Polish Annals of Medicine

Journal homepage: https://www.paom.pl

Research paper

The efficacy and safety of inhaled acetylcysteine in comparison with oral acetylcysteine in chronic obstructive pulmonary disease: A randomized single-center study

Nina Kuzmenko[1], *Oleg Iaremenko*[1], *Dmytro Dobrianskiy*[1], *Roman Ilnytskyi*[1], *Galyna Gumeniuk*[2]

[1] *Bogomolets National Medical University, Kyiv, Ukraine*
[2] *National Institute of Phthisiology and Pulmonology named after F. G. Yanovsky, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine*

Article info

Article history
Received 3 February 2020
Accepted 11 April 2020
Available online 6 August 2020

Keywords
COPD
Mucolytics
Nebulizer

Doi
https://doi.org/10.29089/2020.20.00112

Abstract

Introduction: One-third of all the patients with chronic obstructive pulmonary disease (COPD) additionally take mucolytics as per GOLD recommendation due to complaints of productive cough despite their compliance with the basic treatment regimen.

Aim: To assess the efficacy and safety of inhaled *N*-acetylcysteine (NAC) in comparison with oral NAC in patients with COPD.

Material and methods: The study included 46 patients with stable COPD and difficult expectoration of sputum who were divided into two groups. The first group ($n = 22$) took 600 mg/day NAC orally, and the second one ($n = 24$) inhaled 600 mg/day NAC by a nebulizer for 10 days. In the beginning and after the 10-day treatment the questionnaires (CAT, mMRC, CCQ, SGRQ, SF-36), 6-minute walk distance test and day and night cough symptoms were evaluated, spirometry and sputum analysis were performed.

Results and discussion: In the first group, CCQ showed improvement in the status of patients (by 9.7%). The severity of night cough also decreased. Other indices were not changed statistically. In the other group, positive changes in the CAT (by 13.1%) and SF-36 were reported, night cough decreased. Additionally, forced expiratory volume in 1 s (FEV1) values increased (by 10.3%). No changes in FEV1 2 hours after the first oral usage or inhalation of NAC were found.

Conclusions: Both oral administration and inhalation of NAC by a nebulizer for 10 days has a similar positive effect on the manifestations of COPD, but the inhalation route of the drug is also accompanied by improved quality of life and lung function test (FEV1) as well.

Corresponding author: Nina Kuzmenko, Bogomolets National Medical University, Department of Internal Medicine No 3, 26, P. Zaporozhtsa street, Kyiv, Ukraine. Tel.: +380936167200.
E-mail: reznikova.rnm@gmail.com.

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the most common diseases in the world and the fourth leading cause of death. Moreover, it is predicted to reach the third place by 2020.[1] According to various estimates, at least 4% of the population in Ukraine has COPD, and about 2% of Ukrainians' deaths are caused by this disease.[2] COPD is characterized by the presence of progressive shortness of breath, provoked by physical activities, and chronic cough, which may be accompanied by the secretion of sputum for a long time. One-third of all the patients with COPD complain of a productive cough and its severity can be variable and may precede changes in lung function tests many years before the diagnosis.[1]

Regular use of inhalation therapy (bronchodilators, cholinolytics, glucocorticoids and the combination of them) is recommended for symptomatic treatment of patients with the stable COPD.[1] However, despite the patients' compliance with the basic treatment regimen, some of them continue to complain of cough, often with difficult sputum production. This excess secretion is associated with an increase in the number of goblet cells and submucous glands due to chronic irritation of the respiratory mucosa by various harmful agents, primarily tobacco smoke. The concomitant use of mucoactive drugs is justified for patients who do not receive glucocorticoids by inhalation. Mucolytics enhance the rheological properties of sputum by altering the structure of the mucus gel, thereby reducing its viscosity and elasticity, which, in turn, facilitates the process of clearing the airways.[3] NAC, erdocysteine, and carbocysteine were most commonly studied among mucoactive drugs. Data on the positive effect of oral mucolytics on the frequency and severity of COPD exacerbations were obtained.[4–8] It should be noted that the content and regimen of basic therapy in the population of patients who participated in the studies were heterogeneous. The question of the mechanism of influence of these drugs on the course of COPD remains debatable.[6]

The number of studies examining the efficacy and safety of administration of the mucolytic drugs, in particular NAC and its derivatives, by a nebulizer in patients with respiratory diseases, is small.[9–12] These studies were performed in patients with bronchiectasis, cystic fibrosis, patients undergoing mechanical ventilation and in healthy subjects. Inhalation of NAC resulted in positive outcomes in all these patient categories. For example, patients with bronchiectasis inhaled the drug for 10 minutes daily by a nebulizer during exacerbation, had a significant improvement in airway clearance due to stimulation of expectoration of the sputum, which was accompanied by improved oxygenation.[11] Some authors cite the results of these studies as an argument for the expectation of a similar effect in patients with COPD.[13]

No published data were identified regarding the effect of inhalation of NAC on clinical status, quality of life, results of lung function tests in patients with COPD, as well as a comparison of the effectiveness and safety of inhalation and oral usage of NAC in this category of patients.

2. AIM

To assess efficacy and safety of inhaled NAC in comparison with oral NAC in patients with COPD.

3. MATERIAL AND METHODS

This was a randomized, open label, comparative, parallel group, prospective, interventional, single-center study. This trial was provided on an outpatient basis at the pulmonology department of the Kyiv City Clinical Hospital No 3 from 2018 to 2019.

Eligible patients were men and women aged between 40 and 80 years with a diagnosis of COPD, confirmed by spirometry,[1,14] with a disease duration of at least 12 months. The patients had stable COPD with an unchanged therapeutic regimen for at least 4 weeks and complained of difficulties with expectoration, despite standard inhalation therapy.

Patients with other chronic lung diseases, tuberculosis, neoplastic processes, hemoptysis, pulmonary hemorrhage, gastric or duodenal ulcers, liver and renal failure, allergic reactions to the study drugs and those who unable to perform procedures of the study were excluded. Pregnant or breast-feeding women were also excluded.

In total, 49 patients suffering from COPD were screened for the study. Out of 49 screened patients, 46 patients were recruited in the study and 46 completed the study. The patients were randomized on two groups. In the first group, the patients took NAC orally, in the second one – patients inhaled it by a nebulizer. The active treatment period was 10 days. The patients in the first group received a NAC 200 mg solution orally as a powder (ACC 200, Salutas Pharma GmbH, Germany) pre-dissolved in a glass of water 3 times a day after meal. The patients in the second group received 3 mL of 10% solution (Ingamist, Yuria-Pharm, Ukraine) 2 times a day (in the morning and in the evening) by a nebulizer, the duration of the inhalation session was approximately 10 minutes. In addition to NAC, patients continued receiving baseline therapy in the unchanged mode during participation in the study.

On first day and right after the 10th day of treatment, severity of symptoms and treatment efficacy were evaluated according to the data received from the 6-minute walk distance test[15] and validated questionnaires: for the overall – COPD Assessment Test (CAT)[16] and quantitative breathlessness questionnaire – Modified Medical Research Council Dyspnea Scale (mMRC),[17] for the evaluation of the dynamics of respiratory symptoms – COPD patients Clinical COPD Questionnaire score (CCQ),[18] St. George's Respiratory Questionnaire (SGRQ),[19] 36-Item Short Form Survey (SF-36) to assess patients' quality of life.[20] At the same time, a sputum microscopic analysis was performed, in which the number of leukocytes was expressed by the numbers: 1 (from 10 to 15 cells), 2 (from 20 to 25 cells), 3 (from 30 to 40 cells), 4 (more than 40 leukocytes), and was estimated according to the results of the quartile distribution of

the obtained variation series. The severity of daytime and nighttime cough was evaluated on a 6-point scale.[21] External respiratory function parameters were determined using a SPIROCOM spirometer (KhAI-Medika LLC, Ukraine) according to the standard protocol[14] at the beginning of the study and after the end of the treatment.

The safety assessment of NAC was carried out in accordance with the data of a survey and examination of patients, including the measurement of blood pressure and heart rate, as well as spirometry before and 2 h after the first use of the drug.

The methods of descriptive statistics were used for statistical data processing, the nature of data distribution was evaluated graphically and using the Shapiro–Wilk test. Mean values were presented as mean and standard error of mean ($\pm$ σ), while qualitative values – in percentages. Parametric methods were used to process the data with the normal distribution of the variable series: to compare two independent patient groups – a *t*-test for independent groups, to compare the results of the initial and retest patients – a paired *t*-test. The difference method was used to compare the significance of changes in indicators in the treatment process between groups. Nonparametric statistical methods were used for the nature of data distribution other than normal: the Mann–Whitney criterion for independent samples, and the Wilcoxon criterion for the dependent ones. The difference was considered significant at $P < 0.05$. Statistical analysis was performed using Microsoft Office Excel 2016 (Microsoft Corp., USA) and Statistica 10.0 (StatSoft Inc., USA).

4. RESULTS

The study included 38 men (82.6%) and 8 women (17.4%) at the age from 49 to 78 years (mean 63.30 $\pm$ 6.94 years), with disease duration from 1 to 14 years (7.80 $\pm$ 4.81). According to GOLD classification, the patients belonged to clinical groups B ($n = 23$) and D ($n = 23$). All of them had GOLD 2–4 spirometric grades. Out of these patients, 22 received NAC orally and 24 inhaled by a nebulizer, as specified above. The unchanged basic therapy was the following:
(1) long-acting β2-agonists – 9 patients,
(2) prolonged cholinolytics – 22 patients,
(3) combinations of inhaled glucocorticoids with β_2-agonists – 8 patients
(4) prolonged-cholinolytics with β_2-agonists – 7 patients.

Clinical and demographic data and baseline assessment results of patients are shown in Table 1.

According to the results of the 6-minute walk distance test, all the questionnaires and spirometry, as well as the number of patients belonging to the classification groups B and D, the groups of oral and inhalation usage of NAC were comparable.

With regard to demographic data, in the group of patients receiving NAC by inhalation, the number of female participants was higher compared to the group receiving the drug orally ($P < 0.05$).

The group of patients treated with NAC orally had a significant improvement in the status of patients according to the CCQ (a decrease by 9.7%) and a decrease in the severity of night cough by 36.0% (Table 2). The significant dynamics according to other questionnaires, the results of the 6-minute walk distance test, the severity of daytime cough symptoms, and spirometry indices were not recorded. At the same time, in patients receiving NAC by inhalation, significant positive changes in the CAT questionnaire (13.1% decrease from baseline) and SF-36, based on improved physical and social activity and overall health perception (by 8.8%), as well as decreased symptoms of night cough (by 36.8%) were reported. In this case, the score according to mMRC, CCQ, SGRQ, symptoms of daytime cough did not change significantly. In addition, the average FEV1 values increased by 10.3% ($P = 0.002$) as evidence of a decrease in the severity of bronchial obstruction (Table 3). There was also a significant decrease (by 8.5%) in the number of leukocytes in the sputum after inhalation treatment. Two patients noted a consid-

Table 1. Demographic and baseline characteristics of patients with COPD.

Indicators	Route of NAC administration	
	Orally ($n = 22$)	Inhalation ($n = 24$)
Women, %	4.3	29.2*
Men, %	95.7	70.8
Age, years ($\overline{X} \pm \sigma$)	62.50 ± 7.35	63.80 ± 6.46
Duration of the disease, years ($\overline{X} \pm \sigma$)	7.60 ± 5.24	7.90 ± 4.37
Smoking status, %		
Current	68.2	62.5
Ex-smoker	31.8	37.5
6-minute walk distance test, m ($\overline{X} \pm \sigma$)	250.24 ± 39.20	248.25 ± 41.04
CAT, points	22.18 ± 8.41	23.46 ± 3.66
mMRC, points	2.00 ± 1.00	2.58 ± 0.83
CCQ, points	29.81 ± 12.01	32.17 ± 9.40
SGRQ, points	54.87 ± 13.84	56.78 ± 11.45
SF-36, points	77.64 ± 2.60	76.68 ± 3.18
Physical function	64.14 ± 2.01	61.35 ± 2.92
Role limitation – physical	60.42 ± 3.57	61.47 ± 1.89
Pain	90.44 ± 1.54	92.57 ± 2.31
Health perceptions	69.52 ± 3.24	67.05 ± 3.03
Social function	74.52 ± 1.23	72.04 ± 2.94
Role limitation – mental	90.23 ± 4.25	89.25 ± 4.87
Mental health	94.24 ± 2.35	93.02 ± 4.28
Daytime cough, points	2.18 ± 0.57	2.63 ± 0.88
Night cough, points	1.22 ± 0.81	1.79 ± 1.35
FEV1 L	1.69 ± 0.71	1.17 ± 0.53
FEV1 %	53.46 ± 20.11	42.57 ± 17.04
Group B, %	50	50
Group D, %	50	50

Comments: * $P < 0.05$ for differences in values between groups.

Table 2. Clinical efficacy endpoints in both groups of patients with COPD (X ± σ).

Indicators	Route of NAC administration						P_2
	Orally (n = 22)			Inhalation (n = 24)			
	before treatment	after treatment	P_1	before treatment	after treatment	P_1	
6-minute walk distance test, m	250.24 ± 39.20	251.90 ± 40.56	0.587	248.25 ± 41.04	252.30 ± 40.11	0.127	0.583
CAT, points	22.18 ± 8.41	21.18 ± 7.74	0.235	23.46 ± 3.66	20.38 ± 5.78	0.003	0.592
mMRC, points	2.00 ± 1.00	1.86 ± 0.83	0.103	2.58 ± 0.83	2.21 ± 1.10	0.055	0.328
CCQ, points	29.81 ± 12.01	26.91 ± 12.39	0.021	32.17 ± 9.40	29.54 ± 9.26	0.079	0.439
SGRQ, points	54.87 ± 13.84	54.04 ± 15.39	0.621	56.78 ± 11.45	55.02 ± 15.84	0.547	0.751
SF-36, points	77.64 ± 2.60	78.54 ± 3.05	0.578	76.68 ± 3.18	78.49 ± 3.39	0.197	0.204
Physical function	64.14 ± 2.01	63.28 ± 3.03	0.325	61.35 ± 2.92	65.82 ± 3.25	0.049	0.105
Role limitation – physical	60.42 ± 3.57	59.85 ± 2.98	0.420	61.47 ± 1.89	60.85 ± 2.38	0.547	0.607
Pain	90.44 ± 1.54	91.25 ± 3.08	0.484	92.57 ± 2.31	93.80 ± 2.81	0.647	0.409
Health perceptions	69.52 ± 3.24	70.01 ± 3.06	0.301	67.05 ± 3.03	72.98 ± 2.96	0.012	0.357
Social function	74.52 ± 1.23	76.53 ± 2.75	0.102	72.04 ± 2.94	77.09 ± 3.06	0.042	0.454
Role limitation – mental	90.23 ± 4.25	93.05 ± 3.67	0.090	89.25 ± 4.87	90.47 ± 5.58	0.504	0.658
Mental health	94.24 ± 2.35	95.84 ± 2.81	0.223	93.02 ± 4.28	95.47 ± 2.89	0.207	0.674
Daytime cough, points	2.18 ± 0.57	1.90 ± 0.67	0.240	2.63 ± 0.88	2.25 ± 0.79	0.077	0.352
Night cough, points	1.22 ± 0.81	0.77 ± 0.60	0.032	1.79 ± 1.35	1.13 ± 1.12	0.003	0.436

Comments: P_1 – significance of differences in values before and after treatment in the group; P_2 – significance of differences in changes in values during treatment between groups.

Table 3. Spirometric and laboratory endpoints in both groups of patients with COPD (X ± σ).

Indicators	Route of NAC administration						P_2
	Orally (n = 22)			Inhalation (n = 24)			
	before treatment	after treatment	P_1	before treatment	after treatment	P_1	
FEV1							
L	1.69 ± 0.71	1.66 ± 0.71	0.535	1.17 ± 0.53	1.29 ± 0.56	0.002	0.820
%	53.46 ± 20.11	52.10 ± 20.94		42.57 ± 17.04	46.25 ± 18.51		
Amount of leukocytes in sputum, quartiles	3.12 ± 0.74	3.11 ± 1.23	0.432	3.17 ± 1.87	2.90 ± 1.85	0.048	0.903

Comments: P_1 – significance of differences in values before and after treatment in the group, P_2 – significance of differences in changes in values during treatment between groups.

Table 4. Changes in FEV1 2 hours later after the first use of NAC (X ± σ).

Route of administration	FEV1 before treatment	FEV1 after first administration
Orally		
L	1.69 ± 0.71	1.68 ± 1.21
%	53.46 ± 20.11	52.97 ± 25.21
Inhalation		
L	1.17 ± 0.53	1.15 ± 0.74
%	42.57 ± 17.04	41.18 ± 19.24

Comments: All changes were not statistically significant ($P > 0.05$).

erable reduction in sputum discharge up to the absence of sputum. There was no statistical difference in the dynamics of indicators between the two groups.

To assess the safety, the direct effect of one dose of NAC on the bronchial patency was analyzed in 2 h after the first administration of the drug by spirometry parameters (Table 4). No significant changes in FEV1 due to single oral or inhalation use of NAC were found. This may indicate that the increase in FEV1 values after completion of the course of inhalation of NAC was a cumulative result and was not directly related to the effect on the bronchial patency of the inhalation procedure.

Treatment with NAC in both groups was generally satisfactory. In the group receiving the drug orally, 3 (13.6%) patients noted periodic discomfort in the stomach area, which passed without treatment. Three (12.5%) patients inhaling NAC by a nebulizer reported an increase in cough immediately after inhalation of the drug, which smoothed

away by itself within a few tens of minutes. Most patients complained of an unpleasant odor of the drug, but this did not affect their compliance with the therapy; all patients underwent a full course of treatment. No episodes of arterial hypotension and tachycardia were recorded.

5. DISCUSSION

As can be seen in the obtained results, oral administration of NAC for 10 days improved the quality of life of the patients with COPD, which was evaluated with the CCQ questionnaire, had a positive effect on the cough frequency at night, although it did not affect the severity of COPD symptoms and quality of life determined by CAT data, mMRC, SGRQ and SF-36, exercise tolerance, and daytime cough rate. No changes in spirometric parameters were detected. It should be noted that the daily dose of NAC corresponded to the average therapeutic one did not differ in both groups and was 600 mg. The inhalation route of administration of the drug was also accompanied by a significant reduction in the total severity of COPD according to the CAT questionnaire, reducing cough at night, but, unlike the oral group, also improving the quality of life in the constituents of the SF-36 questionnaire (physical, social activity and general condition of the patient), and values of respiratory function, and decrease in the number of leukocytes in sputum. Thus, NAC in both variants of its use had a positive effect on the clinical condition of patients, but the 10-day course of inhalation by a nebulizer was also associated with an improvement in quality of life and airway patency assessed by spirometry, and a decrease in the number of leukocytes in sputum. No significant differences in the dynamics of the investigated parameters were found between groups, possibly due to the small sample of patients.

Previous studies examining the effect of oral NAC in patients with COPD have shown conflicting data on the efficacy of the drug.[22–24] During its use in medium and high doses,[25] a long-term decrease in the progression of FEV1, a decrease in the incidence of exacerbations of the disease and an improvement in quality of life have been observed over a long period of time. Influence of the drug in average therapeutic doses on the clinical condition of patients and spirometry indicators in short-term treatment (up to 1 month) in patients with COPD outside the exacerbation was not revealed. NAC has significant antioxidant and anti-inflammatory properties, which may explain the positive changes with long-term mucolytic administration. The drug increases the intracellular production of glutathione, a molecule of which is part of the pulmonary antioxidant protection, which reduces the inflammatory manifestations of the disease and, thereafter, the frequency of exacerbations.[5,25] Our findings on the minor effects on COPD manifestations and the absence of changes in spirometry rates are consistent with those of other researchers[24] who used traditional NAC for a short time (up to 8 weeks).

No studies have been found to investigate the feasibility of inhaling NAC using a nebulizer in patients with COPD without exacerbation phase. In patients with chronic bronchitis, the efficacy and safety of NAC in dosed inhalers have been studied.[26] In this study, significant dynamics were not achieved, the use of the drug was safe, the course of treatment was short, so it was impossible to estimate the frequency of exacerbations. The efficacy of inhaled mucolytic therapy in patients with other respiratory diseases, such as cystic fibrosis, bronchiectasis,[27] idiopathic pulmonary fibrosis,[28–30] in patients undergoing mechanical ventilation and in healthy persons has also been investigated.[9] The data obtained confirm the safety of use and the positive effect of the drug on the spirometry. Thus, according to a meta-analysis,[28] NAC slows the rate of decline in predicted vital capacity in patients with idiopathic pulmonary fibrosis, and the combination of the drug with perfinidone can reduce the rate of annual reduction of forced vital capacity.[29] In patients with bronchiectatic disease, inhaled NAC not only improved airway clearance but also significantly increased oxygen saturation.[11]

Our research of the positive effects of a 10-day NAC treatment with a nebulizer, such as reducing disease symptoms, improving quality of life, and reducing the severity of bronchial obstruction, generally coincides with previous studies, although they were performed in a different contingent of patients with a pulmonary profile. Interestingly, similar to our results for cough reduction after NAC treatment were obtained in one of the experimental studies,[31] which researched the effect of the drug on the protective mechanisms of respiratory tract in animals. The argument for the use of NAC with a nebulizer can be connected to the higher potential of its mucolytic action during direct contact with mucus, which due to the presence of a sulfhydryl group in the molecule.[32] It is probably 'opens' the disulfide bonds in the mucus, thereby reducing its viscosity.[33] In addition, the drug has antioxidant and anti-inflammatory properties, which can be more fully realized with local rather than systemic use.

Limitations of the study were the following: small sampling size, single-center trial, and lack of follow-up.

6. CONCLUSIONS

In the patients with COPD, both oral and nebulized usage of NAC for 10 days has a similar positive effect on the manifestations of the disease, including night cough, but the inhalation route of the drug is also accompanied by the improved quality of life and lung function test (FEV1). Oral usage and inhalation of one dose of NAC has no direct effect on the bronchial patency, the safety of the drug is satisfactory, side effects develop infrequently and do not cause interruption of treatment.

Conflict of interest

None declared.

Funding
None declared.

Acknowledgements
We thank Dmytro Fedkov and Liubov Petelytska for comments that greatly improved the manuscript.

Ethics
The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines, and was approved by the Commission on Bioethical Expertise and Research Ethics of the Bogomolets National Medical University (Minutes No 110 of 04/12/2018). Informed consent was obtained from each participant prior to the study.

References
1 Global Initiative for Chronic Obstructive Lung Diseases. *Global strategy for diagnosis, management and prevention of chronic obstructive lung disease. 2018 Report*. https://goldcopd.org/wp-content/uploads/2017/11/GOLD-2018-v6.0-FINAL-revised-20-Nov_WMS.pdf. Accessed: 20.06.2020.

2 Why chronic obstructive pulmonary disease occurs and how to avoid it. https://moz.gov.ua/article/health/chomu-vikikae-hronichne-obstruktivne-zahvorjuvannja-legen-i-jak-jogo-uniknuti. Accessed: 22.11.2018 [in Ukrainian].

3 Rogers DF. Mucoactive agents for airway mucus hypersecretory diseases. *Respir Care*. 2007;52(9):1176–1197.

4 Cazzola M, Rogliani P, Calzetta L, Hanania NA, Matera MG. Impact of mucolytic agents on COPD Exacerbations: A pair-wise and Network meta-analysis. *COPD*. 2017;14(5):552–563. https://doi.org/10.1080/15412555.2017.1347918.

5 Cazzola M, Calzetta L, Page C, et al. Influence of N-acetylcysteine on chronic bronchitis or COPD exacerbations: A meta-analysis. *Eur Respir Rev*. 2015;24(137):451–461. https://doi.org/10.1183/16000617.00002215.

6 Poole P, Chong J, Cates CJ. Mucolytic agents versus placebo for chronic bronchitis or chronic obstructive pulmonary disease. *Cochrane Database Syst Rev*. 2015;7: CD001287. https://doi.org/10.1002/14651858.CD001287.pub5.

7 Stey C, Steurer J, Bachmann S, Medici TC, Tramèr MR. The effect of oral N-acetylcysteine in chronic bronchitis: a quantitative systematic review. *Eur Respir J*. 2000;16(2):253–262. https://doi.org/10.1034/j.1399-3003.2000.16b12.x.

8 Zheng JP, Wen FQ, Bai CX, et al. Twice daily N-acetylcysteine 600 mg for exacerbations of chronic obstructive pulmonary disease (PANTHEON): A randomised, double-blind placebo-controlled trial. *Resp Med*. 2014;2(3):187–194. https://doi.org/10.1016/S2213-2600(13)70286-8.

9 Szkudlarek U, Zdziechowski A, Witkowski K, et al. Effect of inhaled N-acetylcysteine on hydrogen peroxide exhalation in healthy subjects. *Pulm Pharmacol Ther*. 2004;17(3):155–162. https://doi.org/10.1016/j.pupt.2004.01.007.

10 App EM, Baran D, Dab I, et al. Dose-finding and 24-h monitoring for efficacy and safety of aerosolized Nacystelyn in cystic fibrosis. *Eur Respir J*. 2002;19(2):294–302. https://doi.org/10.1183/09031936.02.00025802.

11 Shehzad MI, Mannan MA, Alam M, Rauf A, Sharif MI. Airway clearance in bronchiectasis: A randomized control trial with N-acetylcysteine and 3% hypertonic saline. *J Islamabad Med Dental Coll*. 2019;8(3). https://doi.org/10.35787/jimdc.v8i3.389.

12 Masoompour SM, Anushiravani A, Tafaroj Norouz A. Evaluation of the effect of nebulized N-acetylcysteine on respiratory secretions in mechanically ventilated patients: Randomized clinical trial. *Iran J Med Sci*. 2015;40(4):309–315.

13 Sadowska AM, Verbraecken J, Darquennes K, De Backer WA. Role of N-acetylcysteine in the management of COPD. *Int J Chron Obstruct Pulmon Dis*. 2006;1(4):425–434. https://dx.doi.org/10.2147%2Fcopd.2006.1.4.425.

14 Moore VC. Spirometry: step by step. *Breathe*. 2012;8(3):232–240. https://doi.org/10.1183/20734735.0021711.

15 Celli B, Tetzlaff K, Criner, et al. The 6-minute-walk distance test as a chronic obstructive pulmonary disease stratification tool. Insights from the COPD Biomarker Qualification Consortium. *Am J Respir Crit Care Med*. 2016;194(12):1483–1493. https://doi.org/10.1164/rccm.201508-1653oc.

16 Gupta N, Pinto LM, Morogan A, Bourbeau J. The COPD assessment test: a systematic review. *Eur Respir J*. 2014;44(4):873–884. https://doi.org/10.1183/09031936.00025214.

17 Munari AB, Gulart AA, Dos Santos K, Venâncio RS, Karloh M, Mayer AF. Modified Medical Research Council Dyspnea Scale in GOLD Classification Better Reflects Physical Activities of Daily Living. *Respir Care*. 2018;63(1):77–85. https://doi.org/10.4187/respcare.05636.

18 Kon S, Dilaver D, Mittal M, et al. The Clinical COPD Questionnaire: response to pulmonary rehabilitation and minimal clinically important difference. *Thorax*. 2014;69(9):793–798. https://doi.org /10.1136/thoraxjnl-2013-204119.

19 Müllerova H, Gelhorn H, Wilson H, et al. St George's Respiratory Questionnaire Score Predicts Outcomes in Patients with COPD: Analysis of Individual Patient Data in the COPD Biomarkers Qualification Consortium Database. *Chronic Obstr Pulm Dis*. 2017;4(2):141–149. https://dx.doi.org/10.15326%2Fjcopdf.4.2.2017.0131.

20 Soyyiğit S, Erk M, Güler N, Kilinç G. [The value of SF-36 questionnaire for the measurement of life quality in chronic obstructive pulmonary disease]. *Tuberk Toraks*. 2006;54(3):259–266. https://www.ncbi.nlm.nih.gov/pubmed/17001544 [in Turkish].

21 Decalmer SC, Webster D, Kelsall AA, McGuinness K, Woodcock AA, Smith JA. Chronic cough: How do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring? *Thorax*. 2007;62:329–334. https://doi.org/10.1136/thx.2006.067413.

22 Sanguinetti CM. N-acetylcysteine in COPD: Why, how, and when? *Multidiscip Respir Med*. 2016;11:8. https://doi.org/10.1186/s40248-016-0039-2.

23 Turner RD, Bothamley GH. N-acetylcysteine for COPD: The evidence remains inconclusive. *Resp Med*. 2014;2(4):e3. https://doi.org/10.1016/S2213-2600(14)70054-2.

24 Millar AB, Pavia D, Agnew JE, Lopez-Vidriero MT, Lauque D, Clarke SW. Effect of oral N-acetylcysteine on mucus clearance. *Br J Dis Chest*. 1985;79(3):262–266. https://doi.org/10.1016/0007-0971(85)90046-4.

25 Repine JE, Bast A, Lankhorst I. Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. *Am J Respir Crit Care Med*. 1997;156(2 Pt 1):341–357. https://doi.org/10.1164/ajrccm.156.2.9611013.

[26] Dueholm M, Nielsen C, Thorshauge H, et al. N-acetylcysteine by metered dose inhaler in thetreatment of chronic bronchitis: a multi-centre study. *Resp Med*. 1992;86(2): 89–92. https://doi.org/10.1016/s0954-6111(06)80220-9

[27] Tarrant BJ, Maitre C, Romero L, et al. Mucoactive agents for chronic, non-cystic fibrosis lung disease: A systematic review and meta-analysis. *Respirology*. 2017;22(6):1084–1092. https://doi.org/10.1111/resp.13047.

[28] Tong Sun, Jing Liu, De Wei Zhao. Efficacy of N-acetylcysteine in idiopathic pulmonary fibrosis. *Medicine*. 2016;95(19):1–7. https://doi.org/10.1097/MD.0000000000003629.

[29] Sakamoto S, Muramatsu Y, Satoh K, et al. Effectiveness of combined therapy with pirfenidone and inhaled N-acetylcysteine for advanced idiopathic pulmonary fibrosis: a case-control study. *Respirology*. 2015;20(3):445–452. https://doi.org/10.1111/resp.12477.

[30] Okuda R, Matsushima H, Oba T, et al. Efficacy and safety of inhaled N-acetylcysteine in idiopathic pulmonary fibrosis: A prospective, single-arm study. *Respir Investig*. 2016;54(3): 156–161. https://doi.org/10.1016/j.resinv.2015.10.001.

[31] Pappová L, Kazimierová I, Kocmálová M. Effect of inhaled and oral n-acetylcysteine on airway defense mechanism. *Eur Pharm. J*. 2017;64(1):17–21. https://doi.org/10.1515/afpuc-2017-0002.

[32] Kandasamy J, Carlo WA. Pharmacologic Therapies IV: Other Medications. In: Goldsmith JP, Karotkin E, Suresh G, Keszler M. *Assisted Ventilation of the Neonate*. 6th Ed. Philadelphia, PA: Elsevier;2017:366–379. https://doi.org/10.1016/B978-0-323-39006-4.00034-X.

[33] Jargin SV. On the use of acetylcysteine as a mucolytic drug. *J Invest Biochem*. 2016;52(3):52–53. https://doi.org/10.5455/jib.20160929024258.