traditional CAD risk factors and each other. Using several traditional and novel methods, it was demonstrated that both measures, whether introduced separately or together, substantially enhanced predictive performance for CAD of traditional

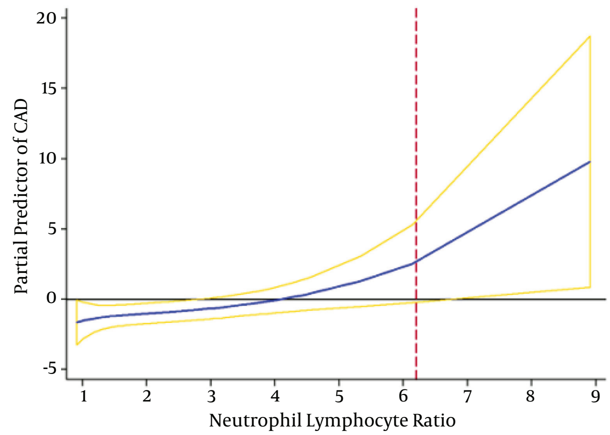

Figure 2. Nonlinear Contribution of the Neutrophil-to-Lymphocyte Ratio to the Log-Odds of Coronary Artery Disease

risk factors. The yield, however, was higher when NLR and fibrinogen were both added to the traditional CAD risk factors simultaneously.

Considering limited health resources, early risk assessment with an estimate of the severity of disease and prognosis is essential for optimized preventive measures. As such, targeted measures could be focused on individuals at high risk for developing acute coronary syndrome, who would most benefit from prevention. The Duke treadmill score (DTS) is the currently accepted standard for risk stratification on treadmill testing (26-30), which is the first step in evaluation of symptomatic patients to predict the presence and extent of coronary artery disease (27, 31, 32). It provides clear decision-making information for patients identified as having low or high risk. The results, however, can be inconclusive when patients are risk stratified as harboring intermediate risk, which is the case for 50% of patients (27, 33). This shortcoming has motivated increased use and development of noninvasive stress imaging technologies (27), or angiography, which are associated with substantially higher costs and complications (34). This study provides easily obtainable and readily available clinical and para-clinical information that, if used efficiently, provides an opportunity to save substantial cost. Better risk-stratification of patients can help avoid unnecessary cardiac catheterization and the complications associated with it (35).

There is currently more than sufficient evidence available to support to the notion that inflammation plays a pivotal role in the initiation and progression of atherosclerosis (36, 37). The white blood cell count, first introduced in 1974 as an independent risk factor for coronary heart disease (12), has been investigated extensively for varying CVD

Table 3. Components, Characteristics, and Predictive Performance Measures for Basic and Enhanced Models[a,b,c]

	Basic Model		Basic + Fibrinogen		Basic + NLR		Basic + NLR + Fibrinogen	
	OR (95% CIs)	P Value	OR (95% CIs)	P Value	OR (95% CIs)	P Value	OR (95% CIs)	P Value
Log-Neutrophil-Lymphocyte ratio	-	-	-	-	3.20 (1.59 - 6.45)	0.001	2.36 (1.16 - 4.81)	0.018
Log-Fibrinogen (In[mg.dL^{-1}])	-	-	9.48 (4.0 - 22.6)	< 0.001	-	-	7.91 (3.3 - 19.2)	< 0.001
Age, y	1.03 (1.01 - 1.05)	< 0.001	1.027 (1.01 - 1.05)	0.005	1.03 (1.02 - 1.05)	< 0.001	1.03 (1.01 - 1.05)	0.003
Gender (male vs. female)	2.06 (1.27 - 3.33)	0.003	2.498 (1.51 - 4.13)	< 0.001	2.04 (1.25 - 3.32)	0.004	2.44 (1.47 - 4.03)	0.001
Smoking (yes vs. no)	2.27 (1.38 - 3.74)	0.001	2.135 (1.27 - 3.58)	0.004	2.04 (1.24 - 3.38)	0.005	1.99 (1.19 - 3.34)	0.009
Diabetes mellitus (yes vs. no)	1.93 (1.17 - 3.20)	0.010	1.837 (1.11 - 3.05)	0.019	2.02 (1.22 - 3.35)	0.007	1.88 (1.13 - 3.14)	0.015
Hypertension (yes vs. no)	2.20 (1.44 - 3.36)	< 0.001	1.825 (1.17 - 2.84)	0.008	2.08 (1.35 - 3.20)	0.001	1.76 (1.13 - 2.75)	0.013
Hyperlipidemia (yes vs. no)	1.03 (1.01 - 1.05)	< 0.001	1.027 (1.01 - 1.05)	0.005	1.03 (1.02 - 1.05)	< 0.001	1.03 (1.01 - 1.05)	0.003
Hosmer-Lemeshow χ^2	9.8	0.459[d]	18.6	0.046[d]	9.3	0.507[d]	5.3	0.868[d]
Akaike information criteria	624.79	NA	599.15	NA	615.37	NA	595.33	NA
Bayesian information criteria	659.26	NA	637.92	NA	654.14	NA	638.41	NA
C statistic (95% CIs)	0.76 (0.73 - 0.80)	NA	0.80 (0.76 - 0.83)	0.004[e]	0.77 (0.74 - 0.81)	0.123[e]	0.80 (0.76 - 0.83)	0.004[e]
D statistic (χ^2)	608.79	< 0.001[d]	581.15	< 0.001[d]	597.37	< 0.001[d]	575.33	< 0.001[d]
G statistic (χ^2)	-	NA	27.6	< 0.001[e]	11.4	< 0.001[e]	33.5	< 0.001[e]
Absolute IDI (95% CIs)	-	NA	0.050 (0.026 - 0.065)	0.000[d]	0.020 (0.005 - 0.025)	0.003[d]	0.050 (0.028 - 0.066)	< 0.001[d]
Relative IDI (95% CIs)	-	NA	0.220 (0.111 - 0.320)	0.000[d]	0.070 (0.021 - 0.123)	0.005[d]	0.220 (0.119 - 0.329)	< 0.001[d]
Cutpoint-based NRI (95% CIs)	-	NA	0.090 (0.019 - 0.169)	0.014[d]	0.030 (-0.028 - 0.092)	0.296[d]	0.130 (0.053 - 0.207)	< 0.001[d]
Cutpoint-free NRI (95% CIs)	-	NA	0.380 (0.214 - 0.543)	< 0.001[d]	0.170 (0.023 - 0.324)	0.024[d]	0.460 (0.303-0.620)	< 0.001[d]

[a]ORs and their 95% CIs were obtained using a series of generalized estimated equations.

[b]statistic is a measure of concordance and is used to examine the discrimination capacity, which refers to the ability of a model to distinguish high risk subjects from low risk subjects. For binary outcomes, C is identical to the area under the receiver operating characteristic (ROC) curve (AROC). As a general rule, if ROC = 0.5, it suggests no discriminatory power, if 0.70 ≤ ROC < 0.80, it suggests acceptable discriminatory power, if 0.80 ≤ ROC < 0.90, it suggests excellent discriminatory power, and if ROC≥0.90, it suggests outstanding discriminatory power. How effectively a model describes the outcome variable is referred to as its goodness of fit. Various measures of goodness of fit were used for this study. Deviance (D statistic) compares the fit of the saturated model to the fitted model. This will be a small value if the model is adequate. For the purposes of assessing the significance of non-linear terms, the values of D with and without the non-linear terms were compared by computing the deviance difference (G statistic). Akaike information criterion (AIC) was used to account for complexity. Difference in AIC > 10 was considered significant (21).

[c]Calibration was examined using the Hosmer-Lemeshow test. Calibration describes how closely the predicted probabilities agree numerically with actual outcomes. New methods have recently been proposed to evaluate and compare predictive risk models. These are based primarily on stratification into clinical categories on the basis of risk, and attempt to assess the ability of new models to more accurately reclassify individuals into higher or lower risk strata. Absolute and relative integrated discriminatory improvement index (IDI) and cut-point-based and cut-point-free net reclassification improvement index (NRI) were used as measures of predictive ability added by the introduction of neutrophil-lymphocyte ratio and/or fibrinogen to that measured by the traditional risk factors of the coronary artery disease.

[d]P Values examine the significance of the relevant statistic.

[e]P Values examine the significance of difference between the enhanced model and the basic model.

outcomes in both sexes and across populations of different ethnicities (13-16). The most comprehensive assessment of specific leukocytes components in CHD was performed by Wheeler et al., who found the neutrophils count to be more strongly associated with CHD than other leukocyte components (17). Results, however, have not been conclusive across studies (18). Most previous studies on inflammation and vascular risk investigated healthy subjects. The prognostic value of inflammatory parameters was previously believed to be apparent only when the level of conventional risk factors is low, but not in those at high-risk of ischemic diseases (38). Grau et al. were the first to demonstrate the predictability of leukocyte count for ischemic events among a high-risk population with recurrent ischemic events (38). Rashidi et al., during a one-year follow-up, observed that elevated WBC count, after elective coronary artery bypass graft surgery, would be a risk factor for recurrent ischemic events independent of traditional CAD risk factors (39). NLR is a simple index of systemic inflammatory burden that correlates with survival following percutaneous coronary intervention. Preoperative NLR has been observed to identify patients at increased risk of death within 2 years of major vascular surgery (40). The predictive ability of the NLR, however, has never been examined among patients with chronic stable angina. This study extended these previous findings to patients with chronic stable angina. This simple index may facilitate targeted preventive measures for high-risk patients.

Positive associations between the risk of CHD and plasma fibrinogen levels has been previously reported by many prospective epidemiological studies (41). There is interest in the possibility that measurement (or modification) of fibrinogen may help in disease prediction (or prevention). This approach is not without some intuitive appeal, and might be reasonable, since fibrinogen levels can be reduced considerably by lifestyle interventions (such as regular exercise, smoking cessation, and moderate alcohol consumption) that also affect levels of traditional risk factors (42-44). This study boasts advanced novel statistical methods that provide the opportunity to investigate the predictive ability of the NLR and plasma fibrinogen levels above and beyond traditional CAD risk factors.

5.1. Clinical Implication

Considering limited health resources, early risk assessment with an estimate of the severity of disease and prognosis is essential for optimized preventive measures. As such, targeted measures could be focused on individuals at high risk for developing acute coronary syndrome, who would most benefit from prevention.

The Duke treadmill score (DTS) is the currently accepted standard for risk stratification on treadmill testing (26-30), which is the first step in evaluation of symptomatic patients to predict the presence and extent of coronary artery disease (27, 31, 32). It provides clear decision-making information for patients identified as having low or high risk. The results, however, can be inconclusive when patients are risk stratified as harboring intermediate risk, which is the case for 50% of patients (27, 33). This shortcoming has motivated increased use and development of noninvasive stress imaging technologies (27), or angiography, which are associated with substantially higher costs and complications (34).

Health care systems boast easily obtainable and readily available clinical and para-clinical information that, if used efficiently, provides valuable opportunities to save substantial cost. Better risk-stratification of patients can help avoid unnecessary cardiac catheterization and complications thereof (35). The results of this study demonstrate that gathering information on NLR and plasma fibrinogen levels can help substantially improve predictive ability for CAD beyond what could be achieved by traditional risk factors.

This study was conducted on a sample of patients with chronic stable angina at intermediated risk for acute coronary syndrome (as detected by exercise electrocardiograph), where uncertainty still continues to exist in determining the appropriate evaluation methods for detecting patients vulnerable to acute coronary syndrome. Obtaining information on NLR or plasma fibrinogen levels may help clinicians decide whether to make reassuring comments or to offer a more invasive test when they encounter these patients.

5.2. Strengths and Limitations

The strength of the current study lies in our ability to define CAD angiographically. The major limitation to our findings is that data on traditional CAD risk factors was obtained on a self-reporting basis. These confounding factors might have affected the results.

5.3. Conclusion

In a sample of individuals with chronic stable angina, with exercise electrocardiographically-detected intermediate risk for acute coronary syndrome, NLR and fibrinogen level predicted varying degrees of angiographically-detected coronary artery stenosis, independent of traditional CAD risk factors and each other. Using several traditional and novel methods, it was demonstrated that both measures, either separately or together, substantially enhanced the predictive performance of traditional risk factors for identifying patients with CAD. The yield, however, was higher when NLR and fibrinogen levels were both added to the traditional CAD risk factors simultaneously.

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Footnotes

Authors' Contribution: Habib Haybar has contributed to conception and design of the study, acquisition, analysis, and interpretation of data. Ahmad Ahmadzadeh, Ahmadreza Assareh, and Nader Afshari revised the manuscript critically for important intellectual content. Mohammadreza Bozorgmanesh conceptualized and designed the study, was involved in analysis and interpretation of data, and drafted the manuscript. All authors have given final approval of the version to be published, and take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.

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