The European Research Journal 2024;10(3):262-267 Original Article

DOI: https://doi.org/10.18621/eurj.1357708 *Chest Diseases*

Predictive value of NLRC3 levels for pulmonary hypertension in patients with chronic obstructive pulmonary disease

Meltem Yılmaz[1], Levent Cem Mutlu[1], Şeref Alpsoy[2], Aydın Akyüz[2], Aliye Çelikkol[3], Özlem Kaymaz[3]

[1]Department of Chest Diseases, Namık Kemal University, Faculty of Medicine, Tekirdağ, Turkey; [2]Department of Cardiology, Namık Kemal University, Faculty of Medicine, Tekirdağ, Turkey; [3]Department of Medical Biochemistry, Namık Kemal University, Faculty of Medicine, Tekirdağ, Turkey

ABSTRACT

Objectives: Chronic obstructive pulmonary disease (COPD) is a medical condition defined by persistent airflow limitation and respiratory symptoms caused by airway and/or alveolar abnormalities. Pulmonary hypertension (PH) is one of the cardiovascular comorbidities associated with COPD. We investigated the correlation of NLRC3 levels in patients with COPD with prognostic and surrogate parameters of PH on echocardiography and examined whether it could be used to predict PH in this patient population.

Methods: A total of 80 patients diagnosed with COPD and 40 healthy volunteers as the control group were included in the study. The COPD group was further divided into two subgroups according to the systolic pulmonary artery pressure (sPAP) as follows: sPAP<35 mmHg and sPAP≥35 mmHg. The enzyme-linked immunosorbent assay (ELISA) method was used to determine the levels of NLRC3 in peripheral blood.

Results: Patients with sPAP≥35 mmHg had a lower mean NLRC3 level than those with sPAP<35 mmHg (P=0.006). The NLRC3 levels showed a significant negative correlation with sPAP, tricuspid regurgitation velocity, right atrium, and pulmonary artery diameter. For NLRC3, the cut-off value was found to be 271,486 ng/L, with a sensitivity of 74%, and specificity of 63% in distinguishing patients with sPAP≥35 mmHg from all patients with sPAP<35 mmHg.

Conclusion: Our study results suggest that NLRC3 levels measured from peripheral blood are predictive of PH in patients with COPD. Although the exact function of NLRC3 in the lungs, COPD, and PH have not been completely understood, we believe these findings will serve as a model for future studies.

Keywords: NLRC3, chronic obstructive pulmonary disease, pulmonary hypertension, echocardiography

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide [1]. Cardiovascular entities are the most frequent comorbidities in patients with COPD. Pulmonary hypertension (PH) is a significant cause of disease prognosis and is associated with an increased risk of exacerbation and decreased survival [2].

Potential mechanisms leading to PH in COPD; hypoxia-hypercapnia, inflammation, endothelial shear

Corresponding author: Meltem Yılmaz, MD.,
Phone: +90 282 250 51 77, E-mail: drmeltemyilmaz59@gmail.com

How to cite this article: Yılmaz M, Mutlu LC, Alpsoy Ş, Akyüz A, Çelikkol A, Kaymaz Ö. Predictive value of NLRC3 levels for pulmonary hypertension in patients with chronic obstructive pulmonary disease. Eur Res J. 2024;10(3):262-267. doi: 10.18621/eurj.1357708

Received: September 11, 2023
Accepted: December 21, 2023
Published Online: December 28, 2023

stress, and endothelial dysfunction cause pulmonary vascular remodeling [2,3].

Nucleotide-oligomerization domain (NOD)-like receptor subfamily C3 (NLRC3) is a recently discovered member of the Nucleotide-binding oligomerization domain-like receptors (NLR) family that negatively regulates inflammatory responses [4, 5]. NLRs are a family of intracellular proteins that play important roles in inflammation and immunity [6]. Some researchers have recently shown that NLRC3 has a role in cell antiproliferation and promoting proapoptotic signals by inhibiting cell proliferation and inflammation [7].

The study aimed to determine the correlation of NLRC3 levels with the prognostic and supportive parameters of PH in echocardiography in COPD patients and to determine its clinical utility in the prediction of PH in these patients.

METHODS

Study Design and Population

This single-center, cross-sectional was conducted at the Department of Chest Diseases of a tertiary care center between June 2020 and November 2020. A total of 80 patients with stable COPD over 40 years of age who were spirometrically diagnosed with COPD according to the 2020 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines and met the inclusion criteria were included. The control group consisted of 40 healthy volunteers. Inclusion criteria for the patient group were age ≥40 years, a diagnosis of COPD, post-bronchodilator FEV1/FVC <70%, and giving consent for participation in the study. Patients having a recent COPD exacerbation or pneumonia within the previous four weeks, having coronary artery disease, obstructive sleep apnea diagnosis by polysomnography, bronchiectasis, asthma, malignancies, diabetes, and known chronic liver and/or kidney disease were excluded from the study. The COPD group was divided into two groups according to the systolic pulmonary artery pressure (sPAP) as follows: 41 patients with sPAP <35 mmHg and 39 patients with sPAP ≥35 mmHg. For the healthy control group, 40 individuals were selected and divided into two groups smokers and non-smokers, including 20 individuals in each group.

Before this cross-sectional study, all participants were informed about the nature of the study and written informed consent was obtained. The study protocol was approved by the the Tekirdağ Namık Kemal University, Non-Interventional Clinical Research Ethics Committee (No: 2019.164.09.24, date: 24/09/2019). The study was conducted on the principles of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study

Blood samples

Blood samples from peripheral veins were centrifuged at 4 °C for 20 min and stored at –80 °C until analysis. On the analysis day, stored samples were brought to room temperature, and NLRC3 serum levels were measured by ELISA using commercially available kits. . (Human NLR Family, CARD Domain Containing 3 ELISA KIT, Bioassay Technology Laboratory Shanghai, China # E6709Hu).

Echocardiography

Echocardiographic measurements were performed by the same cardiologist according to ACC/AHA/ASE 2003 Guideline Update for the Clinical Application of Echocardiography (https://doi.org/10.1161/01.CIR.0000073597.57414.A9).

Statistical Analysis

Statistical analysis was performed using the SPSS for Mac version 22.0 software (IBM Corp., Armonk, NY, USA). The normality of numerical variables was analyzed using the Shapiro–Wilk or Kolmogorov–Smirnov test, where appropriate. Descriptive data were presented in mean ±standard deviation (SD), and median (min–max) for continuous numerical variables, and in number and frequency for categorical variables. To compare the continuous variables between the groups, the independent samples t-test or one-way analysis of variance (ANOVA) was used for normally distributed variables, whereas the Mann–Whitney U analysis or Kruskal–Wallis test was used for non-normally distributed variables. The homogeneity of variance was checked using the Levene test. The Welch test was used to analyze parameters with a non-homogeneous distribution. A P-value <0.05 was considered statistically significant. Tamhane's T2 test was used as the post-hoc test to determine which groups resulted in a significant difference. A P-value

Table 1. Demographic characteristics of patients

Variables	COPD group		Control group	
	sPAP <35 mmHg (n=41)	sPAP ≥35 mmHg (n=39)	Smokers (n=20)	Non-smokers (n=20)
Gender				
Female/Male	8/35	8/31	8/12	9/11
Age (years)	61.34±8.61 (44-80)	65.72±7.20 (55-82)	59.25±8.39 (44-74)	59.45±7.68 (48-72)
Smoking (pack/year)	48.40±24.60 (0-120)	49.06 ±21.05 (0-100)	27.05 ±10.93 (10-45)	0
BMI (kg/m^2)	26.69±3.84 (20.67-37.46)	25.68±4.62 (15.60-35.90)	28.38±3.89 (22.03-37.46)	28.26±5.13 (20.43-41.01)

Data are shown as mean±standard deviation (minimum-maximum). COPD=Chronic obstructive pulmonary disease, sPAP=Systolic pulmonary artery pressure, BMI=Body mass index

<0.008 was considered statistically significant for the post-hoc analysis. The Spearman correlation analysis was used to compare two continuous numerical variables, whereas the Pearson correlation analysis was used to compare categorical variables. The receiver operating characteristic (ROC) analysis was performed to identify the predictors of PH with an area under the curve (AUC) and 95% confidence interval (CI).

RESULTS

A total of 80 COPD patients and 40 healthy individuals were included in this study. There was no statistically significant difference between the groups in terms of age, sex, and body mass index (BMI). The demographic characteristics of the patients are shown in Table 1.

The NLRC3 level was found to be significantly different between the groups (P<0.001). Although NLRC3 level was lower in the group with COPD with sPAB≥35 mmHg, no statistically significant difference was found between COPD without sPAB<35 mmHg in post-hoc analyses (P=0.628). When the whole COPD group was compared with the control group, the NLRC3 level was found to be significantly lower in the COPD group (P=0.002). When the sPAB≥35 mmHg group was compared with the group that included all patients with sPAB<35 mmHg, NLRC3 levels were found to be significantly lower in the group with sPAB≥35 mmHg (P=0.006). NLRC3 results are shown in Table 2. In correlation analysis, a negative correlation was observed between NLRC3 levels and sPAB, tricuspid regurgitation velocity (TRV), right atrium (RA) diameter, and pulmonary artery (PA) diameter (Table 3).

The ROC analysis revealed a cut-off value of 271,486 ng/L, with 74% sensitivity and 63% specificity in distinguishing patients with sPAP ≥35 mmHg from all patients with sPAP <35 mmHg (Table 4).

Table 2. NLRC3 levels

	COPD group		Control group	
	sPAP <35 mmHg	sPAP ≥35 mmHg	Smoker	Non-smoker
NLRC3 (ng/L)	387.52±292.10 (163.67-1256.69)	304.09±220.16 (86.226-1047.36)	317.23±204.47 (117.04-949.05)	800.74±572.23 (264.78-1804.99)

Data are shown as mean±standard deviation (minimum-maximum). COPD=Chronic obstructive pulmonary disease, sPAP=Systolic pulmonary artery pressure

Table 3. The correlation between NLRC3 and echocardiographic parameters in the overall patient group

	NLRC3	
	r	P value
TRV	-0,253	**0.005**
sPAP	-0,299	**0.001**
PA diameter	-0,270	**0.003**
RA	-0,276	**0.002**

sPAP=Systolic Pulmonary Artery Pressure, TRV=Tricuspid regurgitation velocity, PA=Pulmonary artery, RA=Right atrium.

r =correlation coefficient, P<0.05

DISCUSSION

COPD is the leading cause of morbidity and mortality worldwide. In the GOLD 2023 report, it is estimated that the increased prevalence of smoking in LMICs coupled with aging populations in high-income countries will result in over 5.4 million annual deaths from COPD and related conditions by 2060 [1]. Cardiovascular comorbidities are the most common comorbidity in COPD, among which PH is a serious condition that determines the prognosis of the disease and is associated with an increased risk of exacerbations and reduced survival [2]. Although the echocardiographic examination is not a definitive diagnostic method for PH, it is an essential, easily accessible, non-invasive diagnostic method in the follow-up of confirmed patients by screening patients in the PH risk group, and providing support and diagnosing clinically [2, 8].

Our results showed that the mean NLRC3 levels were significantly different between the groups. There was a statistically significant negative correlation between NLRC3 levels and sPAP, TRV, RA diameter, and PA diameter.

Nucleotide-binding oligomerization domain-like receptors (NLRs) belong to an intracellular protein family that has important roles in inflammation and immunity [4, 5]. NLRC3 is a newly discovered member of the NLR family and has been described as a negative regulator of the inflammatory response [6]. It is involved in the inhibition of cellular proliferation and the promotion of pro-apoptotic signals [7]. NLRC3 is expressed in macrophages and lymphocytes. Its expression in lung tissue remains controversial [9, 10].

In a 2018 study by Zha *et al*. [7] involving 40 patients (diagnosed as having PH with RHC) and 20 healthy controls, PH patients were found to have significantly lower NLRC3, which also negatively correlated with mPAP and PVR. However, ECHO parameters did not correlate with NLRC3 levels, and no negative correlations with WHOFC were also found. An NLRC3 cut-off of 2.897 ng/L was found to be 88% sensitive and 85% specific for predicting the presence of PHT. These authors concluded that NLRC3 concentrations could be used as a diagnostic and prognostic tool for PHT and that their observations warranted further study [7].

Our findings are consistent with literature data, indicating significantly lower NLRC3 levels in patients with elevated pulmonary artery pressure. Additionally, negative correlations of NLRC3 with TRV, sPAP, RA diameter, and PA diameter were found.

Right atrial dilation is common in PH patients as a result of right ventricular failure, increased right ventricular diastolic pressure, functional tricuspid failure, and increased right atrial dimensions representing a poor prognostic sign [11]. According to our results, TRV and RA diameter negatively correlate with

Table 4. ROC analysis for NLRC3

Area Under Curve Test result for NLRC3 variable				
Area	Standard error[a]	Asymptotic Sig.[b]	95% Confidence interval for the odds ratio	
			Lower limit	Upper limit
,655	,052	,006	,554	,756

a. Under non-parametric assumptions.

b. Null hypothesis: actual area = 0.5

NLRC3, and we believe that this might be useful in screening and monitoring these patients.

One of the most important mechanisms for the development of PH in COPD patients involves chronic inflammation and vascular remodeling. Activation of phosphoinositide 3-kinase (PI3K) plays a critical role in the vascular remodeling of the pulmonary vasculature in PH [6, 12]. NLRC3 has been shown to inhibit cellular proliferation and inflammation, through the inhibition of the PI3K signals in colorectal cancer [6]. Based on these observations, Zha *et al*. [12] published their study in 2019 that showed reduced levels of NLRC3 in pulmonary arteries as well as in pulmonary arterial smooth muscle cells stimulated with platelet-derived growth factor -BB (PDGF-BB) in animal models of PH. Administration of NLRC3 protein for therapeutic purposes resulted in decreased right ventricular systolic pressure, reduced pulmonary vascular remodeling, and alleviation of proliferation, migration, and inflammation [12]. Our results are consistent with these results and showed reduced NLRC3 levels in patients with elevated pulmonary arterial pressure as compared to other study groups. Since our samples were limited to peripheral blood, we may assume that NLRC3 may be related to the development of PH in COPD patients, although it is obvious that further studies are required to better understand the role of NLRC3 in PH.

Most of the previous publications regarding NLRC3 focused on malignant conditions and immunity. To the best of our knowledge, no previous studies examined the role and effect of NLRC3 in COPD patients or the development of PH in COPD. Therefore, we believe that our study may represent the first of its kind.

The disease burden associated with COPD is continuously increasing. PH in COPD patients is an established risk factor for worse prognosis and increased mortality, underscoring the importance of detecting and screening PH. Our data suggest that NLRC3 levels may be predictive of PH in COPD patients. PH occurs via multiple mechanisms in COPD. Furthermore, the exact function(s) of NLRC3 in COPD and PH are yet to be elucidated. Therefore, we believe that our findings may provide some guidance for future studies.

Limitations

This study had certain limitations. First, as right heart catheterization could not be performed in our patients, the elevation of mean pulmonary artery pressure could not be definitively proven. Second, our study had a single-center design, which precludes the generalizability of the results. Finally, the use of biomarkers regularly in our study would incur a certain expense. Therefore, additional studies are warranted to establish which groups should be screened. In our study, those with sPAP ≥35 mmHg were older on average and had a lower BMI, although no statistically significant difference was present. In addition, DLCO and FEV1 values were lower in this group of patients. For screening, we recommend using our biomarker primarily in these patients.

CONCLUSION

Our results indicate a significant reduction in NLRC3 levels measured in peripheral blood samples from COPD patients presenting with PH. NLRC3 also was found to have a significant negative correlation with sPAP, TRV, RA, and PA diameter, which are ECHO parameters supportive of a diagnosis of PH and which also have prognostic implications. We believe that this biomarker may be predictive of PH in this setting. The role of NLRC3 in lungs, COPD patients, and PH is still unknown, indicating the need for further studies.

Authors' Contribution

Study Conception: MY, LCM; Study Design: MY, LCM; Supervision: LCM; Funding: MY, LCM; Materials: MY, LCM, SA, AA, AÇ, ÖK; Data Collection and/or Processing: MY, LCM, SA, AA, AÇ, ÖK; Statistical Analysis and/or Data Interpretation: MY, LCM; Literature Review: MY; Manuscript Preparation: MY and Critical Review: LCM.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

This study was funded by the Coordination Unit of Scientific Research Projects, Tekirdağ Namık Kemal University (project number: NKUBAP.02.TU.20.249).

REFERENCES

1. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2023. Available from: https://goldcopd.org/2023-gold-report-2/ Accessed on September 1, 2023.
2. Jyothula S, Safdar Z. Update on pulmonary hypertension complicating chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2009;4:351-63. doi: 10.2147/copd.s5102.
3. Seeger W, Adir Y, Barberà JA, et al. Pulmonary hypertension in chronic lung diseases. J Am Coll Cardiol. 2013;62(25 Suppl):D109-116. doi: 10.1016/j.jacc.2013.10.036.
4. Davis BK, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol. 2011;29:707-735. doi: 10.1146/annurev-immunol-031210-101405.
5. Chen G, Shaw MH, Kim YG, Nuñez G. NOD-like receptors: role in innate immunity and inflammatory disease. Annu Rev Pathol. 2009;4:365-398. doi: 10.1146/annurev.pathol.4.110807.092239.
6. Karki R, Man SM, Malireddi RKS, et al. NLRC3 is an inhibitory sensor of PI3K-mTOR pathways in cancer. Nature. 2016;540(7634):583-587. doi: 10.1038/nature20597.
7. Zha LH, Zhou J, Li TZ, et al. NLRC3: a novel noninvasive biomarker for pulmonary hypertension diagnosis. Aging Dis. 2018;9(5):843-851. doi: 10.14336/AD.2017.1102.
8. Chaouat A, Naeije R, Weitzenblum E. Pulmonary hypertension in COPD. Eur Respir J. 2008;32(5):1371-1385. doi: 10.1183/09031936.00015608.
9. Chaput C, Sander LE, Suttorp N, Opitz B. NOD-like receptors in lung siseases. Front Immunol. 2013;4:393. doi: 10.3389/fimmu.2013.00393.
10. Schneider M, Zimmermann AG, Roberts RA, et al. The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-κB. Nat Immunol. 2012;13(9):823-831. doi: 10.1038/ni.2378.
11. Ghio S, Klersy C, Magrini G, et al. Prognostic relevance of the echocardiographic assessment of right ventricular function in patients with idiopathic pulmonary arterial hypertension. Int J Cardiol. 2010;140(3):272-278. doi: 10.1016/j.ijcard.2008.11.051.
12. Zha LH, Zhou J, Li TZ, et al. NLRC3 inhibits MCT-induced pulmonary hypertension in rats via attenuating PI3K activation. J Cell Physiol. 2019;234(9):15963-15976. doi: 10.1002/jcp.28255.