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Journal of Lung, Pulmonary & Respiratory Research

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Neutrophil gelatinase-associated lipocalin, a new biomarker for copd acute exacerbation

Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease which is frequently in acute exacerbations. Neutrophil gelatinase-associated lipocalin (NGAL) is a protein form mediator which is synthesized and emitted from neutrophils. Its level in blood raises in many diseases with inflammation. Purpose of this study is to reveal the importance of NGAL in COPD acute exacerbations.

Methods: The study was conducted prospectively between the dates June 2013 and April 2014. Population of the study consists of 30 patients diagnosed with chronic obstructive pulmonary disease acute exacerbation (COPD-AE) and a control group of 20 healthy people. NGAL, C-reactive protein, leucocyte, fibrinogen and sedimentation levels of the patients were measured on their first and seventh day of their hospitalization.

Results: Age average of 30 COPD-AE diagnosed patients was 67.4±8.6. Majority of the group were male (94% of patient's men). In patients with COPD-AE NGAL levels of patients were significantly higher than control group, respectively 141±64ng/ml and 61±26ng/ml ($p<0.001$). The level of serum NGAL measured on the seventh day of treatment was obviously lower than first day NGAL value (respectively 89±28ng/ml and 141±64ng/ml, $p<0.001$). Also, a positive correlation which was quite significant was detected between NGAL and CRP ($p=0.017$, $r=0.432$).

Discussion: Serum NGAL level rises with COPD-AE and recedes after treatment. It can be used to diagnose with COPD-AE and for its response to treatment. However, this data should be confirmed by comprehensive studies.

Keywords: NGAL, COPD, acute exacerbation

Volume 1 Issue 2 - 2014

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Received: August 13, 2014 | **Published:** September 15, 2014

Abbreviations: COPD, chronic obstructive pulmonary disease; NGAL, neutrophil gelatinase-associated lipocalin; COPD-AE, chronic obstructive pulmonary disease acute exacerbation; AE, acute exacerbations; C-RP, C-reactive protein; ABG, arterial blood gases; PFT, pulmonary function tests; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; WBC, white blood cell

Introduction

Chronic obstructive pulmonary disease (COPD) is a response from airways and lungs against detrimental gases and particles, especially against tobacco and biomass fuel smoke, in the form of chronic inflammation. It is generally a progressive disease which can be prevented and treated (GOLD 2014).[1] During the course of stable COPD, it is possible to observe acute exacerbations (AE) which reduces the quality of life, accelerates the progress of the disease and leads to complications.[2] COPD acute exacerbations are often the result of respiratory tract infections, and it is an important reason of mortality and morbidity.[3] In the case of COPD-AE, levels of various acute phase reactants such as C-reactive protein (C-RP), IL-6 and TNF-alpha and inflammatory cells rise in systemic circulation.[4] C-RP is the most sensitive yet nonspecific biomarker for COPD-AE.[5] Half-life of CRP is quite short. In the attacks emerging due to bacterial infection, it reduces to a normal level following appropriate antibiotic treatment.[6]

Neutrophil Gelatinase-associated Lipocalin (NGAL) is a protein of 25kDA which is also known as lipocalin-2. It is synthesized and stored in the granules of neutrophils and emitted during various clinical cases.[7] Though its function is not certain yet, it is thought that it plays a role in the immune response of the body. It was seen that there was a raise in the NGAL level of blood when the diagnose was acute and chronic inflammatory diseases, ischemic diseases such as stroke and myocardial infarction, metabolic diseases such as obesity and type 2 diabetes mellitus, acute and chronic renal failure, post-period of heart and kidney transplantation, solid tumours such as lung cancer, colon cancer and breast cancer.[8–11] It is used as a biomarker which enables an early diagnosis of renal damage in clinical practice.[12]

Purpose of this study is to demonstrate the importance of NGAL for COPD-AE as a current inflammation marker and reveal it's linked to other acute inflammation indicators such as C-RP, leukocyte and fibrinogen.

Materials and methods

Study design

Patients: The study was conducted in Pulmonary Diseases Department in Faculty of Medicine of Recep Tayyip Erdogan University. Before initiating the study, local clinic ethics committee approval was granted. Patients were informed and their approvals were also taken via signed documents. 30 patients who were over 40 ages diagnosed with COPD-AE and hospitalized between the dates June 2013 and April 2014 were included in the study. CRP, leucocyte, fibrinogen, sedimentation and d-dimer levels of the examinees were measured

twice. The first measurement was made within the 24hours of their hospitalization while the second measurement was made on the seventy day. Arterial Blood Gases (ABG) was also measured in the room air. Besides, kidney function test, liver function tests were made and electrolytes were measured in the blood. Emergency applications and hospitalizations of all patients due to COPD-AE within the last 1year were recorded. Their smoking history was obtained. After three months of their release from the hospital, their pulmonary function tests (PFT) were made in a stable period.

Control group: A control group composed of 20 healthy people with similar age, gender, body mass index (BMI) characteristics was formed. We choose the control groups from similar age groups with patients groups for calculating real statistic results. 10ml venous blood was taken from control group for serum NGAL measurement.

Defining COPD-AE: In addition to increase in difficulty of breathing, it must be accompanied by at least two of the things below for 24hours or longer increase in coughing frequency or severity, increase in purulence or amount of sputum and wheeze.[13] Hospitalization criteria were made according to previous study.[14]

Exclusion criteria: Patients who were being followed in intensive care unit due toacute exacerbations, the ones who died during follow-up, patients with infections apart from lower respiratory tract infections, active cancer, liver cirrhosis, acute or chronic renal failure and decompensed heart failure were excluded from the study.

Measurement of serum NGAL: 10ml venous blood was taken twice for NGAL measurement. The first one was taken within the 24hours of their hospitalization and the latter one was taken on seventy day of their hospitalization. Blood sample was centrifuged within the hour it was taken (3000 revolutions per minute, for 10minutes). The serum which was floating over the part which sank was taken by pipette and put into eppendorf tubes. Samples were collected in deep-freeze at -80 Celsius degree. When the samples were considered enough, NGAL measurements were made. Using Biovendor, Research and Diagnostic Products, Heidelberg, Germany NGAL antibodies, measurements were made quantitatively via enzyme-linked immunosorbent assay (ELISA) method.

Pulmonary function tests measurement: Patients who were stable after at least 3months of their release from the hospital were included. Their lung volume and flow measurements (FEV1, FVC, FEV1/FVC, FEF25-75 and PEF) were carried out in the laboratory of our clinic using Flow handy ZAN 100 USB Pulmonary Spirometerdevice (inspire Health, Inc, GERMANY). Measurements were made while patients were in erect sitting position. At least three measurements were made. The highest value was accepted as the test result. Results were calculated using the most appropriate reference values for patients' ages based on their demographical details by means of computer.

Statistical analysis, Statistical evaluations were made using IBM-SPSS program (SPSS version 21; SPSS Inc., Chicago, IL, USA). Continuous variables were given through average±standard deviation while categorical variables were given in the form of %. T-student was employed to compare two groups while ANOVA and Post-Hoc Tukey HSD analysis methods were employed to compare more than two groups and double comparisons within groups respectively. The correlation between variables were analysed via Pearson correlation analysis. X-Square test was used to compare categorical variables. $P<0.05$ value was considered statistically significant.

Results

The study started with 53 people in total. 33 of them were diagnosed with COPD-AE while the rest 20 were control group. 1 of the patients died during follow up. 2 patients were excluded from the study since they were intubated and transferred to intensive care unit due to respiratory insufficiency. The study was finalized with 30 COPD-AE patients in total. Age average of COPD-AE patients, who were included in the study, was 67.4±8.6. Majority of the patients were males (28men-2women). In our study, there were 10 patients who had positive sputum culture. Six of them had positive sputum culture for streptococcus pneumonia, three for Haemophilus influenza and one for Moraxella catarrhalis. In addition to this, eight patients had new infiltration on chest X-ray when compared their old chest X-ray graphs. Despite the other 12 patients had no positive sputum culture and newly infiltrate on chest X-ray they had increased sputum purulence and sputum amount. It is known that there is positive relation between serum CRP, white blood cell (WBC) and infection. All 30 patients hospitalized with a diagnosis of COPD-Ae were administered systemic corticosteroids and systemic antibiotic therapy. Patients were discharged in a mean 10±4days after admission. 6 of the patients smoker (20%) while 23 of them ex-smoker (77%).One of the patients had never smoker (3%). PFT results of the COPD patients, which were measured during the stable period, were as follows, FVC%, 54±13, FEV1%, 36±11, FEV1/FVC%, 51±8, PEF%, 36±11 and FEF25-75%, 19±10. After the patients were received in to service, ABG were measured in the room air pH, 7.38±0.05, pO2, 56±10mmHg, pCO2, 43±8mmHg. Oxygen saturation was %, 89±5. According to PFT results, 7 of the patients were in very severe state (23%) while 15 were in severe state (50%) and 8 of them were in moderate state (27%). None of the patients in the working group had mild COPD. This was an unplanned situation. A significant difference was revealed between NGAL levels of COPD-AE patients on the first and seventy days. These values were 141±64 and 89±28 respectively ($p<0.001$). COPD-AE group and control group were compared. On the first day, NGAL level was significantly higher than control group ($p<0.001$). On the seventy day, though NGAL level was higher than control group, this difference was not significant ($p=0.080$). Demographical characteristics of patients and control group and NGAL value comparisons are shown in Table 1, Table 2 and Figure 1.

A significant positive correlation was detected between serum NGAL level and C-RP ($p=0.017$, r, 0.432) (Figure 2). However, there was not a significant relationship between NGAL and other inflammation indicators such as leucocyte, fibrinogen and sedimentation. On the other hand, there was a significant relationship between C-RP and other inflammation markers and ABG values (Table 3) Compared to first day of their hospitalization, NGAL and other inflammation symptoms of COPD-AE patients were significantly lower on the seventy day of hospitalization (Table 4). In COPD group, there were patients with additional diseases. 4 patients had hypertension, 2 patients had type 2 diabetes mellitus and a patient had coronary arterial patients. As for control group, there were 2 patients with hypertension and another 2 patients with type 2 diabetes mellitus. All the patients who were diagnosed and hospitalized due to COPD-AE were treated with methyl-prednisolone. The dosage was ranging from 40 to 60mg/day. The treatment period lasted for 5 to 10days. Besides, 25(83%) of the patients were treated with antibiotics in parenteral form. The patients were released from the hospital after 9±6 day on the average.

Citation: Gumus A, Cinarka H, Hazıroglu M, et al. Neutrophil gelatinase-associated lipocalin, a new biomarker for copd acute exacerbation. *J Lung Pulm Respir Res.* 2014;1(2):31–35. DOI: 10.15406/jlprr.2014.01.00008

Table 1 Demographical characteristics of COPD-AE patients and their comparison with control group

	Patients (n=30)	Control (n=20)	p-value
Age (Year)	67.4±8.6	62.2±7.3	0,085
Gender (F/M)	2/28	3/17	0,318
BMI (kg/m²)	24.5±4.8	26.5±5.9	0,301
Smoking (packet-year)	60±20	45±14	0,092

N, the number of patients; F, female; M, male; BMI, body mass index

Table 2 Comparison of serum NGAL level of COPD-AE group and control group

	1.Day (COPD-AE)	7.Day (COPD-AE)	Control	p-value
NGAL (ng/ml)	141±64	89±28	61±26	<0.001*& <0.001**& 0.080***&

Ng/ml, nanogram/millilitre; &: The difference between two groups was shown by Post-Hoc Turkey HSD analysis.
*Comparison of NGAL levels of patients with COPD-AE on the first day and seventh days
**Comparison of NGAL levels of patients with COPD-AE on the first day and control group
***Comparison of NGAL levels of patients with COPD-AE on the seventh day and control group

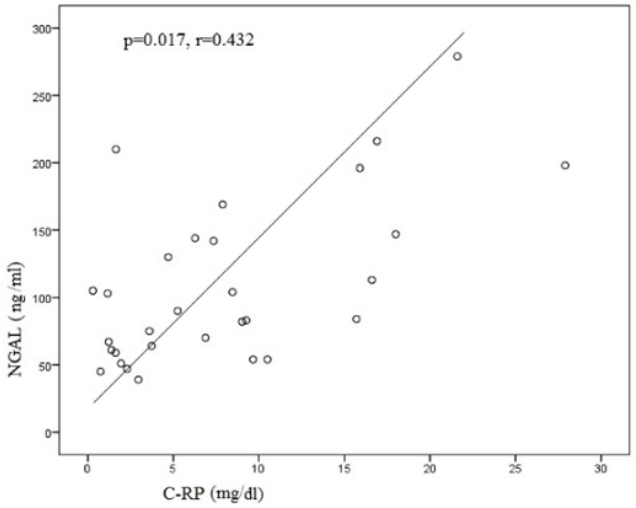

Figure 1 Comparison of serum NGAL level of COPD-AE group and control group in box plot graphics.

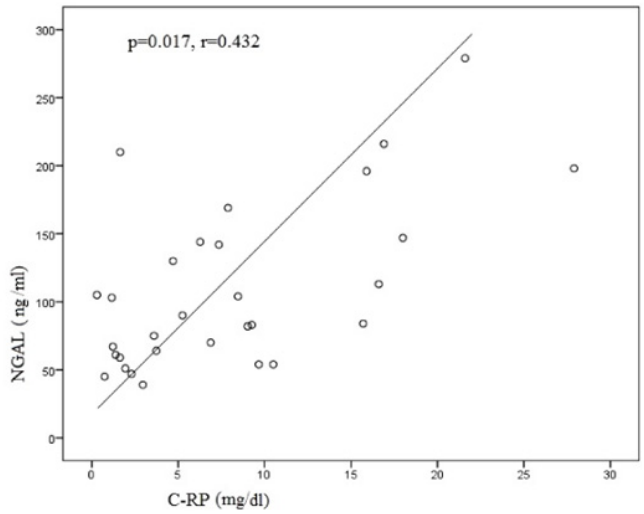

Figure 2 Scatter plot demonstration of positive relationship between NGAL and CRP.

Table 3 Correlation between serum NGAL and C-RP levels with other inflammation markers, ABG and PFT parameters

	NGAL		C-RP	
	r	p-value	r	p-value
NGAL, ng/ml	—	—	0.432	0.017
C-RP, mg/dl	0.432	0.017	—	—
Leukocyte,/ml	0.25	0.510	0.544	0.002
Fibrinogen, mg/dl	0.259	0.167	0.688	<0.001
Sedimentation rate, mm/h	0.183	0.333	0.484	0.007
FEV1, % predicted	-0.199	0.320	-0.072	0.722
FVC, % predicted	-0.147	0.463	-0.109	0.590
pH	0.286	0.140	-0.506	0.006
PaO_2, mmHg	-0.131	0.505	-0.393	0.039
PCO_2, mmHg	-0.271	0.164	0.435	0.021

r = Pearson's Correlation Coefficient

Table 4 The difference in NGAL and other systemic inflammation indicators on the first and seventh days.

	1. Day	7. Day	p-value
NGAL, ng/ml	141±64	89±28	<0.001
C-RP, mg/dl	8,0±7,1	1,6±2,1	<0.001
Leukocyte, /ml	1575±7418	9525±3088	<0.001
Fibrinogen, mg/dl	610±144	447±107	<0.001
Sedimentation rate, mm/h	30±18	17±13	<0.001

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Discussion

Acute exacerbations increase the severity of disease in COPD.[1] During acute exacerbation, many inflammatory biomarkers and cells increase in blood and sputum.[5] Previous studies revealed that systemic biomarker levels increased and this was related to prognosis and mortality.[13,15] C-RP is the most sensitive acute inflammation indicator.[5] Karadeniz et al. stated that high C-RP levels in COPD-AE were the predictors of increased mortality.[14] This study proved that patients had high levels of CRP, leucocyte and fibrinogen initially which rapidly reduced after treatment.

The unique characteristic of this study is that it is the first attempt to examine serum NGAL level in COPD-AE. The most outstanding result of this study was the detection of NGAL as biomarker of acute inflammation in COPD. Serum NGAL levels which were measured on the first day were significantly higher than healthy control grouped. It was also seen that NGAL reduced at a significant rate on the seventy day of the treatment compared to initial value. On the seventy day, NGAL levels of the patients were higher than control group. However, this difference was not significant. These results reveal that serum NGAL levels are both acute inflammation indicators and it can also be an indicator of response to treatment. Another result supporting this hypothesis is the existence of a positive correlation between NGAL and C-RP. However, NGAL had no significant correlation with fibrinogen, leucocyte and sedimentation speed which were other inflammation indicators. In their study, Allegra et al.,[16] stated a positive correlation between serum NGAL level and leucocyte and neutrophil counts. In another study conducted by Eagan et al.,[17] a positive relationship between serum NGAL level and neutrophil counts and CRP in patients with stable COPD.In this study, there was a positive relationship between NGAL and leucocyte count. However, it was not statistically significant. That the number of patients in this study was low may be the reason of this result. It is thought that more comprehensive studies will yield to a significant relationship. According to relevant results, serum NGAL levels can predict COPD-AE and can be used for treatment follow-up. However, it was seen that C-RP was a more sensitive biomarker than NGAL. In this sense, it is not possible to recommend using serum NGAL level instead of C-RP for routine practice. However, there is a need for studies with more comprehensive patient populations to study NGAL in COPD-AE. Besides, there is also a need for studies directed to reveal the effects of NGAL on prognosis and mortality in COPD-AE.

It was seen that NGAL was generally high in patients with chronic inflammatory diseases, particularly renal damage. There are only a limited number of studies investigating the value of NGAL on acute infections. In the study conducted by Axelsson et al. it was seen that serum NGAL level was 10times higher in patients with acute peritonitis compared to control group.[18] In another study carried out by Chakraborty et al. serum NGAL levels were higher in patients with acute pancreatitis in relation to severity of the disease.[9] Recently, a study was conducted with African children who had pneumonia. This study revealed that serum NGAL level had high specificity and sensitivity to distinguish severe pneumonia from mild pneumonia and bacterial pneumonia from non-bacterial pneumonia.[19] COPD-AE is mainly the result of bacterial infections. It was seen that NGAL increased in the early phases and receded after treatment. According to the results, NGAL seems to be a biomarker which can be used to diagnose acute infection or its follow up. It is believed that this characteristic of NGAL must be explored with more comprehensive studies.

In conclusion, NGAL was studied for the first time in patients with COPD-AE. It was revealed that it was an indicator of acute inflammation. Serum NGAL level is reduced by antibiotic treatment such as C-RP. Besides, NGAL level can predict the response to treatment. However, these results need to be confirmed by comprehensive studies. Also, its effect on prognosis needs to be evaluated by prospective studies.

Acknowledgements

None.

Conflict of interest

This study was carried out with the support of Scientific Research Projects of Recep Tayyip Erdogan University.

References

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease; 2014.
2. Hurst JR, Wedzicha JA. Management and prevention of chronic obstructive pulmonary disease exacerbations: a state of the art review. *BMC Med*. 2009;7:40–45.
3. Wedzicha JA, Seemungal TA, MacCallum PK, et al. Acute exacerbations of chronic obstructive pulmonary disease is accompanied by elevations of plasma fibrinogen and serum IL–6 levels. *Thromb Haemost*. 2000;84(2):210–215.
4. Shameem M, Bhargava R, Ahmad Z, et al. Association between serum C– reactive protein levels and other important predictive markers of outcome in COPD. *Acta Med Iran*. 2011;49(1):18–20.
5. Celli BR, Barnes PJ. Exacerbations of chronic obstructive pulmonary disease. *Eur Respir J*. 2007;29(6):1224–1238.
6. Peisajovich A, Marnell L, Mold C, et al. C–reactive protein at the interface between innate immunity and inflammation. *Expert Rev Clin Immunol*. 2008;4(3):379–390.
7. Kjeldsen L, Cowland JB, Borregaard N. Human neutrophil gelatinase–associated lipocalin and homologous proteins in rat and mouse. *Biochim Biophys Acta*. 2000;1482(1–2):272–283.
8. Fjaertoft G, Foucard T, Xu S, et al. Human neutrophil lipocalin (HNL) as a diagnostic tool in children with acute infections:A study of the kinetics. *Acta Paediatr*. 2005;94(6):661–666.
9. Chakraborty S, Kaur S, Muddana V, et al. Elevated serum neutrophil gelatinase– associated lipocalin is an early predictor of severity and outcome in acute pancreatitis. *Am J Gastroenterol*. 2010;105(9):2050–2059.
10. Yndestad A, Landro L, Ueland T, et al. Increased systemic and myocardial expression of neutrophil gelatinase–associated lipocalin in clinical and experimental heart failure. *Eur Heart J*. 2009;30(10):1229–1236.
11. Chakraborty S, Kaur S, Guha S, et al. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. *Biochim Biophys Acta*. 2012;1826(1):129–169.
12. Devarajan P. Neutrophil gelatinase–associated lipocalin (NGAL):a new marker of kidney disease. *Scand J Clin Lab Invest Suppl*. 2008;241:89–94.
13. Bathoorn E, Liesker JJ, Postma DS, et al. Change in inflammation in out–patient COPD patients from stable phase to a subsequent exacerbation. *Int J Chron Obstruct Pulmon Dis*. 2009;4:101–109.
14. Karadeniz G, Polat G, Senol G, et al. C–reactive protein measurements as a marker of the severity of chronic obstructive pulmonary disease exacerbations. *Inflammation*. 2013;36(4):948–953.

15. Karadag F, Karul AB, Cildag O, et al. Biomarkers of systemic inflammation in stable and exacerbation phases of COPD. *Lung*. 2008;186(6):403–409.

16. Allegra A, Alonci A, Bellomo G, et al. Increased serum levels of neutrophil gelatinase–associated lipocalin in patients with essential thrombocythemia and polycythemiavera. *Leuk Lymphoma*. 2011;52(1):101–107.

17. Eagan TM, Damas JK, Ueland T, et al. Neutrophil gelatinase–associated lipocalin: a biomarker in COPD. *Chest*. 2010;138(4):888–895.

18. Axelsson L, Bergenfeldt M, Ohlsson K. Studies of the release and turnover of a human neutrophil lipocalin. *Scand J Clin Lab Invest*. 1955;55(7):577–588.

19. Huang H, Ideh RC, Gitau E, et al. Discovery and validation of biomarkers to guide clinical management of pneumonia in african children. *Clin Infect Dis*. 2014;58(12):1707–1715.

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