VOL. 41, No. 3, Juli 2021

p-ISSN 0853-7704
e-ISSN 2620-3162

JURNAL RESPIROLOGI INDONESIA

Majalah Resmi Perhimpunan Dokter Paru Indonesia
Official Journal of The Indonesian Society of Respirology

Akreditasi RISTEKDIKTI
Surat Keputusan Menteri Riset dan Teknologi Nomor: 200/M/KPT/2020 Tanggal 23 Desember 2020, Peringkat 2
Website: http://www.jurnalrespirologi.org

JURNAL
RESPIROLOGI INDONESIA

Majalah Resmi Perhimpunan Dokter Paru Indonesia
Official Journal of The Indonesian Society of Respirology

SST : Surat Keputusan Menteri Penerangan RI
No.715/SK/DitjenPPG/SST/1980 Tanggal 9 Mei 1980

Alamat Redaksi
PDPI Jl. Cipinang Bunder, No. 19, Cipinang Pulo Gadung
Jakarta Timur 13240 Telp: 02122474845
Email : editor@jurnalrespirologi.org
Website : http://www.jurnalrespirologi.org

Diterbitkan Oleh
Perhimpunan Dokter Paru Indonesia (PDPI)
Terbit setiap 3 bulan (Januari, April, Juli & Oktober)

Jurnal Respirologi Indonesia
Akreditasi Peringkat 2
Sesuai Keputusan Menteri Riset dan Teknologi/Kepala Badan
Riset dan Inovasi Nasional Republik Indonesia
Nomor: 200/M/KPT/2020 Tanggal 23 Desember 2020

JURNAL
RESPIROLOGI INDONESIA

Majalah Resmi Perhimpunan Dokter Paru Indonesia
Official Journal of The Indonesian Society of Respirology

VOLUME 41, NOMOR 3, Juli 2021

DAFTAR ISI

Artikel Penelitian

Tinjauan Pustaka

Exhaled Carbon Monoxide (eCO) and Serum CC16 Levels in Active Smokers

Fitri Indah Sari[1], Tri Wahju Astuti[1], Teguh Rahayu Sartono[1], Garinda Alma Duta[1]

[1] Department of Pulmonology and Respiration Medicine, Faculty of Medicine, Universitas Brawijaya, RSUD Dr. Saiful Anwar, Malang

Abstract

Background: *Toxic particles within tobacco smoke are responsible for several respiratory system problems. Among these toxic particles is Carbon Monoxide (CO), produced from environment and Heme Oxygenase induction. Expiratory CO levels can be measured using CO analyzer. Club Cell Protein 16 (CC16) is a pneumoprotein produced by club cells in distal respiratory tract. In acute condition, CC16 level will increase to maintain homeostasis and anti-inflammation. In chronic condition, i.e.smokers, Club cell destruction would lead to decrease level of CC16. This study aims to determine exhaled CO (eCO) levels and serum CC16 levels in active smokers.*

Methods: *This is a cross-sectional study on 40 healthy active smokers from October 2019-June 2020. Subjects who met the criteria; men, age between 19 until 60 years old, smoked at least 1 year and 1 cigarette/day, smoked in the Universitas Brawijaya and signed the informed consent. Exhaled CO levels are measured using CO analyzer, while ELISA is used to measure serum CC16 evels.*

Results: *Among 40 subjects, mean eCO level is 10.18 ± 7.42 ppm. Mean serum CC16 level is 3.17 ± 1.78 ng/mL, lower than normal value of 6.4 ng/mL. The mean serum CC16 based on the Brinkman Index were heavy smokers 5.24±0.45 ng/mL, medium 3.32±1.87 ng/mL, and light smokers 2.49±1.42 ng/mL. The highest serum CC16 level is 5.56 ng/mL included in the category of CO Analyzer levels smoker-heavily addicted, i.e 28 ppm, a number of 1 subject.*

Conclusion: *eCO levels and serum CC16 decreases in active smokers. This indicates that CO from tobacco smoke could damage the Club cells in the respiratory system, so the resulting of serum CC16 will be reduced.* ***(J Respirol Indones 2021; 41(3): 200–6)***

Keywords: *Active Smokers, Exhaled Carbon Monoxide (eCO), Serum CC16*

Kadar Karbon Monoksida Ekshalasi (eCO) dan Kadar CC16 Serum pada Perokok Aktif

Abstrak

Latar belakang: *Zat toksik yang terkandung di dalam asap rokok dapat menyebabkan masalah pada sistem respirasi. Salah satunya adalah gas karbon monoksida (CO) yang dapat dihasilkan dari lingkungan maupun akibat induksi Heme Oxygenase. Gas CO dapat diukur melalui udara yang diekspirasi menggunakan CO Analyzer.* Club Cell Protein 16 (CC16) *adalah pneumoprotein yang dihasilkan oleh Club cells di saluran nafas bagian distal. Pada kondisi akut CC16 akan meningkat dan berfungsi mempertahankan homestasis dan aktivitas anti-inflammasi. Pada kondisi kronis, seperti pada perokok Kerusakan Club cell dapat menyebabkan penurunan kadar CC16. Tujuan studi ini adalah untuk mengetahui kadar CO ekshalasi (eCO) dan kadar serum CC16 pada perokok aktif.*

Metode: *Studi menggunakan desain penelitian uji potong lintang pada 40 subjek perokok aktif yang sehat pada Oktober 2019-Juni 2020. Subjek yang memenuhi kriteria; laki-laki, usia 19–60 tahun, aktif merokok minimal 1 tahun dan 1 batang/hari, merokok di lingkungan Universitas Brawijaya dan telah menandatangani persetujuan penelitian. Pengukuran kadar eCO menggunakan CO Analyzer dan kadar serum CC16 menggunakan metode ELISA.*

Hasil: *Rerata kadar eCO pada 40 subjek penelitian termasuk kategori smoker-low addicted (10,18 ± 7,42 ppm). Rerata kadar CC16 serum pada penelitian ini yaitu 3,17 ± 1,78 ng/mL. Rerata serum CC16 berdasarkan Indeks Brinkman yaitu pada kelompok berat 5,24 ± 0,45 ng/mL, sedang 3.32 ± 1.87 ng/mL, dan ringan 2.49 ± 1.42 ng/mL. Kadar serum CC16 tertinggi menurut kadar CO Analyzer adalah perokok berat, yaitu 28 ppm sebanyak 1 subjek.*

Kesimpulan: *Pada perokok aktif terjadi peningkatan rerata kadar eCO disertai dengan penurunan rerata kadar serum. Hal ini menunjukkan CO dari asap rokok dapat menyebabkan kerusakan pada Club cell di bronkiolus respiratorius, sehingga produksi serum CC16 akan berkurang.* ***(J Respirol Indones 2021; 41(3): 200–6)***

Kata kunci: *Karbon monoksida ekshalasi (eCO), Perokok aktif, Serum CC16*

Correspondence: Fitri Indah Sari
E-mail: fitrindahs1705@gmail.com

INTRODUCTION

Smoking is one of unhealthy habit that is difficult to eliminate.[1] According to WHO in 2015, the global number of active smokers exceeding 1.1 billion with male predominant.[2] Health issues and death related to smoking are still a burden for the economic losses. Annual data from WHO, there are 7 million people death and 1.4 trillion USD losses due to lessen productivity and health cost related to smoking.[3] In smoked cigarettes contained Carbon Monoxide (CO) gas, which is invisible, odorless but poisonous.[4] The benefit of CO gas analysis is to measure the levels of CO gas exhaled by active and passive smokers on a non-invasive basis and not influenced by the use of nicotine-containing products.[5]

Air pollution, including cigarette smoke, will stimulate the secretion of Club Cell Protein-16 (CC16). In acute conditions, there will be increased production of CC16 by maintaining homeostasis and anti-inflammation activity in the airways exposed to irritants, allergens, and viruses. While in chronic conditions, such as in smokers, CC16 can decrease. This is due to the club cell that produces CC16 is damaged. Decreased production of CC16 in smokers results in increased proinflammation that eventually causes some lung diseases, one of which is PPOK.[6]

Measurement of CO gas exhaled by active smokers is one of the stages of efforts to quit smoking by identifying exhalation CO levels or exhaled carbon monoxide (eCO) as tools for smoking cessation. Previous studies with descriptive statistical design, obtained increased CO levels and decreased serum CC16 levels in smokers, but the association between the two substances is not widely understand. Further study regarding this issue will be conducted in a cross-sectional design and the minimum length of smoking is 1 year.

METHOD

This cross-sectional study was conducted from October 2019–June 2020. Subjects eligible for this study are men, age 19–60 years, minimum smoking is 1 cigarette per day for at least 1 year, and signed the informed consent. Passive or former smokers, history or current treatment of any pulmonary disease, and any kidney or liver disease were not included in this study. There are 40 eligible subjects whom we measured the levels of CO exhalation with a CO Analyzer and the levels of CC16 in serum by ELISA method.

RESULT

In this study, 40 eligible subjects with characteristic male, mean age 34±9.0 years and mean Body Mass Index (BMI) of 22.1±4 kg/m². The mean or median of the smoking duration is 1 to 40 years. The type of cigarettes that are often used are filter 70.0%, then the clove 20%, and mixture 10%. According to Brinkman index degree, this study has a proportion of mild degrees 65.0%, moderate degrees 30%, and heavy degrees 5.0%.

Table 1. Demographics on Research Subjects

Characteristic	Mean±SD or Percentage
Age (years)	34±9
BMI (kg/m²)	22.1±4
Smoking Duration (years)	15.17±9.5
Type of work	
Administration Officer	62.5%
Non-administration	25.0%
College student	12.5%
Type of Cigarette	
Filtered	70.0%
Unfiltered	20.0%
Mix	10.0%
Brinkman Index	
Light	65.0%
Medium	30.0%
Heavy	5.0%
Haematological Parameters	
Haemoglobin (gr/dl)	15.1±1.4
Leukocytes (/µl)	7.132±1,273.9
Haematocrit (%)	44.02±3.25
Platelets (/µl)	297.875±68.33
Serum CC16 (ng/mL)	3.17±1.78
eCO (ppm)	10.18±7.42

The haematological parameters of the study subjects for the haemoglobin average were 15.1±1.4 gr/dl, leukocytes 7.132±1.273,9 /µl, haematocrit 44.02±3.25% and platelets are 297.875±68.33/µl.

Table 2. Distribution Number of Study Subjects Based on Degree of Brinkman Index by Category CO Analyzer Level of eCO

eCO Category (Smokerlyzer)	Brinkman Index (n)			
	Light	Medium	Heavy	Total
Non-smoker	14	2	-	16
Borderline	4	3	-	7
Smoker-low addicted	4	-	1	5
Smoker-moderately addicted	4	7	-	11
Smoker-heavily addicted	-	-	1	1
Total	26	12	2	40

Table 3. eCO Level (ppm) Based on CO Analyzer Category with Serum CC16 Level (ng/mL)

Characteristic	Amount (n)	eCO (ppm)	CC16 Serum (ng/mL)
Non-smoker	16	3.25±1.18	2.93±1.95
Borderline	7	8±1	2.18±0.81
Smoker-low addicted	5	11.6±1.34	4.00±1.62
Smoker-moderately addicted	11	19.36±2.37	3.54±1.84
Smoker-heavily addicted	1	28	5.56

In this study, the average level of serum CC16 in the study subjects was 3.17±1.78 ng/mL, which showed a lower value than the normal value in non-smokers of 6.4 ng/mL based on Prior study by Lomas et al.[7]

While the average carbon monoxide exhalation (eCO) is 10.18±7.42 ppm. This value is based on the CO Analyzer category of eCO levels belonging to the smoker-low addicted category. The number of eCO levels in the study subjects based on the Brinkman Index was the most in the CO Analyser eCO non-smoker category with a light Brinkman Index of 87.5% subjects. The distribution of the number of study subjects by Brinkman index degree by CO Analyzer category eCO levels is described in Table 2.

There is no significant difference between CC16 levels and Brinkman Index (*P*=0.09). There are tiny gap of CC16 levels between every degree of Brinkman Index.

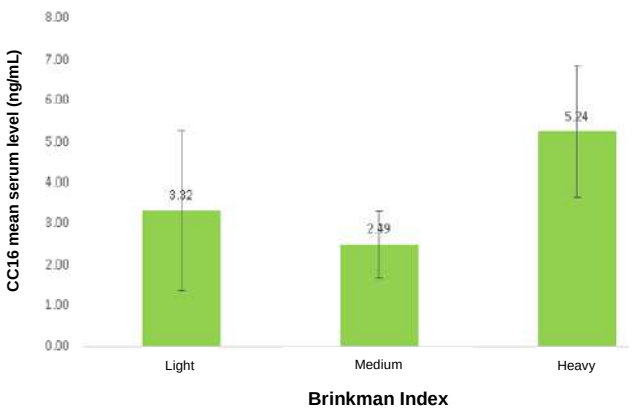

Figure 1. Mean CC16 serum levels based on Brinkman Index

Figure 2 explains the difference in serum CC16 levels to the CO Analyzer category of eCO levels. Heavily addicted smoker group had the highest serum CC16 levels, meanwhile the borderline group had the lowest level of CC16.

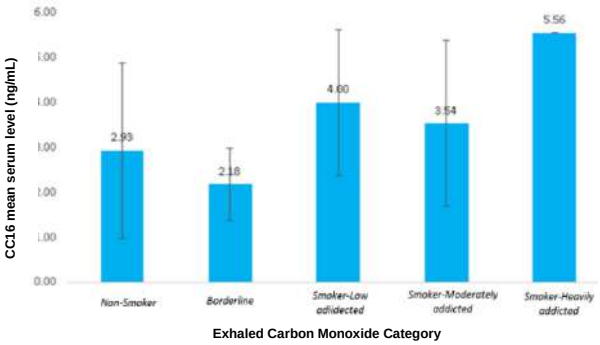

Figure 2. Mean of Serum CC16 Levels Based on Smokerlyzer Category Exhaled Carbon Monoxide (eCO) Levels

DISCUSSION

In this study, the average overall eCO level based on CO Analyzer was 10.18±7.42 ppm, including the smoker-low addicted category of 10-15 ppm.[8] The amount of carbon monoxide (CO) in cigarette smoke inhaled to the alveoli influenced by several factors, namely the type of cigarette, smoking pattern, and the depth of smoking. In addition, eCO can be affected by different environmental CO levels, especially for residents living in urban areas. The depth of smoking can affect carboxyhemoglobin (COHb) levels in a smoker and ultimately affect the expirated CO levels. This is due to tiny amount of CO diluted in

the mouth and larynx. Everytime the blood level COHb increase, the CO has reached the alveoli and diffused through the alveolar-capillary membrane to bind to haemoglobin, forming COHb. Carboxyhemoglobin values themselves have a strong correlation with eCO.[9,10] Physical activity, average ventilation, and respiration diseases such as infectious diseases can also affect eCO levels.[11]

Carbon monoxide is also produced endogenously due to the induction of Heme Oxygenase (HO), which accounts for about 85% of the body.[4] This induction is affected by stressful conditions, such as oxidative stress, hyperthermia, hypothermia, ischemia, hypoxia, inflammation, and ultraviolet (UV) light exposure.[12] Carbon monoxide produced from both endogenous and exogenous sources will go through several mechanisms: expiration, scavenging, and oxidation. These expirated air CO levels can be measured using a CO meter and applied for various clinical purposes.[8]

Based on this study, the average value of eCO levels (10–15 ppm) can be produced from exogenous and endogenous CO. Exogenous CO in the study subjects was influenced by the type of cigarette which were mostly filtered cigarette (70%) and number of cigarettes consumed measured by the Brinkman Index.

Filtered cigarettes provide filters to reduce co-production so that the level of CO that enters the respiratory tract will also be reduced. Smoking patterns and CO gases derived from the environment itself can affect the value of eCO levels in the study subjects. Endogenous CO in this study can certainly influence the value of eCO levels, namely by looking at the value of COHb (%), which correlates with eCO (ppm) values that can be measured using CO Analyzer.

The previous study also showed that the exhaled CO level in the smoker's group was higher 22 (4;48) ppm than the non-smoker gorup 5.83+1.82 ppm. Based on previous study, the results of the current research are not much different. Smoker-heavily addicted has the highest value of eCO, which is 28 ppm, smoker-moderately addicted 19.36±2.37 ppm, smoker-low addicted 11.6±1.34 ppm compared to non-smokers 3.25±1.18 ppm. The most correlated factor to CO levels in exhaled air in smokers is gender, men tend to have higher CO levels than women. The cut-off point for CO levels to determine smoking status in a person is 8 ppm with a sensitivity of 91% and a specificity of 90%.[9] From the previous, it can be concluded that exhaled carbon monoxide level was higher in smoker.

The average value of serum CC16 levels in this study was 3.17±1.78 ng/mL, indicating a lower value than the normal value in non-smokers of 6.4 ng/mL.[7] Other study by Rong et al (2020), the serum CC16 levels in COPD patients with smoking risk factors 3.10±2.23 ng/mL compared to those who had quit smoking 4.35±2.72 ng/mL. Meanwhile the serum CC16 levels in non-smokers could reach 102.5±20.3 ng/mL.[13] Research conducted by Naha et al. 2020 on ceramic workers in India, smokers with early detection of the onset of silicosis, had lower serum CC16 levels (2.6±2.72 ng/mL) compared to those, non-smokers and not exposed to silica (10.2±2.72 ng/mL).[14]

The serum CC16 levels was not affected by the severity of the Brinkman Index. The mean CC16 level on the Brinkman Index is 5.24 ± 0.45 ng/mL. However, the serum CC16 levels from this study are still below the normal value of 6.4 ng/mL according to a study by Lomas et al.[7] According study in 2018 by Lam et al., decreasing in lung function in smokers showed an insignificant relationship between serum CC16 levels and the number of cigarettes consumed packs per year (P=0.126).[15]

CC16 metabolism consists of three mechanisms. First, the increase in the permeability of the epithelial barrier in the lungs, thus causing the diffusion of CC16 into the blood. This is due to the release of vasoactive neuropeptides (tachykinins) through sensory nerves present in the respiratory tract, but the mechanism is reversible. The second is caused by a decrease in CC16 production by club cells due to chronic exposure to toxic substances, which is cigarette smoke. Third is increased creatinine clearance in the kidneys. Serum CC16 half-life is 2 to 3 hours in the serum. The study, according to Park et al, 25% variations in serum

CC16 levels do not differ between healthy subjects and smokers. Variations in CC16 serum levels can be used as specific biomarkers to the integrity of the epithelium of the airways in individuals who did not have impaired renal function.[16]

The club cell will undergo several structural changes after an hour of exposure to toxic substances. Such changes are clumping and margination of nuclear chromatin, oedema of mitochondria, and dilation of the endoplasmic reticulum. After exposure for approximately 24 hours, the club cell will enlarge, and some vacuole will approach the cell membrane. The existence of this process, the Club cell, is believed to provide host defence against toxic substances outside the body. However, the number of Club cells will significantly decrease in smoker with minimum smoking 10 packs per year.[17,18]

Research by Lam et al stated that, the result of an endobronchial biopsy followed by immunohistochemical painting after exposure to cigarette smoke for 96 hours, showed a decrease in mRNA and CC16 expression. But it will increasedagain after the exposure of cigarette smoke is eliminated.[15] Recent study by Laucho-Contreraset al al with immunostain in human bronchi in COPD sufferers, healthy subjects showed a more striking colour in healthy subjects. Similiarly, studies by Laucho-Contreraset al al, mice that have been exposed to cigarette smoke showed immunostaining is more prone.[6]

Decreasing serum CC16 levels are associated with damage to the club cell, as non-ciliated non-mucous secretory cells located in the respiratory bronchioles due to exposure to cigarette smoke. In addition, there is also damage in tracheal cells and the integrity of the pulmonary vascular barrier, and the influence of kidney cleansing. Decreasing serum CC16 levels are associated with damage to the club cell, as non-ciliated non-mucous secretory cells located in the respiratory bronchioles due to exposure to cigarette smoke. In addition, there is also damage in tracheal cells and the integrity of the pulmonary vascular barrier, and the influence of kidney cleansing. The CC16 level of the serum has the same gradient as the one in Club Cell. Even the CC16 content in the serum is 20 times lower. This is due to the permeability of the epithelium and damage to the integrity of the pulmonary vascular barrier so that CC16 can diffuse passively. The increase of Serum Protein (SP)-B, another product of club cell, also affects the increase in epithelial permeability.[13,15,17]

Cigarette smoke can increase epithelial permeability leakage in acute conditions by releasing vasoactive neuropeptides (tachykinins) through sensory nerves present in the respiratory tract, but this is reversible. While chronic conditions increase the initial damage of connective tissue alveoli.[18] Blood sampling will result in a variation in serum CC16 serum by 20% to 25%. This is likely due to circadian rhythms that affect the change of cycles in epithelial bonds in the Club cell resulting in CC16 leakage into blood vessels at certain times.[16]

The average decrease in serum CC16 levels in the study subjects was due to damage from the club cell. Exposure to cigarette smoke with an average smoking length of 15.17±9.5 years is enough to cause progressive damage. The CC16 capability produced by club cells in protecting the epithelium of the distal airway is reduced which could reduce its effectiveness. Similarly, in research subjects with a heavy Brinkman Index, although it has a higher mean value. However, the rate is still below the normal level. This may be due to the presence of a number of club cells that produce CC16 in acute conditions without causing impaired pulmonary function. This is based on anamnesis, physical examination, and normal chest x-ray from the subjects with heavy Brinkman Index not obtained abnormalities.

In this study, the entire study subjects were active smokers with a minimum smoking duration of 1 year. Elevated eCO levels in subjects were affected by the last time smoking, cigarrette type, and smoking patterns. Smokers who last smoked 2 hours earlier had a higher value than the last one who smoked 72 hours earlier. From this study, no respiratory disease could affect the value of eCO. The cause of the decrease in serum CC16 levels

caused by several factors, including exposure to cigarette smoke that cause damage in club cells, kidney cleansing in healthy subjects. The value of serum CC16 levels will also decrease rapidly due to the short half-life of serum CC16, which is 2 to 3 hours. Serum CC16 could influenced the circadian rhythms. In this study, the timing of samples obtained from each subject are different due to the availability of the subjects.

The results of this study showed that increased eCO levels would lower serum CC16 levels. Exhaled carbon monoxide and serum CC16 levels have different effects, especially the half-life. The increasing level of eCO is temporary and will decreased even the subjects has stopped smoking. While the level of serum CC16, if in active smoker is still actively smoking for a long time, will cause permanent damage to the club cell as a result, the production of CC16 will decrease even the subject has stopped smoking. However, club cells in active smokers in acute conditions can still produce CC16, but not exceed the levels in non-smokers. Based on the results of this study, the levels of eCO and serum CC16 are expected to be indicators and specific biomarkers in efforts to stop smoking.

CONCLUSION

The mean eCO levels in active smokers was 10.18±7.42 ppm (CO Analyzer smoker-low addicted category) following with low level of serum CC16 (3.17±1.78 ng/mL). This study showed the CO resulting from continuous exposure to cigarette smoke could cause damage to the club cells that produce CC16, thus resulting reduced CC16 levels.

REFERENCE

1. World Health Organization. Tobacco. World Health Organization. 2018.
2. World Health Organization. WHO. The Tobacco Atlas. 2016.
3. Kementerian Kesehatan. Rokok: Akar Masalah Jantung dan Melukai Hati Keluarga - Direktorat P2PTM. Kementerian Kesehatan. 2018.
4. Ryter SW, Choi AMK. Carbon monoxide in exhaled breath testing and therapeutics. J Breath Res. 2013;7(1):17111.
5. Herbeć A, Perski O, Shahab L, West R. Smokers' Views on Personal Carbon Monoxide Monitors, Associated Apps, and Their Use: An Interview and Think-Aloud Study. Int J Environ Res Public Health. 2018;15(2):288.
6. Laucho-Contreras ME, Polverino F, Gupta K, Taylor KL, Kelly E, Pinto-Plata V, et al. Protective role for club cell secretory protein-16 (CC16) in the development of COPD. Eur Respir J. 2015;45(6):1544–56.
7. Lomas DA, Silverman EK, Edwards LD, Miller BE, Coxson HO, Tal-Singer R. Evaluation of serum CC-16 as a biomarker for COPD in the ECLIPSE cohort. Thorax. 2008;63(12):1058–63.
8. Bedfont Scientific LTD. Micro+ smokerlyzer operating manual. Kent: Bedfont Scientific LTD; 2017.
9. Inayatillah IR, Syahrudin E, Susanto AD. Kadar Karbon Monoksida Udara Ekspirasi pada Perokok dan Bukan Perokok serta Faktor-Faktor yang Mempengaruhi. J Respirol Indones. 2014;34(4):180–90.
10. Moscato U, Poscia A, Gargaruti R, Capelli G, Cavaliere F. Normal values of exhaled carbon monoxide in healthy subjects: comparison between two methods of assessment. BMC Pulm Med. 2014;14(1):204.
11. Shie H-G, Pan S-W, Yu W-K, Chen W-C, Ho L-I, Ko H-K. Levels of exhaled carbon monoxide measured during an intervention program predict 1-year smoking cessation: a retrospective observational cohort study. NPJ Prim care Respir Med. 2017;27(1):59.
12. Queiroga CSF, Vercelli A, Vieira HLA. Carbon monoxide and the CNS: challenges and achievements. Br J Pharmacol. 2015;172(6):1533–45.
13. Rong B, Fu T, Gao W, Li M, Rong C, Liu W, et al. Reduced Serum Concentration of CC16 Is Associated with Severity of Chronic

Obstructive Pulmonary Disease and Contributes to the Diagnosis and Assessment of the Disease. Int J Chron Obstruct Pulmon Dis. 2020;15:461–70.

14. Naha N, Muhamed JCJ, Pagdhune A, Sarkar B, Sarkar K. Club cell protein 16 as a biomarker for early detection of silicosis. Indian J Med Res. 2020;151(4):319–25.
15. Lam DC-L, Kwok H-H, Yu W-C, Ko FW-S, Tam C-Y, Lau AC-W, et al. CC16 levels correlate with cigarette smoke exposure in bronchial epithelial cells and with lung function decline in smokers. BMC Pulm Med. 2018;18(1):47.
16. Park HY, Churg A, Wright JL, Li Y, Tam S, Man SFP, et al. Club cell protein 16 and disease progression in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2013;188(12):1413–9.
17. Rokicki W, Rokicki M, Wojtacha J, Dżeljijli A. The role and importance of club cells (Clara cells) in the pathogenesis of some respiratory diseases. Kardiochir Torakochirurgia Pol. 2016;13(1):26–30.
18. Robin M, Dong P, Hermans C, Bernard A, Bersten AD, Doyle IR. Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. Eur Respir J. 2002;20(5):1152–61.