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Neutrophil Gelatinase-Associated Lipocalin for Predicting Intensive Care Unit Admission and Mortality in Patients with Pneumonia

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Pneumonia is one of the most common causes of hospital admissions and mortality, and it is responsible for significant socioeconomic burden worldwide. Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein, which is involved in iron trafficking and has chemostatic and bacteriostatic effects. NGAL is also known as an early marker of many inflammatory diseases. However, little is known about the role of NGAL in the management of pneumonia. Thus, this study aimed to investigate whether plasma NGAL levels can predict intensive care unit (ICU) admission and in-hospital mortality in patients with pneumonia. This retrospective observational study included 241 adults hospitalized with pneumonia who underwent NGAL measurement. We compared the prognostic values of plasma NGAL with pneumonia severity index (PSI) for prediction of ICU admission and in-hospital mortality. Of 241 patients, 47 (19.5%) died during hospital admission. There was no significant difference between NGAL and PSI for predicting ICU admission (area under the receiver operating characteristic curve [AUC] of log NGAL vs. PSI, $P > 0.999$). Although log NGAL was useful in predicting in-hospital mortality, its ability was inferior to that of PSI (AUC of log NGAL vs. PSI, $P = 0.008$). Multivariable analysis revealed that log NGAL was significantly associated with ICU admission (adjusted odds ratio = 10.76, $P < 0.001$) and in-hospital mortality (adjusted odds ratio = 5.04, $P = 0.004$). These results suggest that plasma NGAL level is a useful biomarker for predicting ICU admission and mortality in hospitalized patients with pneumonia.

Keywords: biomarker; death; intensive care units; lipocalin-2; pneumonia
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Introduction

Pneumonia is one of the most common causes of hospital admissions and mortality, and it is responsible for significant socioeconomic burden worldwide (GBD 2015 LRI Collaborators 2017; Thomas et al. 2012). Despite advances in the treatment of pneumonia, patients with severe pneumonia, especially those who require hospital admission or intensive care unit (ICU) admission(s), continue to have high mortality rates (Shindo et al. 2015; Li et al. 2016). Therefore, it is clinically important to appropriately select patients who have a higher probability of clinical deterioration necessitating ICU management. Unfortunately, however, few biomarkers are available for this purpose.

Blood biomarkers may be clinically useful to guide clinical decisions and to predict treatment outcomes in patients with pneumonia (Ebrahimi et al. 2018). Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein which is involved in iron trafficking (Bao et al. 2010) and has chemostatic (Schroll et al. 2012) and bacteriostatic effects (Goetz et al. 2002). This protein is actively secreted by neutrophils and respiratory epithelial cells (Cowland and Borregaard 1997; Friedl et al. 1999), and is associated with acute kidney injury (Devarajan 2008). NGAL is also known to be associated with many types of cancers (Bolignano et al. 2010; Chakraborty et al. 2012). Regarding pulmonary diseases, it has been suggested that blood or bronchoalveolar lavage fluid NGAL may reflect

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inflammation in many pulmonary diseases such as chronic obstructive pulmonary disease (COPD) (Betsuyaku et al. 1999; Eagan et al. 2010), idiopathic pulmonary fibrosis (IPF) (Ikezoe et al. 2014), and ventilator-associated lung injury (Xiao and Chen 2017).

However, few data are available whether NGAL could predict treatment outcomes in patients with pneumonia, especially in those with severe pneumonia requiring hospitalization (Yeh et al. 2013; Kim et al. 2016). Thus, we aimed to evaluate whether NGAL can predict ICU admission and mortality in hospitalized patients with pneumonia.

Materials and Methods

Study design and population

A retrospective cohort study, including 241 patients aged ≥ 19 years, who had pneumonia and who underwent NGAL measurement at Hanyang University Hospital, a tertiary university hospital with 828 beds in Seoul, South Korea, between November 2016 and December 2017, was performed. Patients aged < 19 years and those who had end-stage renal disease and received renal replacement therapy were excluded. This investigation was approved by the Institutional Review Board of Hanyang University Hospital (2018-03-024-004). Given the retrospective nature of the study and the use of anonymized patient data, the requirement for informed consent was waived.

Primary and secondary outcomes

The primary objectives of this study were to evaluate whether NGAL could predict ICU admission and in-hospital all-cause mortality. The secondary endpoint was to evaluate the correlation of NGAL and PSI.

Data collection

Data on demographic characteristics including age, sex, smoking history, and underlying chronic diseases, and laboratory findings including plasma concentration of NGAL measured within 24 h after the diagnosis of pneumonia, serum concentrations of C-reactive protein (CRP), white blood cell count (WBC), creatinine, and procalcitonin, were collected by reviewing medical charts. Pneumonia was defined as a new infiltrate in a chest radiograph with respiratory symptoms compatible with pneumonia, such as fever (> 38.0℃), cough, sputum, hemoptysis, and dyspnea. Data about each component of PSI were collected by reviewing medical charts, and PSI was calculated as previously reported (Fine et al. 1997). Briefly, in the calculation of PSI, various demographic, clinical, laboratory, and radiographic factors were considered, including age, nursing home resident, presence of neoplastic disease, chronic liver disease, congestive heart failure, cerebrovascular disease, altered mental status, pleural effusion on X-ray, respiratory rate, systolic blood pressure, body temperature, heart rate, and laboratory test results (arterial pH, blood urea nitrogen, sodium level, glucose level, hematocrit, and PaO2).

Measurement of plasma levels of NGAL

Plasma levels of NGAL were measured using a particle-enhanced turbidimetric immunoassay (NGAL TEST™, BioPorto Diagnostics A/S, Hellerup, Denmark) on a Beckman Coulter AU 5822 (Beckman Coulter, USA) according to the manufacturer's instructions. Briefly, plasma samples from the subjects were mixed with reaction buffer and incubated for a short time. Subsequently, an immunoparticle suspension (polystyrene microparticles coated with mouse monoclonal antibodies to NGAL) were added to cause aggregation of the immunoparticles. After quantifying the degree of aggregation using a light absorption test, plasma levels of NGAL were calculated based on an established calibration curve (Lippi et al. 2011).

Statistical analyses

Data are presented as mean (standard deviation), median (interquartile range [IQR]), or as number (percentages), as appropriate. Categorical variables were compared using the Pearson's chi-squared test or Fisher's exact test. Continuous variables were compared using the Student's t-test or Mann-Whitney U test. The prognostic values of plasma biomarkers and risk scores for the prediction of ICU admission and in-hospital mortality were evaluated by constructing receiver operating characteristic (ROC) curves and the area under the ROC curves (AUCs) were determined to ascertain the efficacy of the biological markers. The methods of Hanley & McNeil were used for the calculation of the standard error of the AUC and of the difference between the two AUCs. Bonferroni correction for multiple comparisons was performed. The optimal cutoff values for the prediction of ICU admission and in-hospital mortality were determined by ROC curve analysis with consideration of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The patients were divided into high NGAL and low NGAL groups according to the optimal cut-off value of log NGAL was used for the prediction of in-hospital mortality, and the difference of in-hospital mortality between the two groups was compared using Kaplan-Meier survival analysis. A multivariable logistic regression analysis was used to evaluate the independent predictors of ICU admissions and in-hospital mortality. All data were analyzed using Stata version 15.0 (Stata Corporation, College Station, TX, USA).

Results

Study population

The baseline characteristics of the 241 patients are summarized in Table 1. The median age was 75 years (IQR, 63-80 years) and 61% were male. Common comorbidities included diabetes mellitus (33.2% [n = 80]), malignancy (17.0% [n = 41]) including lung cancer (7.1% [n = 17]), chronic kidney disease (15.4% [n = 37]), previous history of pulmonary tuberculosis (9.5% [n = 23]), and interstitial lung disease (4.2% [n = 10]). There were 60 patients (24.9%) with hospital-acquired pneumonia and 181 patients

Table 1. Baseline characteristics.

	Total (n = 241)	Patients who survived (n = 194)	Patients who died (n = 47)	*P* value[a]
Age (median [IQR])	75.0 (63.0-80.0)	73.0 (61.0-80.0)	77.0 (69.0-81.0)	0.019
Male sex (%)	147 (61.0)	111 (57.2)	36 (76.6)	0.023
Smoking history (%)				0.741
Never-smokers	165 (68.5)	134 (69.1)	31 (66.0)	
Current smoker	52 (21.5)	40 (20.6)	12 (25.5)	
Ex-smoker	24 (10.0)	20 (10.3)	4 (8.5)	
Comorbidities (%)				
Diabetes mellitus	80 (33.2)	65 (33.5)	15 (31.9)	0.972
Malignancy[b]	41 (17.0)	29 (14.9)	12 (25.5)	0.008
Chronic kidney disease	37 (15.4)	24 (12.4)	13 (27.7)	0.017
Chronic obstructive pulmonary disease	33 (13.7)	30 (15.5)	3 (6.4)	0.165
Previous history of tuberculosis	23 (9.5)	19 (9.8)	4 (8.5)	1.000
Interstitial lung disease	10 (4.2)	5 (2.6)	5 (10.6)	0.039
Others	16 (6.6)	13 (6.7)	3 (6.4)	1.000
Types of pneumonia (%)				0.153
Hospital acquired pneumonia	60 (24.9)	44 (22.7)	16 (34.0)	
Community acquired pneumonia	181 (75.1)	150 (77.3)	31 (66.0)	
PSI (mean ± SD)	108.3 ± 39.5	98.0 ± 33.5	150.5 ± 34.5	< 0.001
PSI class (%)				< 0.001
I	58 (24.1)	57 (29.4)	1 (2.1)	
II	62 (25.7)	59 (30.4)	3 (6.4)	
III	61 (25.3)	51 (26.3)	10 (21.3)	
IV	60 (24.9)	27 (13.9)	33 (70.2)	

IQR, interquartile range; PSI, pneumonia severity index; SD, standard deviation.
[a]Obtained using the chi-squared test or Fisher's exact test for categorical variables and the Student's t-test or Mann-Whitney U test for continuous variables, as appropriate.
[b]Seventeen had lung cancer.

(75.1%) with community-acquired pneumonia. The mean PSI was 108.3 ± 39.5.

Compared with survivors, non-survivors were more likely to be older (median 77 years vs. 73 years; $P = 0.019$), male (76.6% vs. 57.2%; $P = 0.023$), and have malignancy (25.5% vs. 14.9%; $P = 0.008$), chronic kidney disease (27.7% vs. 12.4%; $P = 0.017$), and interstitial lung disease (10.6% vs. 2.6%; $P = 0.039$) as comorbidities. The mean PSI was significantly higher in non-survivors versus survivors (mean 150.5 vs. 98, respectively; $P < 0.001$) with significantly higher proportion of patients with PSI class IV ($P < 0.001$).

Comparison of laboratory findings and ICU admission between survivors and non-survivors

As shown in Table 2, laboratory findings, including those for serum creatinine, CRP, procalcitonin, and plasma NGAL, were significantly higher in non-survivors than survivors (creatinine, median 1.4 mg/dL vs. 0.8 mg/dL, $P < 0.001$; CRP, median 17.6 mg/dL vs. 11.2 mg/dL, $P = 0.006$; procalcitonin, median 2.5 ng/mL vs. 0.4 ng/mL, $P < 0.001$; NGAL, median 463.6 ng/mL vs. 146.0 ng/mL; $P < 0.001$). The proportion of patients who were admitted to the ICU was significantly higher in non-survivors than survivors (89.4% [42/47] vs. 18.6% [36/194]; $P < 0.001$).

Comparison of log NGAL values according to PSI quartile

There was significant correlation between log NGAL values and PSI (Pearson's correlation = 0.423, $P < 0.001$). The median level of log NGAL significantly increased according to the PSI class ($P < 0.001$) (Fig. 1).

ROC analysis for the prediction of ICU admission

The ROC curves for CRP, log NGAL, and PSI for the prediction of ICU admission in patients with pneumonia is shown in Fig. 2. The AUCs for CRP, log NGAL, and PSI for the prediction of ICU admission were 0.595 ($P < 0.001$), 0.791 ($P < 0.001$), and 0.779 ($P < 0.001$), respectively. While the AUCs of PSI and log NGAL concentration for the prediction of ICU admission were not different ($P = 0.999$), the AUCs of PSI and log NGAL for the prediction of ICU admission were significantly higher than that for CRP ($P < 0.001$ for both PSI and log NGAL). The optimal log NGAL cut-off was 2.33, with a sensitivity of 73.1%, a specificity of 72.4%, PPV of 15.1%, and NPV of 44.1%.

Table 2. Comparison of laboratory findings, ICU admission, and hospital stay in patients who survived versus those who died.

	Total (n = 241)	Patients who survived (n = 194)	Patients who died (n = 47)	*P* value[a]
Laboratory findings (median [IQR])				
WBC, /mm^3	115.0 (78.0-167.0)	112.0 (78.0-156.0)	125 (78-178)	0.369
ANC, /mm^3	8,320 (5,330-12,420)	8,020 (5,360-12,180)	10,420 (4,970-14,660)	0.343
Creatinine, mg/dL	0.9 (0.6-1.4)	0.8 (0.6-1.1)	1.4 (1.0-2.2)	< 0.001
C-reactive protein, mg/dL	11.9 (3.9-21.4)	11.2 (3.1-18.9)	17.6 (5.7-27.7)	0.006
Procalcitonin, ng/dL	0.6 (0.1-5.2)	0.4 (0.1-3.2)	2.5 (0.4-8.9)	< 0.001
NGAL, ng/mL (median [IQR])	180.8 (95.4-365.8)	146.0 (79.4-275.5)	463.6 (208.6-914.4)	< 0.001
log NGAL (mean ± SD)	2.3 ± 0.5	2.2 ± 0.4	2.6 ± 0.5	< 0.001
ICU admission (%)	78 (32.4)	36 (18.6)	42 (89.4)	< 0.001
Hospital stay, days (median [IQR])	12.0 (8.0-18.0)	13.0 (9.0-19.0)	9.0 (5.0-15.0)	0.001

IQR, interquartile range; WBC, white blood cell; ANC, absolute neutrophil count; NGAL, Neutrophil gelatinase-associated lipocalin; SD, standard deviation; ICU, intensive care unit.
[a]Obtained using the chi-squared test or Fisher's exact test for categorical variables and the Student's t-test or Mann-Whitney U test for continuous variables, as appropriate.

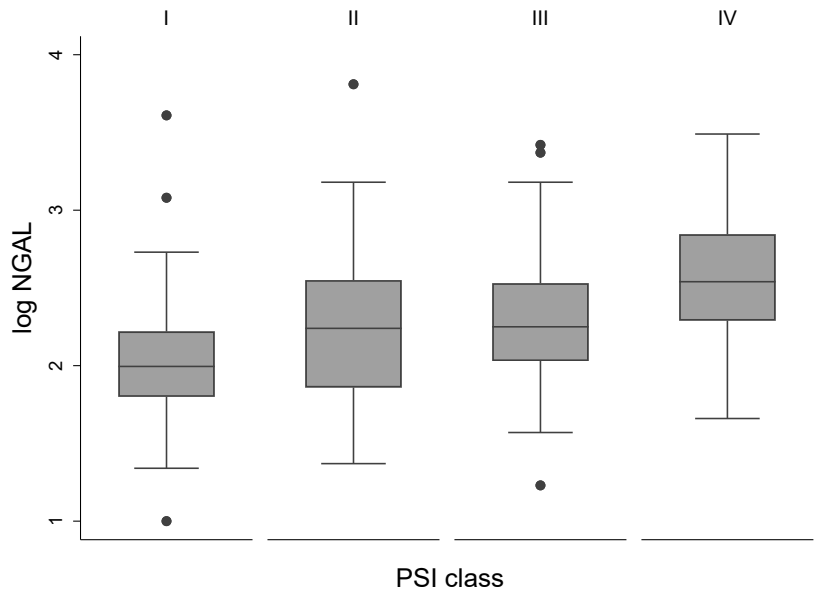

Fig. 1. log NGAL level according to PSI class.
NGAL, neutrophil gelatinase-associated lipocalin; PSI, pneumonia severity index.

ROC analysis for the prediction of in-hospital mortality

The ROC curves for CRP, log NGAL, and PSI for the prediction of in-hospital mortality in patients with pneumonia are shown in Fig. 3. The AUCs for CRP, log NGAL, and PSI for the prediction of in-hospital mortality were 0.629 ($P < 0.001$), 0.743 ($P < 0.001$), and 0.863 ($P < 0.001$), respectively. Although the AUC of log NGAL for the prediction of in-hospital mortality was lower than that of PSI ($P = 0.008$), it was significantly higher than that of CRP ($P < 0.001$). The optimal log NGAL cut-off was 2.35, with a sensitivity of 74.5%, a specificity of 67.5%, PPV of 8.4%, and NPV of 64.3%.

Association between log NGAL and ICU admission

As shown in Table 3, in univariable analysis, log NGAL (odds ratio [OR] 17.01, 95% confidence interval (CI) 7.38-39.62]), creatinine (OR 2.28, 95% CI 1.61-3.24), CRP (OR 1.02 [95% CI 1.01-1.05]), chronic kidney disease (OR 2.96, 95% CI 1.45-6.05), and PSI class III-IV (OR 11.14, 95% CI 4.16-33.54) were factors associated with ICU admission.

In multivariable analyses, log NGAL (adjusted OR 10.76, 95% CI 3.54-32.73) and PSI class III-IV (adjusted OR 10.95, 95% CI 4.08-29.38) were significantly associated with ICU admission in patients with pneumonia.

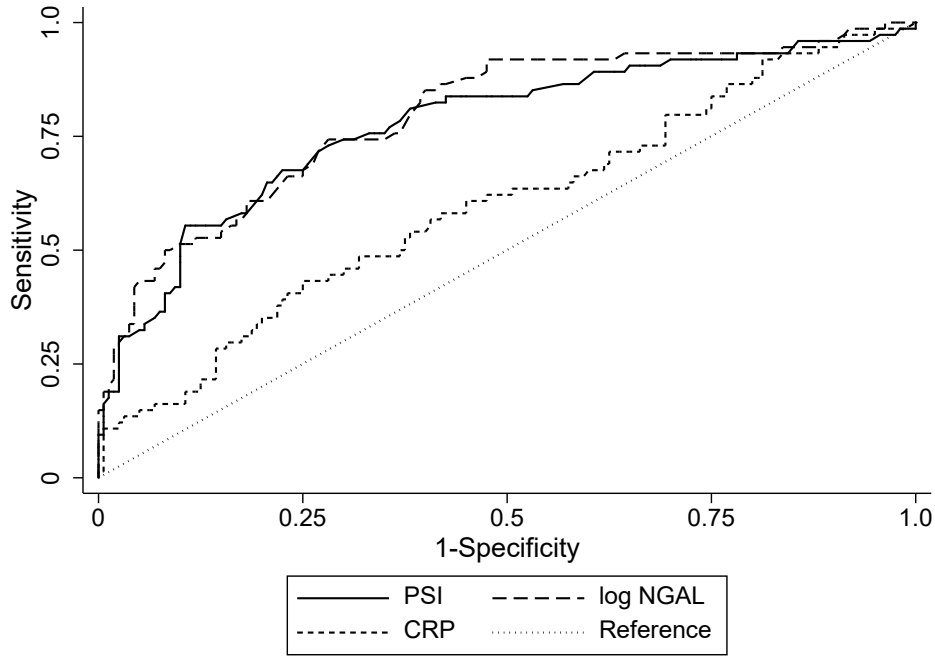

Fig. 2. ROC curves of C-reactive protein and NGAL concentrations, and PSI for the prediction of ICU admission.
ROC, receiver operating characteristic; NGAL, neutrophil gelatinase-associated lipocalin; PSI, pneumonia severity index; ICU, intensive care unit.

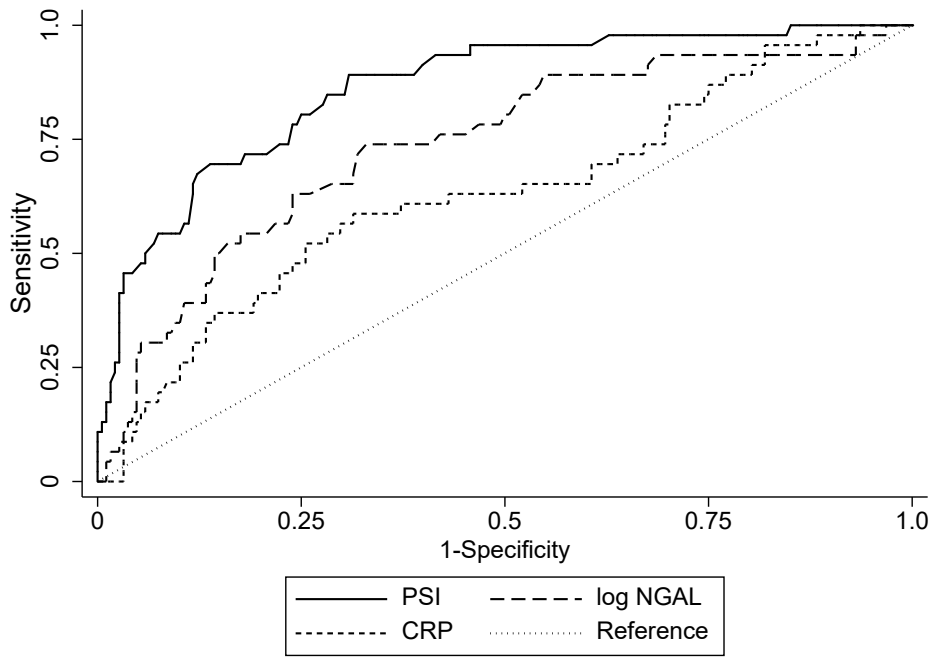

Fig. 3. ROC curve of C-reactive protein and NGAL concentrations, and PSI for the prediction of in-hospital mortality in patients with pneumonia.
ROC, receiver operating characteristic; NGAL, neutrophil gelatinase-associated lipocalin; PSI, pneumonia severity index.

Association between log NGAL and in-hospital mortality

As shown in Table 4, in univariable analysis, log NGAL (OR 6.61, 95% CI 3.05-14.30), age (OR 1.03, 95% CI 1.01-1.06), female sex (OR 0.41, 95% CI 0.21-0.85), creatinine (OR 1.40, 95% CI 1.10-1.78), CRP (OR 1.03, 95% CI 1.01-1.06), the presence of interstitial lung disease (OR 4.48, 95% CI 1.24-16.16), chronic kidney disease (OR 2.71, 95% CI 1.26-5.84), and PSI class III-IV (OR 15.99, 95% CI 6.17-54.70) were significantly associated with in-hospital mortality.

In multivariable analyses, log NGAL (adjusted OR 5.04, 95% CI 1.66-15.31), female sex (adjusted OR 0.34,

Table 3. Factors associated with intensive care unit admission.

	Univariate analysis		Multivariable analysis	
Factor	Unadjusted OR	95% CI	Adjusted OR	95% CI
log NGAL	17.01	7.38-39.62	10.76	3.54-32.73
Age	1.00	0.98-1.02	0.95	0.93-0.98
Female sex	0.76	0.43-1.33	0.77	0.33-1.78
Smoking history				
Never-smokers	Reference		Reference	
Current or ex-smoker	1.13	0.63-2.01	0.78	0.34-1.79
Laboratory findings				
White blood cell	1.00	1.00-1.00	1.00	1.00-1.00
Creatinine	2.28	1.61-3.24	1.14	0.76-1.71
C-reactive protein	1.02	1.01-1.05	1.00	0.97-1.03
Comorbidities				
Interstitial lung disease	2.15	0.60-7.66	1.35	0.29-6.28
Malignancy	0.97	0.33-2.78	0.74	030-6.28
Chronic kidney disease	2.96	1.45-6.05	0.77	0.29-2.04
PSI class				
I-II	Reference		Reference	
III-IV	11.14	4.16-33.54	10.95	4.08-29.38

CI, confidence interval; NGAL, neutrophil gelatinase-associated lipocalin; OR, odds ratio; PSI, pneumonia severity index.

Table 4. Factors associated with in-hospital mortality.

	Univariate analysis		Multivariable analysis	
Factor	Unadjusted OR	95% CI	Adjusted OR	95% CI
log NGAL	6.61	3.05-14.30	5.04	1.66-15.31
Age	1.03	1.01-1.06	1.00	0.97-1.04
Female sex	0.41	0.21-0.85	0.34	0.13-0.95
Smoking history				
Never-smoker	Reference		Reference	
Current-or ex-smoker	1.15	0.59-2.27	0.67	0.27-1.67
Laboratory findings				
White blood cell	1.00	1.00-1.00	1.00	0.99-1.00
Creatinine	1.40	1.10-1.78	0.96	0.67-1.38
C-reactive protein	1.03	1.01-1.06	1.03	1.00-1.06
Comorbidities				
Interstitial lung disease	4.48	1.24-16.16	8.18	1.71-38.93
Malignancy	1.95	0.91-4.19	1.18	0.44-3.17
Chronic kidney disease	2.71	1.26-5.84	1.18	0.43-3.24
PSI class				
I-II	Reference		Reference	
III-IV	15.99	6.17-54.70	12.21	3.34-44.66

CI, confidence interval; NGAL, neutrophil gelatinase-associated lipocalin; OR, odds ratio; PSI, pneumonia severity index.

95% CI 0.13-0.95), CRP (adjusted OR 1.03, 95% CI 1.00-1.06), the presence of interstitial lung disease (adjusted OR 8.18, 95% CI 1.71-38.93), and PSI class III-IV (adjusted OR 12.21, 95% CI 3.34-44.66) were significant factors associated with in-hospital mortality.

Survival analysis according to NGAL level

As shown in Fig 4, patients in the high NGAL group

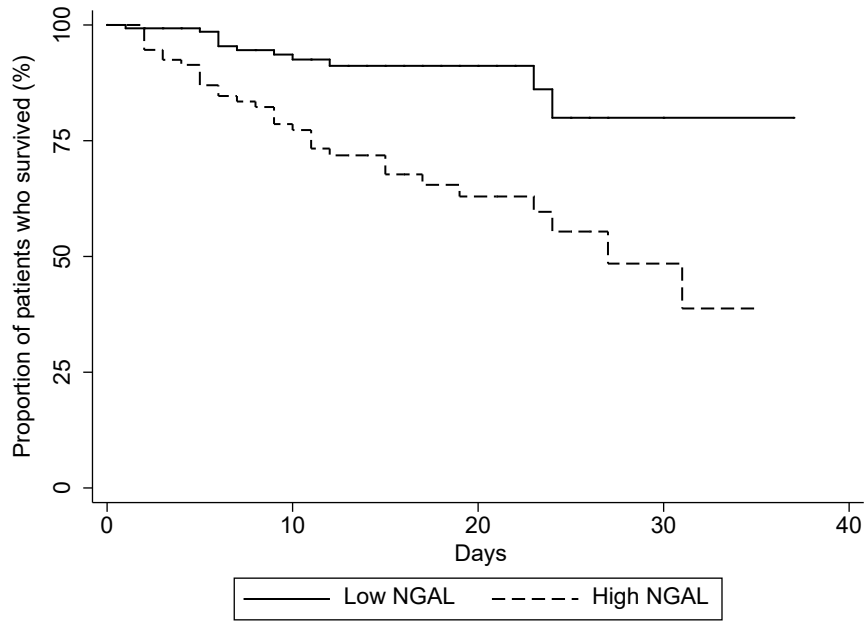

Fig. 4. Survival analysis according to log NGAL level. The cut-off value of the log NGAL cut-off was 2.35. NGAL, Neutrophil gelatinase-associated lipocalin.

(i.e., log NGAL ≥ 2.35) were more likely to die compared with those in the low NGAL group (log NGAL < 2.35) (35.7% [35/98] vs. 8.4% [12/143]; $P < 0.001$).

Discussion

The present study evaluated the association between plasma levels of NGAL, ICU admission, and in-hospital mortality in 241 hospitalized patients with pneumonia. Patients who were admitted to the ICU had significantly higher NGAL levels compared with those who were not. Similarly, non-surviving patients were more likely to have higher NGAL levels than surviving patients. NGAL was as useful as PSI for predicting ICU admission. Although NGAL was useful in predicting in-hospital mortality, its ability was slightly inferior to that of PSI. Even after adjusting for several important covariables, NGAL was found to be an independent biomarker predicting ICU admission and in-hospital mortality.

Although NGAL is extensively studied in renal diseases, recent studies have suggested that NGAL levels in the blood or bronchoalveolar lavage fluid can reflect disease severity in many pulmonary diseases such as COPD and IPF (Betsuyaku et al. 1999; Eagan et al. 2010; Ikezoe et al. 2014). Compared with controls, COPD patients were more likely to exhibit higher levels of NGAL, and elevated NGAL level was significantly associated with severe disease progression (i.e., more frequent exacerbations) (Eagan et al. 2010). Another study reported that emphysema is associated with elevated levels of NGAL (Betsuyaku et al. 1999). Studies evaluating the association between NGAL and IPF have also shown that NGAL is highly expressed in lung tissues and associated with disease severity in patients with IPF (Ikezoe et al. 2014).

Accumulating evidence suggests that NGAL is associated with infectious conditions. According to an *in vivo* study using NGAL-deficient mice, NGAL was shown to limit bacterial growth via iron sequestration in the innate immune system (Flo et al. 2004). Several *in vitro* studies have also demonstrated that NGAL can inhibit the growth of specific strains of bacteria, including *Escherichia coli* and *Mycobacterium tuberculosis* (Martineau et al. 2007; Mori et al. 2016; Saiga et al. 2008). Furthermore, two recent studies demonstrated a significant association between NGAL and the severity of community-acquired pneumonia. In these studies, plasma NGAL concentrations were strongly correlated with clinical pneumonia severity scoring systems such as the PSI and CURB-65 (Kim et al. 2016; Yeh et al. 2013). Furthermore, higher NGAL concentration was an independent predictor of mortality in these populations (Kim et al. 2016). We extended the results of previous studies by evaluating patients with more severe disease (i.e., hospitalized patients) and showed that NGAL could be a useful biomarker in predicting treatment outcomes in patients with severe pneumonia. Moreover, a particular strength of our study was that it included patients diagnosed with hospital-acquired as well as community-acquired pneumonia.

ICU admission is an important factor associated with high mortality rates in patients with pneumonia (Chalmers 2009). Considering the relative lack of medical resources, careful selection of patients with pneumonia who are likely to experience a more severe clinical course is very important. Unfortunately, however, to date, there have been no suitable parameters or biomarkers that can be used for this

purpose (Chalmers 2009). Although the PSI or CURB-65 can be used for this purpose, PSI is complex and challenging to use in emergent situations, and CURB-65 may be better suited to community settings (Singanayagam et al. 2009). From this perspective, our study provided informative data in that NGAL level was highly correlated with clinical decisions on ICU admission, with a similar performance of PSI, suggesting that NGAL may be a promising biomarker for this issue.

There were several limitations to this study. First, this study was performed in a referral hospital in Korea, which may limit the generalizability of the results. Second, the retrospective design of our study may confer several inherent biases. Third, other inflammatory conditions associated with high levels of NGAL, such as COPD or interstitial lung disease, were more frequently presented in non-surviving than in surviving patients. Although we adjusted for some of these factors in the multivariable analyses, the possibility that these conditions may have affected higher NGAL concentration in non-survivors remains.

In conclusion, this study demonstrated that increased NGAL concentration was independently associated with a higher likelihood of ICU admission and mortality in hospitalized patients with pneumonia. These results suggest that NGAL is a useful biomarker for assessing the severity of pneumonia and predicting mortality in hospitalized patients with pneumonia.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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