

Relationship between neutrophil gelatinase-associated lipocalin, cardiac biomarkers, inflammation index and renal parameters in cardiovascular disease

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Abstract: The plasma neutrophil gelatinase-associated lipocalin (NGAL) level is elevated in myocardial infarction (MI) and affected by inflammation and kidney function. The aim of this study was to determine which of these conditions more critically affects the plasma NGAL level in MI. Patients with MI were evaluated by measuring the NGAL concentration and its corrected values. No significant association was observed between plasma NGAL concentration and cardiac biomarkers. However, the NGAL/inflammation index ratio (NGAL/Inf ratio) was positively correlated with troponin-I ($r=0.289$, $p<0.001$), and the NGAL/serum creatinine ratio (NGAL/sCr ratio) was significantly correlated with creatine kinase-MB ($r=0.251$, $p<0.001$). After adjusting for inflammation and kidney function, increased NGAL concentrations returned to baseline levels, which were not different from those of healthy individuals. The percent difference between NGAL and the NGAL/Inf ratio was 35.6%, significantly higher than that between NGAL and the NGAL/sCr ratio (15.4%; $p<0.001$). The severity of inflammation seems to play a more crucial role than renal and myocardial dysfunction in affecting plasma NGAL levels in MI. Plasma NGAL levels need to be corrected using the inflammation index and sCr levels for exactly evaluating patients with MI.

Keywords: neutrophil gelatinase-associated lipocalin; inflammation index; myocardial infarction; renal dysfunction; cardiac biomarkers

INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL) is a glycosylated protein belonging to the lipocalin family, expressed by activated granulocytes and a variety of epithelial cells [1]. As NGAL increases within two hours of renal damage, particularly before an elevation in serum creatinine (sCr), it has been used as an early predictor of acute kidney injury [2]. In addition, recent studies have reported that NGAL is involved in vascular inflammation and affects plaque instability and vascular remodeling after coronary heart disease [3,4]. Evidence supports the role of NGAL in the pathophysiology of cardiovascular diseases [5-7]. Despite the emerging role of NGAL in cardiovascular diseases, the significance of elevated NGAL levels remains unclear. There have been inconsistent results on its predictive value. One study demonstrated that the expression of NGAL is increased

in atherosclerosis and myocardial infarction (MI) [8]. In contrast, other studies reported that underlying renal insufficiency is a stronger determinant than myocardial dysfunction [9,10].

Inflammation plays a critical role in the development of atherosclerosis. Systemic inflammation contributes to endothelial dysfunction and the generation of atherosclerotic plaque [11]. Furthermore, inflammation is an important risk factor for decreased renal function [12]. Cardiovascular diseases are closely associated with impaired renal function. Heart and kidney diseases often coexist, and patients with cardiac and renal failure have high mortality [13]. Although NGAL is specifically induced in damaged kidney, it is influenced by a variety of inflammatory conditions [14]. Hence, it is difficult to interpret the significance of increased NGAL levels in patients with both coro-

nary heart disease and concurrent renal dysfunction, particularly in cases of systemic inflammation.

Few studies have closely examined the contribution of myocardial injury, inflammation severity or impaired renal function to augmented plasma NGAL levels in acute MI. In the present study, we calculated the corrected values of NGAL, including the NGAL/inflammation index ratio (NGAL/Inf ratio), the NGAL/sCr ratio and the NGAL/inflammation index and sCr ratio (NGAL/Inf-sCr ratio). Based on these parameters, we estimated the effect of myocardial damage on plasma NGAL levels and investigated the efficacy of the parameters for predicting renal dysfunction in patients with MI.

MATERIALS AND METHODS

Ethics statement

The study protocol was reviewed and approved by the institutional review board, and written informed consents were obtained from all subjects. This study was conducted in accordance with the guidelines of the Helsinki Declaration. All blood samples were collected after a sufficient explanation of the study procedure.

Study population

A total of 117 patients with acute MI were evaluated. Their ages ranged from 53 to 78 years (median age, 65 years), and 79 patients were males (67.5%). Basic demographic data and medical history are summarized in Supplementary Table S1. Age-matched healthy subjects (n=35), who had no evidence of heart diseases, inflammation and renal dysfunction, were enrolled as the control group. Diagnosis of MI was performed based on the criteria for acute MI [15]. Heart failure was defined as plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) concentration ≥ 400 pmol/L, which was based on European guidelines for the diagnosis and treatment of acute and chronic heart failure [16]. Subjects with infectious disease (n=2), sepsis (n=1), and multiple trauma (n=1) were excluded from the study. Patients who had incomplete data (n=3), a recent operation (n=1), or a medication history of antiinflammatory drugs (n=6) were also excluded from the analysis.

Measurement of laboratory parameters

On admission, blood samples were collected to estimate troponin-I, creatine kinase-MB (CK-MB), NT-proBNP, sCr and complete blood cell counts. The blood samples were obtained prior to treatment, such as primary percutaneous coronary intervention, lipid-lowering medications and the administration of antithrombotic and antihypertensive drugs. Plasma NGAL levels were measured by fluorescence immunoassay using the Triage NGAL Test (Alere, Inc., San Diego, CA, USA). The upper normal limit of plasma NGAL was set at 150 ng/mL [17]. All assays for cardiac biomarkers were performed using an Elecsys 2010 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The cardiac biomarkers were measured by an electrochemiluminescence immunoassay using the Elecsys anti-CK-MB, anti-cardiac troponin-I and anti-NT-proBNP antibodies (Roche Diagnostics GmbH). High-sensitivity C-reactive protein (hsCRP) was analyzed by an immunonephelometry assay (Dade Behring, Inc, Deerfield, IL, USA). An increased level of hsCRP was defined as >0.3 mg/dL, which was based on the cutoff point with a 95% confidence interval (CI) for the hsCRP of healthy individuals. The estimated glomerular filtration rate (eGFR) was computed using the Modification of Diet in Renal Disease formula. A renal impairment was defined as having an eGFR of lower than 60 mL/min/1.73 m² [18].

Corrected values of NGAL

The NGAL/Inf ratio was calculated using the following equation:

$$\text{NGAL/Inf ratio} = \text{plasma NGAL concentration} / \text{inflammation index.}$$

The inflammation index was obtained from the sum of the scores that were given to patients on the basis of corrected erythrocyte sedimentation rate (cESR) and hsCRP levels, as described previously [19]. In brief, scores (0.5, 1.0, 1.5, and 2.0) were assigned to each patient by the hsCRP and cESR levels: patients with hsCRP <0.3 mg/dL (score 0.5), 0.3-5.0 mg/dL (score 1.0), 5.1-10.0 mg/dL (score 1.5) and >10.0 mg/dL (score 2.0); patients with cESR <15.0 mm/h (score 0.5), 15.0-30.0 mm/h (score 1.0), 30.1-60.0 mm/h (score 1.5) and >60.0 mm/h (score 2.0). As the score

of 0.5 is given to each of the patients with a hsCRP concentration <0.3 mg/dL and with a cESR level <15.0 mm/h, the sum of the scores in the patients who were within the reference interval in hsCRP and cESR becomes 1 (0.5 plus 0.5). Thus, in patients without evidence of inflammation, the corrected NGAL levels are the same values as the uncorrected NGAL levels.

The cutoff limit of the NGAL/Inf ratio for assessing the risk of renal dysfunction in patients with MI was defined as 145 ng/mL. The cutoff was based on the highest sensitivity and specificity value for identifying renal dysfunction in receiver operating characteristic (ROC) curve analysis. The NGAL/sCr ratio was computed by the following formula:

NGAL/sCr ratio=plasma NGAL level/sCr concentration.

For subjects with sCr<1.0 mg/dL, 1.0 mg/dL of sCr was used to avoid the overestimation due to the decimal point. The NGAL/Inf-sCr ratio was determined by the following equation:

NGAL/Inf-sCr ratio=(NGAL/Inf ratio)/sCr concentration.

Percent difference for NGAL was calculated by the following equation:

$$[(\text{NGAL-corrected NGAL})/\text{NGAL}]\times 100.$$

Categorization of subjects

The subjects were classified into two groups: patients with MI (n=117) and healthy controls (n=35). To investigate the effects of renal dysfunction and inflammation severity on plasma NGAL level, the patients were compared to healthy controls after alternately excluding patients with eGFR<60 mL/min/1.73 m² (n=38) and hsCRP>0.3 mg/dL (n=83) from the study population.

Statistical analysis

Continuous variables were expressed as the mean± standard deviation, and asymmetrically distributed data were presented as the median and interquartile range. Categorical variables were described using frequencies and percentage. The Student's t-test and

Mann-Whitney U test were used. The ROC curve was analyzed to determine the diagnostic efficacy of NGAL and the NGAL/Inf ratio for identifying impaired renal function in patients. Multivariate linear regression analysis was performed to determine the association between cardiac biomarkers and lipocalin levels after adjusting for potential confounders. The association between the NGAL/Inf ratio and the presence of renal dysfunction was assessed by multivariate logistic regression analysis. The data were analyzed using SPSS software (version 19.0; SPSS Inc., Armonk, NY, USA). P values<0.05 were considered as statistically significant.

RESULTS

NGAL and its corrected values

Plasma NGAL levels were significantly higher in patients with MI than in healthy controls (148.5 ng/mL versus 67.4 ng/mL; p<0.001). Of the 117 patients, 38 (32.4%) patients had impaired renal function, 34 (29.1%) had heart failure and 83 (70.1%) had elevated hsCRP. The percent difference between NGAL and the NGAL/Inf ratio was 35.6%, whereas the percent difference between NGAL and the NGAL/sCr ratio was 15.4% (p<0.001). The NGAL/Inf-sCr ratio of the patients did not differ from that of healthy individuals (72.3 ng/mL vs. 65.9 ng/mL; p=0.527), and the percent difference between the two median values was 9.7% (Supplementary Table S1).

NGAL according to hsCRP and eGFR

The effects of impaired renal function (eGFR<60 mL/min/1.73 m²) and active inflammation (hsCRP>0.3 mg/dL) on the plasma NGAL concentration of patients with MI were assessed. After excluding subjects with eGFR<60 mL/min/1.73 m² from the study population, the plasma NGAL concentration of the patients was still high compared with that of the healthy controls (106.0 ng/mL versus 67.4 ng/mL; p<0.05). However, after excluding subjects with hsCRP>0.3 mg/dL, no significant difference in the plasma NGAL level was noted between the groups (Table 1).

Table 1. Plasma NGAL levels after excluding patients with renal dysfunction and active inflammation.

| | Patients with MI (n=117) | | Healthy controls (n=35) |
|------------------------------------|---|--|-------------------------|
| | After excluding patients with eGFR < 60 mL/min/1.73 m ² (n=79) | After excluding patients with hsCRP > 0.3 mg/dL (n=34) | |
| Age (year) | 64 (56-75) | 67 (57-74) | 63 (51-79) |
| Sex (male, %) | 25 (65.7) | 54 (65.1) | 24 (68.6) |
| BMI (kg/m ²) | 23.4±2.6 | 23.2±2.5 | 22.9±3.7 |
| Systolic BP (mmHg) | 130.7±20.3 | 129.6±21.8 | 124.8±20.3 |
| Diastolic BP (mmHg) | 82.1±12.2 | 80.7±13.4 | 79.6±12.5 |
| Heart rate (/min) | 72±13 | 76±15 | 74±13 |
| Lipocalin level | | | |
| NGAL (ng/mL) | 106.0 (61.2-210.5) ^a | 75.6 (53.4-203.1) | 67.4 (52.0-105.1) |
| Inflammatory parameter | | | |
| hsCRP (mg/dL) | 1.31 (0.08-6.94) ^b | 0.07 (0.04-0.15) | 0.16 (0.06-0.25) |
| Renal parameters | | | |
| sCr (mg/dL) | 1.02±0.54 ^b | 1.25±1.20 ^a | 0.84±0.19 |
| eGFR (mL/min/1.73 m ²) | 83.8±22.7 ^b | 68.3±29.2 ^a | 89.4±18.4 |
| Cardiac biomarkers | | | |
| Troponin-I (µg/L) | 1.31 (0.59-4.08) ^a | 1.36 (0.65-4.72) ^a | 0.10 (0.10-0.16) |
| CK-MB (ng/mL) | 7.3 (4.8-11.5) ^a | 7.5 (5.1-12.4) ^a | 2.2 (0.8-4.1) |
| NT-proBNP (pmol/L) | 309.4 (176.2-631.7) ^a | 312.3 (180.4-642.8) ^a | 21.4 (9.3-37.9) |

Data are expressed as the mean±SD, median (interquartile range) or frequency (percentage).

^a Significant (p<0.05), compared to healthy controls.

^b Significant (p<0.05), compared to the patients after excluding patients with hsCRP>0.3 mg/dL.

MI – myocardial infarction; BMI – body mass index; BP – blood pressure; NGAL – neutrophil gelatinase-associated lipocalin; sCr – serum creatinine; hsCRP – high-sensitivity C-reactive protein; eGFR – estimated glomerular filtration rate; CK-MB – creatine kinase-MB; NT-proBNP – N-terminal pro-brain natriuretic peptide.

Table 2. Univariate and multivariate linear regression analysis of NGAL and its corrected values in relation to cardiac biomarkers.

| | Univariate | | Multivariate* | |
|--------------------|------------|---------|---------------|---------|
| | Standard β | p value | Standard β | p value |
| NGAL | | | | |
| Troponin-I (µg/L) | 0.163 | 0.142 | 0.131 | 0.352 |
| CK-MB (ng/mL) | 0.148 | 0.259 | 0.122 | 0.316 |
| NT-proBNP (pmol/L) | 0.173 | 0.139 | 0.140 | 0.238 |
| NGAL/Inf ratio | | | | |
| Troponin-I (µg/L) | 0.325 | <0.001 | 0.289 | <0.001 |
| CK-MB (ng/mL) | 0.165 | 0.139 | 0.127 | 0.307 |
| NT-proBNP (pmol/L) | 0.294 | <0.001 | 0.253 | <0.001 |
| NGAL/sCr ratio | | | | |
| Troponin-I (µg/L) | 0.305 | <0.001 | 0.256 | <0.001 |
| CK-MB (ng/mL) | 0.293 | <0.001 | 0.251 | <0.001 |
| NT-proBNP (pmol/L) | 0.178 | 0.142 | 0.139 | 0.258 |

* Adjusted for age, gender, BMI, systolic BP, smoking habit and diabetes. NGAL – neutrophil gelatinase-associated lipocalin; NT-proBNP – N-terminal pro-brain natriuretic peptide; CK-MB – creatine kinase-MB; NGAL/Inf ratio – ratio of NGAL to the inflammation index; NGAL/sCr ratio – ratio of NGAL to serum creatinine.

Linear regression analysis

In multivariate linear regression analysis adjusted for potential confounders, no significant association was observed between plasma NGAL concentration and the levels of troponin-I and CK-MB. However, the NGAL/Inf ratio was positively correlated with troponin-I (r=0.289, p<0.001) and the NGAL/sCr ratio was significantly correlated with CK-MB (r=0.251, p<0.001) (Table 2). Correlation between the NGAL/Inf ratio and troponin-I is illustrated in Fig. 1.

Multivariate logistic regression analysis

In logistic regression analysis, an elevated NGAL/Inf ratio (>145 ng/mL) was significantly associated with the presence of renal dysfunction in patients with MI following adjustment for potential confounders, such as age, gender, BMI, systolic blood pressure, smoking habit and diabetes (odds ratio, 1.35; 95% CI, 1.07-2.52; p<0.001) (Table 3).

ROC curve analysis

The diagnostic ability of the NGAL/Inf ratio for identifying impaired renal function in patients with MI was investigated by ROC curve analysis. The area under the curve (AUC) of the NGAL/Inf ratio (0.811; 95% CI, 0.729-0.893) for identifying renal dysfunction was significantly larger than that of plasma NGAL concentration (0.694; 95% CI, 0.599-0.790; p<0.001) (Fig. 2).

DISCUSSION

In the present study, the relationship between plasma NGAL level, cardiac biomarkers, kidney function and the severity of inflammation in MI was investigated. The median plasma NGAL concentration of patients with MI was significantly higher than that of healthy individuals. Our results are in agreement

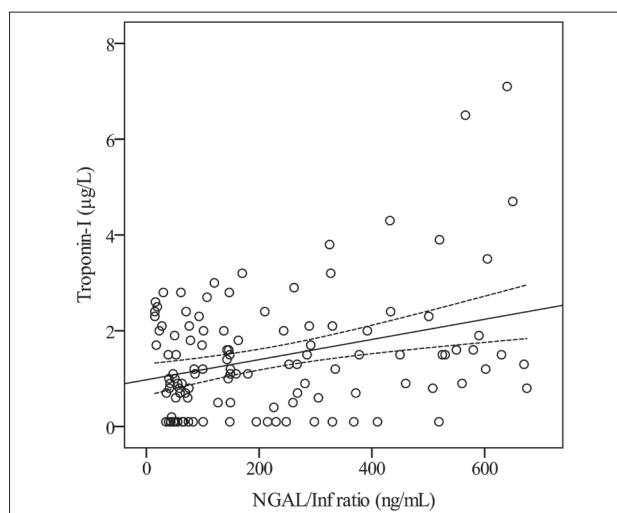


Fig. 1. Correlation between the NGAL/Inf ratio and troponin-I in patients with MI. The NGAL/Inf ratio correlates positively with troponin-I level ($y=0.002x+0.977$; $r^2=0.105$; $p<0.001$). NGAL/Inf ratio – ratio of NGAL to the inflammation index; MI – myocardial infarction.

Table 3. Multivariate logistic regression analysis for the presence of renal dysfunction in relation to the NGAL/Inf ratio.

| NGAL/Inf ratio > 145 ng/mL as a categorical variable | Presence of renal dysfunction | |
|---|-------------------------------|---------|
| | Odds ratio (95% CI) | p value |
| Unadjusted | 1.76 (1.26-3.50) | <0.001 |
| Adjusted for age, gender, and BMI | 1.51 (1.15-3.41) | <0.001 |
| Adjusted for age, gender, BMI, and systolic BP | 1.43 (1.14-3.29) | <0.001 |
| Adjusted for age, gender, BMI, systolic BP, and smoking | 1.48 (1.20-3.43) | <0.001 |
| Adjusted for age, gender, BMI, systolic BP, smoking, and diabetes | 1.35 (1.07-2.52) | <0.001 |

NGAL/Inf ratio – ratio of NGAL to the inflammation index; BMI – body mass index; BP – blood pressure; CI – confidence interval.

with those of a previous study, which demonstrated the enhancement of NGAL expression in patients with acute MI and the increase of serum NGAL level in cases of coronary heart disease [20].

NGAL is an acute phase reactant that participates in various antibacterial immune responses [21]. In normal populations, plasma NGAL levels are largely determined by the granulocyte count and hsCRP concentration, whereas in patients with impaired renal function, plasma NGAL levels are mainly determined by the eGFR [22]. The activation of granulocytes

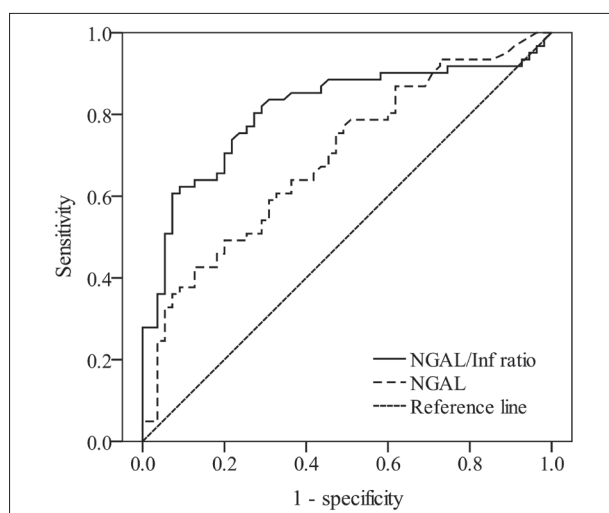


Fig. 2. ROC curve analysis showing the diagnostic efficacy of NGAL and the NGAL/Inf ratio for identifying renal dysfunction in patients with MI. The AUC of the NGAL/Inf ratio was significantly larger than that of plasma NGAL concentration (0.811; 95% CI, 0.729-0.893 versus 0.694; 95% CI, 0.599-0.790; $p<0.001$). ROC – receiver operating characteristic; NGAL – neutrophil gelatinase-associated lipocalin; NGAL/Inf ratio – ratio of NGAL to the inflammation index; MI – myocardial infarction; AUC – area under the curve.

and the subsequent release of NGAL are crucial in the development of inflammatory reactions in the course of cardiovascular diseases [23]. A previous study reported that elevated NGAL concentrations reflect the inflammatory status in various stages of coronary artery disease [24]. In our study, 32.4%, 29.1%, and 70.1% of the patients had renal dysfunction, heart failure and elevated hsCRP, respectively. Therefore, the elevated plasma NGAL levels of our patients may reflect the overall effect of several adverse conditions, such as myocardial injury, activated neutrophils due to systemic inflammation and impaired renal function associated with MI.

A study has proposed that elevated NGAL level in cardiovascular diseases is caused by the enhanced NGAL expression in the cardiomyocytes of the injured myocardium in response to proinflammatory cytokines [25]. In our study, to adjust the effect of inflammation and kidney function on plasma NGAL levels, corrected values of plasma NGAL were calculated. The NGAL/Inf-sCr ratio of patients with MI exhibited an increasing trend compared to that of healthy individuals; however, the differences were not

statistically significant. After adjusting for inflammation and kidney function, the elevated NGAL levels returned to baseline levels, which were not significantly different from those of healthy individuals, suggesting that enhanced NGAL production in MI may be caused by inflammation and renal dysfunction rather than failing myocardium.

A study reported that augmented NGAL expression in acute MI may be considered as a mediator of postischemic inflammation [26]. In contrast, another study demonstrated that an increased plasma NGAL level, which was observed in coronary artery disease, may be largely attributed to impaired renal function [27]. In the current study, we examined which of the conditions (inflammation or kidney function) would more critically affect plasma NGAL levels in MI. When patients with impaired renal function were excluded from the subject populations, the plasma NGAL level remained significantly higher than that of the healthy individuals. However, when patients with elevated hsCRP were excluded from the study population, the plasma NGAL level of the patients did not differ from that of the healthy controls. Additionally, the percent difference between NGAL and the NGAL/Inf ratio of patients with MI was 35.6%, which was significantly different from that (15.4%) between NGAL and the NGAL/sCr ratio. These results suggest that inflammation and renal dysfunction contributed to approximately 35.6% and 15.4% of the elevated NGAL levels, respectively, in patients with MI. The results also suggest that the severity of inflammation could play a more crucial role than impaired renal function in affecting plasma NGAL levels, at least for the population in this study.

As MI is frequently accompanied by systemic inflammation and renal dysfunction, it is difficult to assess to what extent myocardial damage contributes to NGAL production. In our study, after adjusting for inflammation and kidney function, the median percent difference of the NGAL/Inf-sCr ratio between patients and healthy controls was 9.7%. Based on these results, it can be estimated that myocardial injury accounted for approximately 9.7% of the increase in the total plasma NGAL level of patients with MI. Overall, our results indicated that the effect of myocardial injury on plasma NGAL levels was smaller than that of inflammation and impaired renal function.

Renal insufficiency is an important independent predictor of poor prognosis in patients with cardiovascular diseases [28]. A group of researchers reported that measurement of plasma NGAL is useful for evaluating renal dysfunction during hospitalization in cases of cardiac diseases [29]. In the present study, the diagnostic efficacy of NGAL, which identifies renal dysfunction in MI, was compared to that of the NGAL/Inf ratio. The NGAL/Inf ratio demonstrated better results in ROC curve analysis. Moreover, in logistic regression analysis, compared to a decreased NGAL/Inf ratio, an elevated NGAL/Inf ratio resulted in a 1.35-fold increase in the risk of renal dysfunction. The findings of our study indicated that the NGAL/Inf ratio was superior to NGAL as a predictor of renal insufficiency in MI. The corrected value of calculated NGAL ratio seems to reduce the impact of the inflammatory condition on the plasma NGAL concentration in patients with renal dysfunction in conjunction with inflammatory diseases.

In a previous study, there were no significant relationships between plasma NGAL concentration and cardiac function in patients with MI [25]. Similarly, in our study, the plasma NGAL level was not significantly associated with the levels of troponin-I and CK-MB. However, the NGAL/Inf ratio was closely associated with troponin-I, and the NGAL/sCr ratio was significantly associated with CK-MB. A possible explanation for these findings is that the overestimated plasma NGAL level, attributed to concomitant inflammation and renal dysfunction, was corrected by adjusting for the inflammation index and sCr levels. Our results suggest that plasma NGAL levels need to be amended using the inflammation index and sCr levels of patients with MI, particularly when systemic inflammation is presented with renal impairment.

There are several limitations in this study. Our results were based on observational data in a cross-sectional study. Therefore, we could not prove a cause-and-effect relationship between NGAL and MI. In addition, we did not measure plasma NGAL levels in serial samples to assess the changes in NGAL in relation to the progression of disease. Despite these limitations, the results of our study are significant. To our knowledge, this is the first study to investigate the NGAL/Inf ratio as an indicator of renal dysfunction in MI. Our results may contribute to the evaluation of MI,

particularly in patients with concurrent inflammation and renal dysfunction. However, further validation is needed in larger, randomized prospective studies.

CONCLUSION

This study demonstrates that the severity of inflammation plays a more crucial role than myocardial injury and impaired renal function in affecting plasma NGAL levels in patients with MI. The diagnostic efficacy of the NGAL/Inf ratio showed a better performance than that of plasma NGAL, suggesting that the measurement of the NGAL/Inf ratio may be an additional benefit in predicting worsening renal function in patients with MI.

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Author contributions: Jong Weon Choi designed the study, organized the research, analyzed the data, and wrote the manuscript. Moon Hee Lee analyzed the data, prepared the tables and reviewed the drafts of the manuscript. Tatsuyoshi Fujii performed the statistical analyses, analyzed the data, searched the literature data and edited the manuscript.

Conflict of interest disclosure: The authors declare that they have no conflict of interest.

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Supplementary Material

The Supplementary Material is available at: http://serbiosoc.org.rs/NewUploads/Uploads/4341_Supplementary%20Table%20S1.pdf