Serum neutrophil gelatinase-associated lipocalin levels are correlated with the complexity and the severity of atherosclerosis in acute coronary syndrome

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Abstract

Objective: Neutrophil gelatinase-associated lipocalin (NGAL) is a novel inflammatory marker that is released from neutrophils. In this study, we evaluated the correlation between serum NGAL level and clinical and angiographic risk scores in patients diagnosed with non-ST elevation acute coronary syndrome (NSTE-ACS).

Methods: Forty-seven random NSTE-ACS patients and 45 patients with normal coronary arteries (NCA) who underwent coronary angiography were enrolled in the study. GRACE risk score and SYNTAX and Gensini risk scores were used, respectively, for the purpose of clinical risk assessment and angiographic risk scoring. Serum NGAL level was measured via ELISA in peripheral blood samples obtained from the patients at the time of admission.

Results: Serum NGAL level was significantly higher in the NSTE-ACS group compared to the control group (112.3 ± 49.6 ng/mL vs. 58.1 ± 24.3 ng/mL, $p<0.001$). There was a significant positive correlation between serum NGAL levels and the GRACE ($r=0.533$ and $p<0.001$), SYNTAX ($r=0.395$ and $p=0.006$), and Gensini risk scores ($r=0.575$ and $p<0.001$). The intermediate-high SYNTAX (>22) group had statistically significantly higher serum NGAL levels compared to the low SYNTAX (≤22) group (143 ± 29.5 ng/mL vs. 98.7 ± 43.2 ng/mL, $p=0.001$).

Conclusion: NGAL level was positively correlated with lesion complexity and severity of coronary artery disease in patients with NSTE-ACS. Serum NGAL levels on admission are associated with increased burden of atherosclerosis in patients with NSTE-ACS.

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Keywords: neutrophil gelatinase-associated lipocalin, non-ST elevation acute coronary syndrome, GRACE, SYNTAX, Gensini

Introduction

Acute coronary syndrome (ACS) represents one of the most significant clinical endpoints of coronary atherosclerosis. Cardiac injury induced by acutely impaired coronary flow threatens both the patient's quality of life and the life span. The lack of a curative treatment for this common and dangerous disease and the heterogeneous treatment outcomes have taught us the importance of risk assessment. A large number of markers have been and are still being tested. Due to the strong correlation between atherosclerosis and inflammation, the investigators believe that inflammatory markers could be better predictors for atherosclerosis. In fact, C-reactive protein (CRP), cytokines, interleukins, leukocyte count, and many other inflam-matory markers have been demonstrated to be predictors of atherosclerosis (1).

Neutrophil gelatinase-associated lipocalin (NGAL) is a gly-coprotein of 25 kDa that was initially isolated from the neutro-phils and covalently bound to matrix metalloproteinase-9a (MMP-9) (2). NGAL includes bacteriostatic and anti-apoptotic effects and enhanced proliferation of renal tubules, which con-stitute possible pathways of NGAL-mediated kidney protection in acute injury. Its production in various cells has been demon-strated, including renal tubular cells, endothelial cells, cardio-myocytes, and macrophages in the atherosclerotic plaque (3-5). Recent studies show increased plasma level of NGAL in coro-nary artery disease (CAD) and that such an increase could be involved in the atherosclerotic process (6, 7). Similarly, HDL-C

Anatol J Cardiol 2015; 15: 450-5

Soylu et al.
Serum NGAL level in NSTE-ACS *451*

level, which is a protective cardiovascular risk factor, is found to be negatively correlated with serum NGAL levels (6). It was demonstrated that high NGAL levels were associated with increased mortality in patients with STEMI (8, 9). On the other hand, the association between increased NGAL level in ACS patients and the severity, complexity, and clinical risk scores in coronary disease is not clear yet. Thus, in this study, we investigated the correlation between serum NGAL level and clinical and angiographic risk scores in patients diagnosed with non-ST elevation acute coronary syndrome (NSTE-ACS).

Methods

Study population

This study was designed as a case-control study; 47 random patients, who were admitted to Ondokuz Mayıs University Medical Faculty Hospital, were diagnosed with non-ST elevation acute coronary syndrome (NSTE-ACS) and found to have >50% stenosis in at least one coronary artery on coronary angiography and were included in the study. Diagnosis of NSTE-ACS was established upon the presence of characteristic chest pain lasting more than 20 minutes with associated ST-segment depression ≥0.1 mV and/or T-wave inversion in two continuous leads on the electrocardiogram and increased levels of troponin T. The control group involved 45 random patients who underwent coronary angiography (CAD) upon suspected presence of coronary artery disease (CAD) but were found not to have atherosclerotic plaques. Patients with coronary ectasia or abnormality, coronary vasospasm, acute or chronic inflammatory disease, malignancy, pregnancy, acute or chronic renal failure (creatinine-based estimated GFR <90 mL/min/1.73 m², as calculated by the Cockcroft-Gault formula), myocarditis, or significant valvular heart disease were excluded from the study. All patients were administered coronary angiography using the Judkins technique (ACOM.PC; Siemens AG, Germany). The study was conducted in accordance with the ethical principles described by the Declaration of Helsinki.

Clinical risk assessment

The clinical risk assessment of NSTE-ACS was performed using the Global Registry of Acute Coronary Events (GRACE) risk scoring (10). GRACE score is a predictive logistical model that uses 8 prognostic variables (age, systolic blood pressure, heart rate, plasma creatinine, Killip class, ST-segment depression, elevation in myocardial necrosis marker, and cardiac arrest on admission).

Angiographic scoring

Gensini (11) and SYNTAX (12) scoring methods were used to evaluate the severity and lesion complexity of coronary artery disease. The images were assessed by two experienced cardiologists blinded to the NGAL data. In the Gensini scoring system, larger segments are more heavily weighted, ranging from 0.5 to 5.0. The narrowing of the coronary artery lumen is rated 2 for 0% to 25% stenosis, 4 for 26% to 50%, 8 for 51% to 75%, 16 for 76% to 90%, 32 for 91% to 99%, and 64 for 100%. The Gensini index is

the sum of the total weights for each segment. In the SYNTAX scoring system, each coronary lesion with a stenosis diameter of 50% or greater in vessels of 1.5 mm or more in diameter was scored. The latest online updated version (2.11) was used in the calculation of the SYNTAX scores (www.syntaxscore.com) (13). The SYNTAX score was classified as follows: low SYNTAX score (≤22) and intermediate-high SYNTAX score (>22). Moreover, the diseased vessel scores ranged between 0 and 3. The criteria for 1-, 2-, or 3-vessel disease was a ≥50% reduction in the internal diameter of the left anterior descending, right, or left circumflex coronary artery. A ≥50% reduction in the internal diameter of the left main coronary artery was considered 2-vessel disease.

Blood samples

Peripheral blood sampling was performed in enrolled patients at the time of admission and after 8 to 12 hours of fasting, respectively, for lipocalin-2/NGAL measurements. The levels of fasting glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, and serum creatinine were measured (Abbott Laboratories, USA). The hematologic parameters were studied using laser and impedance methods with the Abbott CellDyn 3700 (Abbott Laboratories, USA) device. High-sensitivity C-reactive protein (hs-CRP) measurement was conducted in separated sera using the immunonephelometric method (BN ProSpec System protein analyzer, Siemens Healthcare Diagnostics, Deerfield, IL, USA).

Serum lipocalin-2/NGAL levels were determined via ELISA method with the Triturus® ELISA analyzer (Grifols International, Barcelona, Spain) device using the human lipocalin-2/NGAL kit (BioVendor-Laboratorni medicana a.s, Brno, Czech Republic). Applying the four-parameter calculation method, a standard curve was established, and the results were calculated based on this curve in terms of ng/mL.

Statistical analysis

All data were loaded into SPSS 15 (SPSS; Inc., Chicago, Illinois, USA) software. Subsequently, the normal distribution of the data was tested using the Kolmogorov-Smirnov test. Student's test was also used to compare the two groups of values demonstrating normal distribution, while groups of values without normal distribution were compared using the Mann-Whitney U test. Comparison of categorical values was carried out using the chi-square test. Any correlation between data was tested via Spearman and Pearson correlation analyses.

While continuous data were expressed in terms of mean±SD (standard deviation), categorical data were expressed as percentage values, and a p value of <0.05 was accepted as statistically significant.

Results

Forty-seven NSTE-ACS patients and 45 patients with normal coronary arteries (NCAs) were included in the study. The demographic, clinical, and laboratory characteristics of the patients

452 Soylu et al.
Serum NGAL level in NSTE-ACS

Anatol J Cardiol 2015; 15: 450-5

Table 1. Baseline characteristics

	NST-ACS group (n=47)	Control group (n=45)	P
Clinical and hemodynamic data			
Age, years	57.4±10.2	54.1±10.3	0.135
Men, n, %	39 (83)	33 (73.3)	0.317
Body mass index, kg/m^2	28±2.4	27.5±1.8	0.373
Hypertension, n, %	38(80.9)	34 (75.5)	0.617
Diabetes mellitus, n, %	11 (23.4)	9 (20)	0.692
Smoking (current), n, %	25 (53.2)	28 (62.2)	0.406
Left ventricular ejection fraction, %	54.7±6.6	61.2±4.5	<0.001
Biochemical and hematological data			
Total cholesterol, mg/dL	187.7±41.9	186.3±40.6	0.870
Low-density lipoprotein, mg/dL	120.4±36.3	112.4±25.4	0.228
High-density lipoprotein, mg/dL	37.8±7.6	41.1±7.4	0.037
Triglyceride, mg/dL	153.3±70.6	158.5±65.1	0.713
Creatinine, mg/dL	0.92±0.19	0.88±0.17	0.247
Fasting glucose, mg/dL	104.4±22.6	108.3±19.9	0.383
Hemoglobin, gr/dL	14.0±1.2	13.7±1.4	0.255
White blood cell count, 10^3/mm^3	9.63±1.6	6.52±1.4	<0.001
Neutrophil, 10^3/mm^3	6.74±1.15	4.57±1.0	<0.001
Platelet, 10^3/mm^3	245.1±47.1	239.7±47.9	0.592
hs-CRP ng/mL	6.92±2.83	0.68±0.38	<0.001
NGAL, ng/mL	112.3±49.6	58.1±24.3	<0.001
Other parameters			
GRACE risk score	104.3±30.8		
SYNTAX score	13.9±9		
GENSINI score	41.8±25.1		
Number of diseased vessels, n, %			
0		45 (100)	
1	20 (42.6)		
2	19 (40.4)		
3	8 (17)		
Location of stenosis, n, %			
LMCA	3 (6.3)		
LAD	29 (61.7)		
LCx	20 (42.5)		
RCA	26 (55.3)		

hs-CRP - high-sensitivity C-reactive protein; LAD - left anterior descending artery; LCx - left circumflex artery; LMCA - left main coronary artery; NGAL - neutrophil gelatinase-associated lipocalin; RCA - right coronary artery

are provided in Table 1. The ejection fraction (EF) (p<0.001) and high-density lipoprotein values (p<0.037) were significantly lower in the NSTE-ACS group compared to the control group (Table 1).

Table 2. Correlations (Spearman) of NGAL levels with baseline clinical, biochemical, hemorheological. and other parameters

Variable	NGAL levels in NST-ACS Group	
	r	P
Left ventricular ejection fraction	0.001	0.998
Fasting glucose	0.622	<0.001
Serum creatinine	0.233	0.115
High-density lipoprotein	-0.638	<0.001
Low-density lipoprotein	0.047	0.751
Hemoglobin	0.113	0.448
White blood cell	0.569	<0.001
Neutrophil	0.563	<0.001
hs-CRP	0.542	<0.001
GRACE score	0.533	<0.001
SYNTAX score	0.395	0.006
GENSINI score	0.575	<0.001
Number of diseased vessels	0.591	<0.001

hs-CRP - high-sensitivity C-reactive protein; NGAL - neutrophil gelatinase-associated lipocalin

Serum NGAL levels (58.1±24.3 ng/mL versus 112.3±49.6 b, p<0.001) and hs-CRP levels (0.68±.38 ng/mL versus 6.92±2.83 b, p<0.001) were found to be significantly higher in the NSTE-ACS group compared to the control group. The increased NGAL level in the NSTE-ACT group exhibited a positive correlation with hs-CRP level (r=0.542 and p<0.001). In addition, fasting NGAL level correlated positively with fasting blood glucose and correlated negatively with HDL-C level (Table 2).

Some circulating inflammatory cells were higher in patients with NSTE-ACS compared to subjects in the control group. We found a significantly higher count of leukocytes (9.63±1.6 vs. 6.52±1.4b, p<0.001) and neutrophils (6.74±1.15 vs. 4.57±1.0b p<0.001) in the NSTE-ACS group. The level of NGAL was correlated positively with the levels of all types of inflammatory cells (for leukocytes: r=0.569, p<0.001; for neutrophils: r=0.563, p<0.001) (Table 2).

Serum NGAL levels showed a significant positive correlation with the GRACE, (r=0.533 and p<0.001), SYNTAX (r=0.395 and p=0.006), and GENSINI risk scores (r=0.575 and p<0.001) (Fig. 1-3). In addition, the Gensini and SYNTAX scores were found to correlate highly with each other (r= 0.836 and p<0.001). The intermediate-high SYNTAX (>22) group had statistically significantly higher serum NGAL levels compared to the low SYNTAX (≤22) group (143±29.5 vs. 98.7±43.2 ng/mL, p=0.001) (Fig. 4).

Discussion

In our study, NSTE-ACS patients were found to have markedly higher NGAL levels compared to patients with normal coronary arteries. In addition, the increased NGAL level was significantly correlated with clinical and angiographic risk scores. This
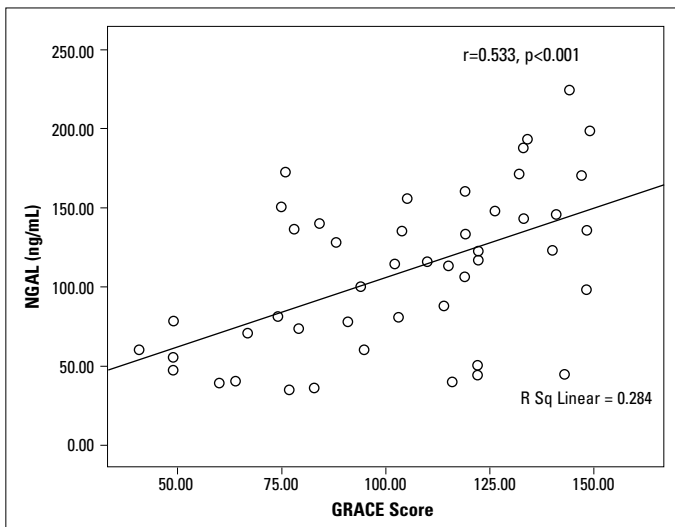
Anatol J Cardiol 2015; 15: 450-5

Soylu et al.
Serum NGAL level in NSTE-ACS *453*

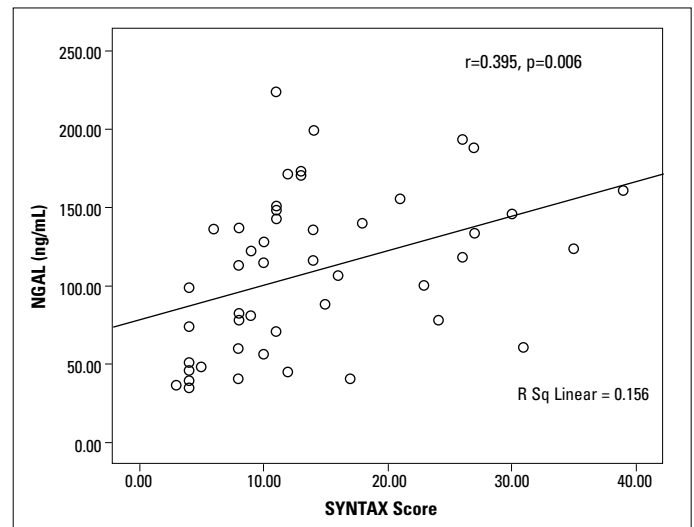**Figure 1. Correlation between GRACE risk score and serum NGAL levels**

Figure 3. Correlation between SYNTAX risk score and serum NGAL levels

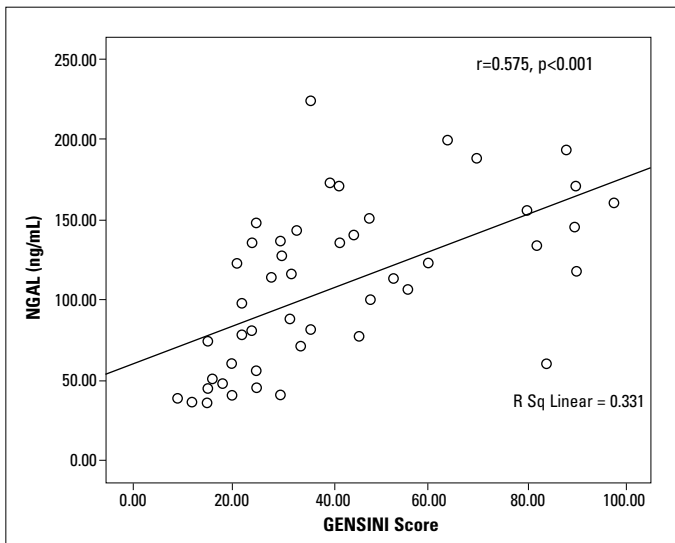**Figure 2. Correlation between Gensini risk score and serum NGAL levels**

Figure 4. Comparison of NGAL levels according to calculated SYNTAX score

is the first study to investigate the correlation between NGAL level and the Grace and SYNTAX risk scores in NSTE-ACS patients.

NGAL is a glycoprotein that was initially isolated from neutrophils and covalently bound to matrix metalloproteinase (MMP)-9. Its impact on atherosclerosis is closely related to MMP activity. As a result of endothelial injury, particularly MMP-9, produced by vulnerable plaques, was demonstrated to be potentially involved in plaque rupture and intra-plaque hemorrhages (14, 15). As for MMP-9 activity, it is inhibited by a stromal factor, called tissue inhibitor of metalloproteinase (TIMP-1). Binding of NGAL to MMP-9 increases the MMP-9/TIMP-1 rate by blocking this inhibition (16). Thus, increased NGAL activity results in an increase in MMP activity and plaque instability.

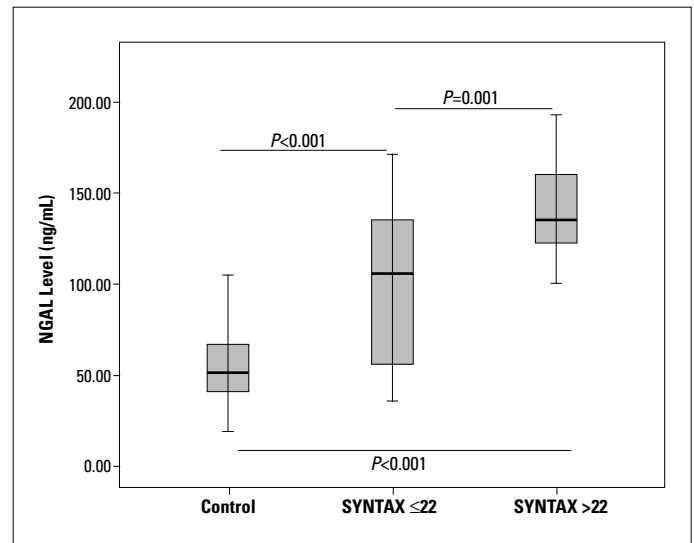In our study, we detected a correlation between increased NGAL level in NSTE-ACS patients and the number of diseased vessels as detected by angiography and the Gensini and SYNTAX scores. In a previous study by Zografos et al. (7), serum NGAL level was shown to be increased in the presence of coronary artery disease. Furthermore, the severity of coronary artery disease was observed to be positively correlated with NGAL level. In another study, Şahinarslan et al. (17) demonstrated higher NGAL levels in patients with acute coronary syndrome compared to patients with stable coronary artery disease. Nevertheless, the correlation between coronary lesion complexity and NGAL level had not been studied previously.

SYNTAX scoring is a significant grading method in terms of determining the complexity and prognosis for coronary artery stenosis (18, 19). Since an increased SYNTAX score indicates a more complex coronary lesion, it also guides in choosing the revascularization technique. The positive correlation of NGAL with SYNTAX score can be explained in two ways: the first is

that while the SYNTAX score is an indicator of lesion complexity, the presence of an association with plaque load and the number of stenotic vessels is inevitable. Thus, a higher SYNTAX score indicates a higher atherosclerotic plaque load. Indeed, the SYNTAX score showed a strong correlation with the Gensini score, a score that indicates the severity of coronary artery disease. The second is the fact that the local inflammatory response in the plaque site is higher in complex lesions compared to simple lesions. In studies by Karakaş (20) and Griva (21), a positive correlation of SYNTAX score with increased inflammatory markers has been demonstrated. Thus, the increased SYNTAX score observed in this study may be related to a higher inflammatory condition.

The Grace risk score is an important predictor for intra-hospital and 6-month mortality and myocardial infarction among patients presenting with acute coronary syndrome (10, 22). This study is the first to demonstrate the correlation between the Grace score, a clinical risk score, and NGAL level. Elneihoum et al. (23) showed that NGAL level was associated with cardiovascular risk factors, including age, hypertension, and smoking. On the other hand, the correlation of NGAL with renal dysfunction is a well-investigated subject (24-26). Therefore, factors, such as age, blood pressure, and creatinine, which are used for calculating the Grace risk score, are also stimulants for NGAL expression. In addition, NGAL may have different roles in the atherosclerotic process that we are not yet aware of.

NGAL is an inflammatory marker released by neutrophils and is also an adipokine secreted by the liver and adipocytes (8, 9). Therefore, the relation of serum NGAL levels with metabolic parameters has been reported (27). Choi et al. (6) reported a positive correlation between serum NGAL levels and weight, fasting insulin, and insulin resistance index and a negative correlation with serum HDL-C. Additionally, in the study of Wang et al. (28), serum NGAL levels were higher in diabetic and obese persons and showed a negative correlation with HDL-C levels. In this study, while serum NGAL levels showed a positive correlation with plasma glucose, they showed a negative correlation with serum HDL-C levels. According to the results of this study, it might be considered that the importance of NGAL in atherosclerosis could be caused by its actions on both metabolic and inflammatory processes.

Study limitations

The main limitation of our study is the fact that clinical endpoints were not investigated. Therefore, the importance of the increase in NGAL level was underlined only via clinical and angiographic risk scores. Another limitation is the lack of an adequate representation of all risk groups due to the small volume. Only 17% of the patients included in our study had three-vessel disease. Thus, the results of this study predominantly reflect NSTE-ACS patients of low-intermediate risk.

Conclusion

NGAL level was significantly higher in NSTE-ACS patients compared to control subjects. In addition, increased NGAL level correlated with clinical and angiographic risk scores in these patients. Therefore, we believe that NGAL level, measured in a patient with NSTE-ACS on admission to the hospital, could beneficial in the clinical and angiographic risk assessment. Particularly, the results to be obtained by prospective follow-up studies would further elucidate this topic.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

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Anatol J Cardiol 2015; 15: 450-5

Soylu et al.
Serum NGAL level in NSTE-ACS   *455*

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