European Review for Medical and Pharmacological Sciences 2022; 26: 3192-3199

Factorial analysis of N-acetylcysteine and propolis treatment effects on symptoms, life quality and exacerbations in patients with Chronic Obstructive Pulmonary Disease (COPD): a randomized, double-blind, placebo-controlled trial

V. KOLAROV[1,2], J. KOTUR STEVULJEVIĆ[3], M. ILIĆ[1,2], M. BOGDAN[2], B. TUŠEK[2], A. AGIC[4], M. DUGAJLIĆ[2], K. TOT VEREŠ[2], S. KUTLEŠIĆ STEVIĆ[5], B. ZVEZDIN[1,2]

[1]Medical Faculty Novi Sad, University of Novi Sad, Novi Sad, Serbia
[2]Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia
[3]Department for Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[4]AbelaPharm, Belgrade, Serbia
[5]Department of Physical Medicine and Rehabilitation "Dr Miroslav Zotović" Banja Luka, Republika Srpska

Abstract. – OBJECTIVE: Standard treatment for chronic obstructive pulmonary disease (COPD) includes inhalation therapy along with mucoactive drugs. The aim of this study was to assess the efficacy and safety of orally administered mucolytic N-acetylcysteine and propolis (NACp) in COPD patients.

PATIENTS AND METHODS: A randomized, double-blind, prospective, interventional, 6 months study was conducted at the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia. Effects of daily NACp administration (600 mg, 1200 mg or placebo) on exacerbation, life quality (St. George's Respiratory Questionnaire-SGRQ), symptoms (COPD assessment test-CAT; Visual analogue cough scale-VAS; Leicester Cough Questionnaire-LCQ; Medical Research Council Dyspnoea scale-mMRC) and spirometric parameters in 120 COPD patients were assessed. Tests were conducted at three-time points: baseline, after three months and after 6 months of NACp treatment.

RESULTS: Repeated measures ANOVA showed that pulmonary function parameters, 6-minute walk test and mMRC score did not significantly change during the study. Cough VAS and CAT scores were significantly different between groups as within experimental groups. LCQ and SGRQ scores did not differ between placebo, and both examined groups, but within each examined group statistically significant difference was confirmed in observed parameters during therapy. Factorial analysis and subsequent binary logistic regression revealed "Symptoms related factor" as the strongest predictor of exacerbation for supplemented groups ($p<0.01$).

CONCLUSIONS: Treatment with high NACp for 6 months is safe and beneficial for cough and expectoration symptoms and improves the life quality. NACp significantly reduces acute exacerbation frequency in COPD patients by controlling COPD related symptoms.

Key Words:
COPD, NAC, Propolis, Exacerbation, Cough, Life quality.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common, preventable, and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation[1]. The Global Initiative for COPD (GOLD) highlights the importance of mucolytics in the treatment and control of COPD, besides the standard therapy[1,2]. It was shown that mucolytic agents can modulate the production and reduce the viscosity of mucus, leading to easier expectoration of secretions, which subsequently decreases the possibility of infection[3,4] and improve health status[1].

Nowadays, few different mucolytic agents have been formulated[5], although evidence of their effects in prevention of acute exacerbation and improvement of life quality are diverse[6]. New studies showed that N-acetylcysteine has antioxidant and anti-inflammatory effects which could significantly decrease the risk of acute exacerbations and improve life quality in patients with moderate and severe COPD[6-10].

Scientific progress in pharmaceutical industry has enabled mucolytic agents, which in

Corresponding Author: Violeta Kolarov, MD; e-mail: violeta.kolarov@mf.uns.ac.rs

addition to N-acetylcysteine, contain propolis. Antimicrobial, antioxidant, antiviral, antifungal and anti-inflammatory effects of propolis have been shown, as well as its beneficial effects on tissue regeneration[11,12]. Previous research has shown that 13% aqueous propolis extract can reduce nighttime breathlessness and ameliorate lung function in patients with mild to severe asthma[13] by reduction of oxidative stress and decreased production of pro-inflammatory agents. Altogether, due to the crucial part that oxidative stress and chronic inflammation of the airways have in COPD pathogenesis, the aim of this study was to assess the efficacy and safety of orally administered N-acetylcysteine and propolis in patients suffering from chronic obstructive pulmonary disease.

Patients and Methods

Patients

A randomized, double-blind, prospective, intervention study was conducted at the Institute for Pulmonary Diseases of Vojvodina in Sremska Kamenica, Serbia, from January 2019 to January 2020. A total of 120 patients diagnosed with COPD were enrolled in the study. Four of them withdrew on their personal initiative, while 116 patients fulfilled the whole study protocol. The Ethical Board of Institute for Pulmonary Diseases of Vojvodina gave permission for performing this study, permission number 80-VIII/13. Before enrollment, patients were obliged to read important information about research and sign their informed consent according to the Good Clinical Practice (GCP).

Study included outpatients of both sexes, aged from 40 to 70 years, with confirmed COPD diagnosis and disease history of at least two years, not allergic to N-acetylcysteine and/or propolis. The disease stage was evaluated spirometrically, according to the GOLD recommendations, corresponding to stage I-IV.

Exclusion criteria were participation in other ongoing clinical study and confirmed diagnosis of asthma or bronchial hyperreactivity.

Methods

Patients were divided into three groups: Group I (37 patients) received N-acetylcysteine and propolis combination (NACp) in the form of powder, dosage of 600 mg, once daily; group II (37 patients) received NACp 1200 mg (2x600 mg); group III (42 patients) placebo.

In regard to patients' maintenance therapy for COPD, each group was divided into subgroups. Subgroups Ia, IIa and IIIa consisted of patients receiving dual bronchodilator maintenance therapy (long lasting β2 agonist-LABA and long-lasting muscarinic antagonist- LAMA). Subgroups Ib, IIb and IIIb consisted of patients receiving triple therapy (long lasting β2 agonist-LABA, long lasting muscarinic antagonist-LAMA and inhaled corticosteroid-ICS). All patients used NACp during study period (6 months) and then were monitored for 6 months.

At the beginning of the study, patients were questioned about their demographics, complete medical history, information concerning the course of the disease, associated diseases, maintenance therapy and exacerbations during the previous year before the study beginning.

Each patient underwent the following tests: COPD Assessment Test-CAT consisting of eight questions which can be scored from 0-40. A higher score indicated more abundant respiratory symptoms[14]. Each patient went through a 6-minute walk test in order to determine their aerobic capacity and endurance[15]. Subjective cough score was gained using Visual Analog Scale-VAS with the score ranging from 0 to 10, wherein a higher score represents a higher intensity of cough[16]. In addition, Leicester Cough Questionnaire-LCQ was used, consisting of 19 questions which can be scored from 1 to 7, giving the estimation of cough symptoms, as well as estimation of physical, psychological and social influence of cough on life[17]. Degree of dyspnoea was evaluated using a modified Medical Research Council Dyspnoea scale-mMRC with the scoring system from 0 to 4, wherein a higher score corresponds to a higher degree of dyspnea[18]. Life quality was assessed using St. George's Respiratory Questionnaire-SGRQ wich consists of 50 questions, scored from 0 to 100, where higher scores correspond to greater limitation[19].

Tests were conducted at three-time points: baseline (measurement performed at the beginning of the study), second visit (medical visit after three months of NACp treatment), third visit (medical visit after 6 months of NACp treatment).

During the study period, outpatient and hospital cases of COPD exacerbations were recorded, either through medical visits or through telephone calls with patients. Exacerbations were recorded during the treatment period and in the 6-months follow-up period. Safety assessment was conducted by recording every side effect throughout the one-year period.

Table I. Basic socio-demographic and clinical data for patients with COPD.

Parameters	Group I n=37	Group II n=37	Placebo n=42	*p*-value
Age	69.11 ± 7.37	69.78 ± 6.15	65.31 ± 7.72	$p>0.05$
Sex				
♂	54.1% (20)	70.3% (26)	57.1% (24)	$p>0.05$
♀	45.9% (17)	29.7% (11)	42.9% (18)	
Smoking habit				
yes	40.54% (15)	43.24% (16)	21.43% (9)	
no	16.22% (6)	13.51% (5)	9.52% (4)	$p>0.05$
quitted	43.24% (16)	43.25% (16)	69.05% (29)	
GOLD stage				
I	2.7% (1)	0% (0)	2.4% (1)	
II	43.2% (16)	51.4% (19)	42.9% (18)	$p>0.05$
III	2.7% (1)	5.4% (2)	9.5% (4)	
IV	51.4% (19)	43.2 (16)	45.2% (19)	
Duration of COPD (years)	7.47 ± 6.76	10.59 ± 9.03	7.87 ± 6.70	$p>0.05$
Comorbidities n (%)	11 (29.7)	16 (43.2)	12 (28.6)	$p>0.05$
LABAs/LAMAs	51.35% (19)	59.46% (22)	52.38% (22)	$p>0.05$
LAMAs/LABAs/ICS	48.65% (18)	40.54% (15)	47.62% (20)	$p>0.05$

Statistical Analysis

Results are presented as count (%) or means ± standard deviation depending on data type. Groups are compared using parametric (ANOVA and repeated measures ANOVA) and nonparametric (Chi-square, Mann-Whitney U test, Cochrane Q, Friedman) tests. Principal component analysis-PCA on variables at the second visit with varimax rotation, eigenvalues >1 and variables with factor loadings larger than 0.5 were selected for factors construction. Scores were calculated from PCA extracted factors and used in subsequent univariate binary logistic regression analysis. All *p*-values less than 0.05 were considered significant.

All data were analyzed using SPSS 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA). Results were displayed as tables and graphics.

Randomization was carried out in relation to disease severity and maintenance therapy by random selection method through softer "Random number generated".

Results

116 patients completed the whole protocol. Active smokers represented 34.48% (40), non-smokers 12.93% (15) and subjects who quit smoking 52.59% (61). Comorbidities in Group I were present in 29.7% (11), in Group II 43.2% (16) and in Group III in 28.6% (12) patients. Values between groups did not show significant statistical difference ($p>0.05$). There were no statistically significant differences between groups ($p>0.05$) when comparing sex, age, smoking habit, GOLD stage, COPD duration and number of comorbidities (Table I).

The comparison of pulmonary function parameters at three-time points showed no statistically significant differences between and within groups ($p>0.05$). Respiratory symptoms (expectorations and cough VAS) during treatment with NACp were statistically significantly different between groups ($p<0.05$). By comparing expectoration, significant changes were noted at the start of the study and in the second and third measurements within groups I and II (Cochran's Q=19.600; $p<0.001$) but not in the placebo group. Results of VAS score showed differences between the first and second, as well as between the first and third measurement in group I as in group II (Friedman $\chi^2=15.525$; $p<0.001$). In group III there was no significant difference between all three measurements. Comparison of the overall CAT score showed a statistically significant difference between groups ($p<0,05$), as within group I and II between baseline and second measurement, as between baseline and third measurement ($p<0,05$). The subjective degree of dyspnea (mMRC scale) was compared using Friedman test; no statistically significant differences between groups have been found (Group I - Friedman $\chi^2=0.383$; $p=0.826$; Group II - Friedman $\chi^2=2.811$; $p=0.245$; Group III - Friedman

Table II. Respiratory symptoms, spirometry, results of 6-minute walk test in tested groups during the treatment periods.

Parameters	Group I			Group II			Placebo		
	Baseline	Second visit	Third visit	Baseline	Second visit	Third visit	Baseline	Second visit	Third visit
Expectoration	89.2% (33)	62.2% (23) aa	45.9% (17) aaa,bb	83.8% (31)	64.9% (24) aa	45.9% (17) aaa,b	95.2% (40)	88.6% (33)	87.6% (20)
Cough VAS	4.51 ± 1.77	4.30 ± 2.04 a	3.27±1.97 aaa,bb	4.78 ± 2.15	4.14 ± 2.00 aa	3.30±2.17 aaa,bb	5.10 ± 2.00	5.07 ± 2.05	5.24 ± 1.92
Dyspnoea mMRC	2.30 ± 0.94	2.24 ± 0.83	2.43 ± 0.87	2.22±1.40	2.11 ± 1.13	2.03 ± 1.55	2.12 ± 1.11	2.14 ± 1.05	2.07 ± 1.00
CAT score	23.54 ± 7.83	19.78 ± 7.40 aaa	19.35 ± 7.22 aaa	18.62 ± 7.91	16.30 ± 7.24 aaa	15.49 ± 7.53 aaa	22.95 ± 8.42	22.76 ± 8.17	22.64 ± 8.29
6-min walk test	423.4 ± 99.3	437.7 ± 100.5	445.6 ± 99.3	428.7 ± 82.9	435.2 ± 87.3	448.5 ± 89.6 a	448.1 ± 91.4	445.6 ± 88.3	455.5 ± 84.0
FVC %	86.35 ± 20.35	84.01 ± 21.15	82.78 ± 19.13	81.22 ± 18.46	84.15 ± 15.69	84.73 ± 14.65	75.53 ± 19.11	78.96 ± 23.71	78.01 ± 26.20
FEV_1 %	54.53 ± 19.85	53.37 ± 22.74	52.72 ± 23.03	53.12 ± 19.75	55.55 ± 17.30	55.36 ± 16.72	48.36 ± 19.29	50.99 ± 24.60	48.97 ± 23.72
FEV_1/FVC	50.18 ± 15.56	50.95 ± 14.38	50.52 ± 15.19	51.89 ± 12.31	51.99 ± 11.37	52.25 ± 12.25	53.12 ± 17.45	53.32 ± 15.46	53.88 ± 17.41

Statistical significance was tested by repeated measures ANOVA: a, aa, aaa – p<0.05, 0.01, 0.001 *vs.* baseline, respectively; b, bb – p<0.05, 0.01 *vs.* second visit, respectively.

χ^2=4.536; p=0.104). Analysis of 6-minute walk test results revealed a statistically significant difference between initial measurement values and measurement values after six months of NACp treatment, unrelated to the used dosage of NACp (F=5.562; p=0.007; Eta_{part}^2=0.047) (Table II).

Variance analysis of repeated measurements determined a statistically significant difference between overall LCQ score during treatment within each group (F=35.915; p<0.001; Eta_{part}^2=0.241). However, no statistically significant difference between groups has been established (F=2.133; p=0.095; Eta_{part}^2=0.036) (Figure 1). Variance analysis of repeated measurements showed the existence of a statistically significant difference of the overall score on SGRQ questionnaire within each group (F=15.906; p<0.001; Eta_{part}^2=0.123), which is not the case when SGRQ score values between groups are compared between tested groups (F=1.370; p=0.251; Eta_{part}^2=0.024) (Figure 1A and 1B).

Table III shows the total number of exacerbations during NACp treatment and 6 months after the end of treatment LAMAs/LABAs/ICS or LAMAs/LABAs. A statistically significant difference in the number of exacerbations corresponds to the usage of ICS in NACp 1200 group (p<0.05). Subsequent comparison among NACp 1200 group regarding drugs combination (dual *vs.* triple therapy) showed significantly less exacerbations The principal component analysis involved symptoms, clinical data and pulmonary function parameters performed at the end of this study, after the NACp treatment. Total variability explained by selected parameters and grouped in 3 factors is 63.2%. Factors with the largest percentage of variability are "symptoms related factor", then "pulmonary function related factor" and "clinical status related factor" as the least significant (Table IV).

Scores calculated from significant factors produced in PCA analysis were used as independent variables in logistic regression analysis for exacerbation status prediction. Univariate logistic regression analysis showed that the strongest predictor of exacerbation status was Factor 1 (symptoms related factor) for supplemented group of patients (p<0.05) compared to placebo. The same analysis showed that Factor 2 (pulmonary function related factor) was also a significant predictor of total exacerbation status for the treatment group (Table V).

Discussion

Previous studies[12] have not sufficiently established quality standards for pharmacological products that contain propolis, which should depend on their biochemical characteristics. Many studies[20,21] suggest multiple beneficial activities of combined NACp treatment. Due to the recognized mucolytic, antibacterial and anti-inflammatory properties, we chose to investigate the effect of NACp on respiratory symptoms, lung function, exacerbations and life quality of COPD patients.

Examination of dyspnoea, using mMRC and 6-minute walk test showed no statistically significant difference between and within placebo, groups I and II. These results are in accordance with the findings of HIACE study[22]. A possible

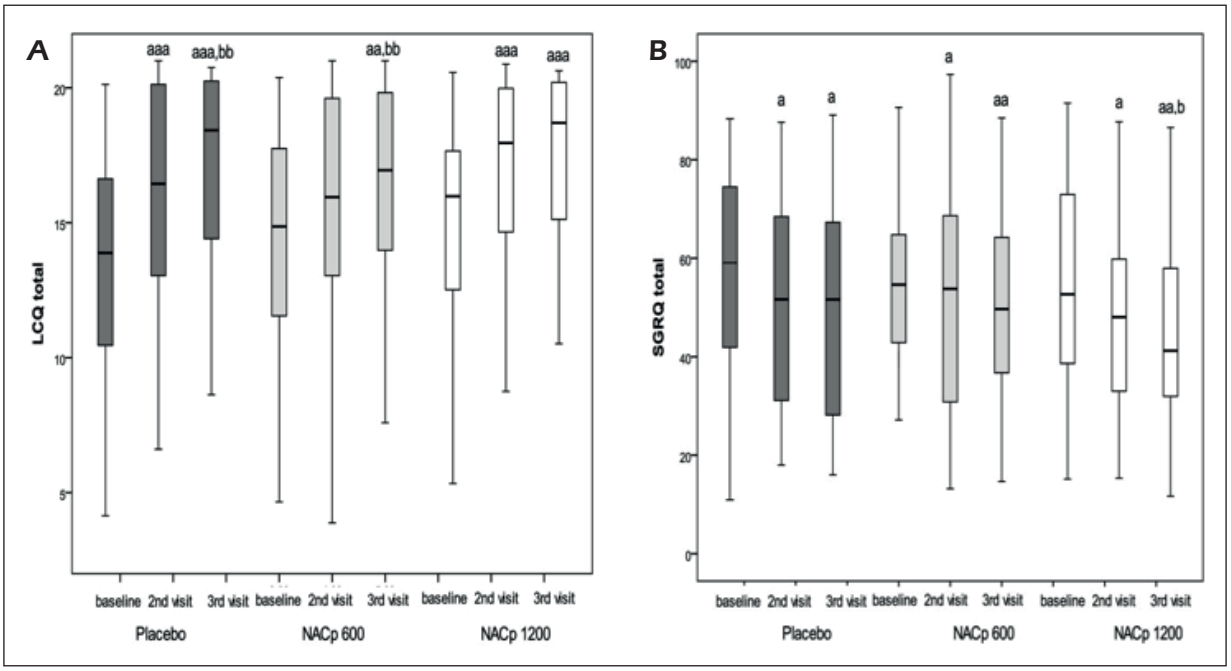

Figure 1. LCQ and SGRQ total scores according to study groups and visits.
Data are presented as box-plot graphs; a, aa, aaa $p<0.05$, 0.01, 0.001, respectively *vs.* baseline; b, bb $p<0.05$, 0.01, respectively *vs.* second visit. (**A**) LCQ total scores according to study groups and visits; (**B**) SGRQ total scores according to study groups and visits.

explanation can be the uniformity of patients regarding duration and severity of COPD. Also, differences in smoking habits did not exist, further increasing the homogeneity of groups and reducing the impact on research results. CAT scores, cough VAS and expectoration showed significant differences between examined groups in all three measurements as well as within these measurements in groups I and II. These results are based on the effect of propolis, which allows the reduction in neutrophil migration and regulation of the inflammatory process by modulating stress signals, toxins and microbiotics[11]. This reduces and modifies the inflammation, dilutes the secretion, and thus facilitates the cleaning of the airways. Within the placebo group there was no significant difference between the three measurements. However, by observing values of LCQ scores we noticed a significant difference between overall LCQ score during treatment within each group, but no statistically significant difference between groups has been established.

A statistically significant difference in walking distance during 6-minute walk test between groups has likewise not been found. Furthermore, walking distances after 6 months of treatment with high dosage of NACp (1200 mg) were significantly higher than that at the beginning of the study. This leads to conclude that NACp treatment during longer period of time reduces fatigue and facilitates everyday activities, in accordance with the results of HIACE study[22].

A statistically significant difference in spirometry parameters has not been found between and within the groups. Previous research has shown that older COPD patients, which took NAC during an extended period, reported a smaller drop in the value of FEV_1 when compared to placebo. However, positive effects of NAC on lung function were not verified in the latter randomized studies. In a substantial three-year-long study BRONCUS, Decramer et al[23] showed that NAC is ineffective at reducing the rate of decay of FEV_1 values in COPD patients. Poole et al[22] conducted a sizeable study which led to the conclusion that mucolytic agent treatments do not produce any significant clinical improvement of pulmonary function[24], which is in accordance with our results. On the other hand, the 1-year, double-blind, randomized, placebo-controlled HIANCE trial offered the conclusion that NAC in a dose of 1200 mg notably improves the function of small airways while having no effect on FEV_1 value. This improvement in ventilator function during NACp usage can contribute to overall reduction in the level of the pro-inflammatory cytokines such as TNF, IL-1,

Table III. Total number of exacerbations during and after the treatment with N-acetylcysteine and propolis regarding maintenance therapy.

NACp 600	Total number of exacerbations		*P*
	LAMA/LABA	LAMA/LABA/ICS	
During treatment	0.479 ± 0.273	0.267 ± 0.205	0.115
After treatment	0.436 ± 0.905	0.436 ± 0.556	0.920
Total	0.915 ± 1.938	0.603 ± 1.121	0.068
NACp 1200			
During treatment	0.227 ± 0.583	0.053 ± 0.042	0.019
After treatment	0.173 ± 0.250	0.100 ± 0.214	0.667
Total	0.400 ± 0.539	0.153 ± 0.133	0.016

IL-6, and the rise of anti-inflammatory ones (IL-4, IL-10)[13].

By observing values of total SGRQ scores and scores of each individual domain of this questionnaire, we noticed that no significant differences have been found between placebo and treated groups. However, statistically significant differences in life quality within each group were found, leading to conclude that the life quality of the patients considerably improved due to the NACp treatment. Given that within the placebo group a statistically significant difference in SGRQ scores has been observed, we can assume that the psychological component has a vital role in subjective assessment of symptoms.

In 1994, Hansen et al[25] conducted a double-blind, placebo-controlled trial that included 129 COPD patients and stated that oral administration of NAC in a dose of 300 mg twice daily during winter considerably enhances general health condition of patients, even though statistically significant difference between placebo and treatment groups has not been noted. Just five years after, in 1999, Pela et al[26] obtained different results for the same doses of NAC, proving the reduction of COPD exacerbations (41%) and improvement of pulmonary function. Further studies like BRONCUS showed that the overall score of SGRQ does not fluctuate when NAC is taken daily in a dose of 600 mg during a period of three years. In contrast, the PANTHEON study[8] pointed out that daily use of NAC in a dose of 1200 mg for one year's period drastically improves score of SGRQ domain concerned with disease symptoms in COPD patients, which is in congruence with our results.

Table IV. Principal component analysis (PCA) and extracted factors in a whole group of COPD patients.

Factors	Included variables with loadings	Factor Variability
1. Symptoms related factor	Cough VAS 3 (0.837) CAT 3 (0.797) Expectoration 3 (0.727)	33.2%
2. Pulmonary function related factor	FVC (%) 3 (0.883) FEV_1 (%) 3 (0.869) 6-min walk test (0.519)	17.0%
3. Clinical status related factor	Comorbidities (0.773) Duration of COPD (years) (0.691)	13.0%
Total variability		63.2%

In our study the mean number of exacerbations was in group I 1.51 ± 2.05, group II 0.54 ± 0.90 and in group III 3.43 ± 1.50, respectively. By analysis of outpatient and hospital exacerbations during out trial, we noticed statistically significant difference between placebo and both groups that received doses of 600 mg or 1200 mg NACp during treatment, but also 6 months after treatment. That is in accordance with recommendations from Australia and New Zealand [27] and results from meta-analysis done by Cazzola et al[10] in 2015.

Examination of the total number of exacerbations during treatment and follow-up period regarding dual and triple maintenance therapy revealed statistically significant difference between groups treated with the highest dose of NACp (1200 mg).

In 2010 Burgel et al[28] performed PCA analysis to get new COPD classification based on multiple clinical variables. That study got similar percent of variability (61%) explained by selected variables, as in this current study. Subsequent cluster analysis revealed 3 main factors among which the most important included dyspnea score, SGRQ score, exacerbation/year, anxiety and depression scores, which is similar to our PCA conception (Table IV). Similar findings were noticed in other papers[29,30] that some of the variables represent the main determinant of extracted factors as in our study (CAT, FEV% and FVC%). But also, there are some differences which could be explained by supplementation implemented in our study.

Table V. Univariate logistic regression analysis of predictive capability of factors extracted in PCA analysis towards exacerbation status (after the supplementation).

Parameter	Placebo group			Supplemented group		
	Wald	OR (95th CI)	p	Wald	OR (95th CI)	p
Symptoms related factor	0.427	1.24 (0.653-2.35)	0.514	8.03	2.81 (1.38-5.75)	0.005
Pulmonary function related factor	6.243	0.30 (0.12-0.77)	0.012	4.66	0.45 (0.22-0.93)	0.031
Clinical status related factor	0.145	1.14 (0.57-2.29)	0.703	1.59	0.69 (0.39-1.23)	0.207

OR – odds ratio; CI – confidence interval.

Logistic regression analysis performed with factors extracted by PCA showed significant predictive capability towards exacerbation of Factor 1 only in supplemented group. This could explain potential NACp influence on several COPD characteristics like coughing, expectoration and those characteristics measured by CAT score. This may be explained by mucolytic and anti-inflammatory effects properties of NACp combination. Previous literature reports a very low incidence of side effects. During our trial, no side effects of NACp have been reported.

Limitations

We recognize and acknowledge the study limitations with a relatively small sample and a short study duration, as well as a follow-up period. Explanation is that the epidemiological situation caused by the SARS CoV-2 disabled a longer duration of the study as well as the inclusion of a larger number of patients. Perhaps the most important limitation of the study was that patients used NACp at home, so we could not with certainty comprehend or verify whether they used it regularly, or whether the compliance was satisfactory.

Conclusions

We can conclude that multiple aspect approach to each individual COPD patient is of greatest importance in disease control and treatment. Peroral administration of NACp in high doses is safe, improves life quality and reduces severity of symptoms such as cough and expectoration. Due to its mucolytic, anti-inflammatory and antioxidant effect, NACp combined with standard inhaled therapy, can reduce frequency of acute exacerbations in patients suffering from moderate to severe COPD. However, previous literature highlights the importance of awareness of heterogeneity of trial population, dosing of therapy, complexity of comorbidities and various smoking habits. The aforementioned problems prevent precise identification of the target population of COPD patients who would benefit from antioxidant agents.

Conflict of Interest

The authors report no conflicts of interest in this work.

Ethics Approval

The Ethical Board of Institute for Pulmonary Diseases of Vojvodina gave permission for performing this study, permission number 80-VIII/13.

Informed Consent

Before enrollment, patients read important information about research and signed the informed consent according to the Good Clinical Practice (GCP).

References

1) Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease (GOLD) 2021 REPORT. https://goldcopd.org/wp-content/uploads/2020/11/GOLD-REPORT-2021-v1.1-25Nov20_WMV.pdf
2) Poole P, Sathananthan K, Fortescue R. Mucolytic agents versus placebo for chronic bronchitis or chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2019; 5: CD001287.
3) Tarrant BJ, Le Maitre C, Romero L, Steward R, Button BM, Thompson BR, Holland AE. Mucoactive agents for chronic, non-cystic fibrosis lung disease: a systematic review and meta-analysis. Respirology 2017; 22: 1084-1092.
4) Turner RD, Bothamley GH. N-Acetylcysteine for COPD: the evidence remains inconclusive. Lancet Respir Med 2014; 2: e3.
5) Rogliani P, Matera MG, Page C, Puxeddu E, Cazzola M, Calzetta L. Efficacy and safety profile of mucolytic/antioxidant agents in chronic obstructive pulmonary disease: a comparative analysis across

erdosteine, carbocysteine, and N-acetylcysteine. Respir Res 2019; 20: 104.

6) Shawn DA. Mucolytics for COPD: negotiating a slippery slope towards proof of efficacy. Eur Respir J 2017; 50: 1701465.

7) Fowdar K, Chen H, He Z, Zhang J, Zhong X, Zhang J, Li M, Bai J. The effect of N-acetylcysteine on exacerbations of chronic obstructive pulmonary disease: A meta-analysis and systematic review. Heart Lung 2017; 46: 120-128.

8) Zheng JP, Wen FQ, Bai CX, Wan HY, Kang J, Chen P, Yao WZ , Ma LJ, Li X, Raiteri L, Sardina M, Gao Y, Wang BS, Zhong NS. Twice daily N-acetylcysteine 600 mg for exacerbations of chronic obstructive pulmonary disease (PANTHEON): a randomised, double-blind placebo-controlled trial. Lancet Respir Med 2014; 2: 187-194.

9) 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: 552-563.

10) Cazzola M, Calzetta L, Page C, Jardim J, Chuchalin AG, Rogliani P, Matera MG. Influence of N-acetylcysteine on chronic bronchitis or COPD exacerbations: a meta-analysis. Eur Respir Rev 2015; 24: 451-461.

11) Król W, Bankova V, Sforcin JM, Czuba Z, Kuropatnickiet AK. Propolis: Properties, Application, and Its Potential. Evid Based Complement Alternat Med 2013; 2013: 807578.

12) Almeida CE, Menezes H. Anti-inflamatory activity of propolis extracts: A review. J Venom Anim Toxins 2002; 8: 191-212.

13) Khayyal MT, El-Ghazaly MA, El-Khatib AS, Hatem AM, De Vries PJF, El-Shafei S, Khattab MM. A clinical pharmacological study of the potential beneficial effects of a propolis food product as an adjuvant in asthmatic patients. Fund Clin Pharmacol 2003; 17: 93-102.

14) Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Leidyet NK. Development and first validation of the COPD Assessment Test. Eur Respir J 2009; 34: 648-654.

15) Casanova C, Cote CG, Marin JM, Torres JPD, Aguirre-Jaime A, Mendez R, Dordelly L, Celliet BR. The 6-min walking distance: long-term follow up in patients with COPD. Eur Respir J 2007; 29: 535-540.

16) Schmit KM, Coeytaux RR, Goode AP, Mccrory DC, Yancy WS, Kemper AR, Hasselblad V, Heidenfelder BL, Sanders GD. Evaluating cough assessment tools: a systematic review. Chest 2013; 144: 1819-1826.

17) Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MDL, Pavordt ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). Thorax 2003; 58: 339-343.

18) Mahler DA, Ward J, Waterman LA, Bairdet JC. Longitudinal Changes in Patient-reported Dyspnea in Patients with COPD. COPD 2012; 9: 522-527.

19) Jones P, Quirk F, Baveystock C. The St George's Respiratory Questionnaire. Resp Med 1991; 85: 25-31.

20) Chirumbolo S. Anti-inflammatory property of propolis. J Clin Biochem Nutr 2015; 56: 163-164.

21) Borrelli F, Maffia P, Pinto L, Ianaro A, Russo A, Capasso F, Ialenti A. Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. Fitoterapia 2002; 73: 53-63.

22) Tse HN, Raiteri L, Wong KY, Yee KS, Ng LY, Wai KY, Loo CK, Chan MH. High-dose N-acetylcysteine in stable COPD: the 1-year, double-blind, randomized, placebo-controlled HIACE study. Chest 2013; 144: 106-118.

23) Decramer M, Mölken MR, Dekhuijzen PN, Troosters T, Herwaarden C, Pellegrino R, Schayck OV, Olivieri D, Donno MD, Backer WD, Lankhorst I, Ardiaet A. Effects of N-acetylcysteine on outcomes in chronic obstructive pulmonary disease (Bronchitis Randomized on NAC Cost-Utility Study, BRONCUS): a randomized placebo-controlled trial. Lancet 2005; 365: 1552-1560.

24) Poole P, Chong J, Cates CJ. Mucolytic agents versus placebo for chronic bronchitis or chronic obstructive pulmonary diseases. Cochrane Database Syst Rev 2015: CD001287.

25) Hansen NC, Skriver A, Brorsen-Riis L, Balsløv S, Evald T, Maltbaek N Gunnersen G, Garsdal P, Sander P, Pedersen JZ, Ibsen TB, Rasmussen FV. Orally administered N-acetylcysteine may improve general well-being in patients with mild chronic bronchitis. Respir Med 1994; 88: 531-535.

26) Pela R, Calcagni AM, Subiaco S, Isidori P, Tubaldi A, Sanguinetti CM. N-acetylcysteine reduces the exacerbation rate in patients with moderate to severe COPD. Respiration 1999; 66: 495-500.

27) Yang IA, Brown JL, George J, Jenkins S, McDonald CF, McDonald V, Phillips K, Smith BJ, Zwar NA, Dabscheck E. The COPD-X Plan: Australian and New Zealand Guidelines for the Management of Chronic Obstructive Pulmonary Disease: 2017 update. MJA 2017; 207: 436-442.

28) Burgel PR, Paillasseur JL, Caillaud D, Tillie-Leblond I, Chanez P, Escamilla R, Court-Fortune, Perez T, Carré P, Roche N. Clinical COPD phenotypes: a novel approach using principal component and cluster analyses. Eur Respir J 2010; 36: 531-539.

29) Kim S, Lim MN, Hong Y, Han SS, Lee SJ, Kimet WJ. A cluster analysis of chronic obstructive pulmonary disease in dusty areas cohort identified three subgroups. BMC Pulm Med 2017: 17: 209.

30) Criner GJ, Bourbeau J, Diekemper RL, Ouellette DR, Goodridge D, Hernandez P, Curren K, Balter MS, Bhutani M, Camp PG, Celli BR, Dechman G, Dransfield MT, Fiel SB, Foreman MG, Hanania NA, Ireland BK, Marchetti N, Marciniuk DD, Mularski RA, Ornelas J, Road JD, Stickland MK. Prevention of acute exacerbation of COPD: American College of Chest Physicians and Canadian Thoracic Society Guideline. Chest 2015; 147: 883-893.