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The relationship between nasal mucociliary clearance time and the degree of smoking dependence in smokers with obstructive sleep apnea syndrome

Abstract

Introduction: The aim of this study was to investigate the relationship between nasal mucociliary clearance time (NMCT), degree of smoking dependence, cumulative smoking burden and OSAS severity in smokers.

Material and methods: 123 patients (Group 1) with OSAS and 92 healthy controls (Group 2) were included in the study. Group 1 was divided into smokers (Group 1a) and non-smokers (Group 1b). In Group 1a, cumulative smoking burden and Fagerström nicotine dependence test (FNDT) were questioned. Saccharin test was applied to Groups 1 and 2. Student-t, Mann-Whitney-U, Anova, Kruskal-Wallis tests were used to compare the means.

Results: NMCT was higher in Group 1 than Group 2 ($p = 0.005$). The duration of NMCT was higher in Group 1A than Group 1B ($p = 0.002$). In Group 1a, NMCT values of mild and moderate OSAS patients were longer than in Group 1b ($p = 0.02$, $p = 0.01$, respectively). NMCT values of patients with mild dependence were shorter than those with moderate or severe dependence ($p = 0.032$, $p < 0.001$, respectively).

Conclusion: Mucociliary clearance time was higher in smokers with OSAS than non-smokers. While OSAS has a negative effect on mucociliary clearance, smoking also exacerbates the condition.

Key words: smoking, obstructive sleep apnea, nasal mucociliary clearance time, saccharine test

Adv Respir Med. 2021; 89: 353–358

Introduction

Mucociliary clearance is the most important mechanism in the protection of upper and lower airways against pathogens, foreign bodies and toxins [1]. Therefore, an effective nasal mucociliary clearance (NMC) depends on the relationship between epithelial structure integrity, ciliary stroke frequency and mucus quantity and quality [2, 3]. Disorders in these defence mechanisms are effective in the pathogenesis of the inflammation and obstruction of small airways, increased susceptibility to respiratory tract infections, lung damage, tissue repair problems and progression of chronic respiratory diseases [4].

For the evaluation of nasal mucociliary clearance, an *in vivo* technique of saccharin clearance was described by Andersen *et al.* [5] in 1974 and was modified by Rutland and Cole [6]. The mean period of clearance varies between 7 and 15 minutes, and transport time longer than 30 minutes indicates that nasal mucociliary clearance is impaired. Simple, effective and reproducible are important for clinical ease of use [5–7].

Obstructive sleep apnea syndrome (OSAS) is a sleep-related respiratory distress disorder [7] that, despite an ongoing effort to breathe, causes airflow reduction or complete cessation of relaxation of the pharyngeal muscles and narrowing or obstruction of the upper airways [8]. The preva-

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lence of OSAS is 13–33% in middle-aged men and 6–19% in women. It is thought that the prevalence will continue to increase due to the obesity epidemic in middle and high income countries [9]. OSAS disrupts the quality of life with excessive daytime sleepiness. Because of increasing cardiovascular morbidity and mortality, it is very important in terms of quality of life [10, 11].

Smoking habits and the number of cigarettes smoked per day are reported to be associated with impairment of nasal mucociliary clearance time (NMCT) [12]. Smoking was also associated with OSAS [1–3, 8, 13–15]. OSAS was found to be related with a decrease in NMCT [16]. Although the interactions between smoking, OSAS and NMCT were clear, the studies that examined NMCT values based on the degree of cigarette dependence, cumulative smoking load and OSAS severity were limited. The aim of this study was to investigate the relationship between NMCT, the degree of smoking dependence, cumulative smoking load and OSAS severity in smokers with OSAS.

Material and methods

This study is controlled, single blind, prospective study and approved by Local Ethical Committee (Ref: 2011-KAEK-25 2018/04-17).

Patients

Patients diagnosed with OSAS in our hospital between April 2018 and April 2019 were examined. All patients aged 18–65 who underwent polysomnography in the sleep laboratory of our hospital were evaluated, and all patients who agreed to participate in the study and did not meet the exclusion criteria were included in the study group (Group 1). A control group was formed according to the demographic characteristics of Group 1 from healthy individuals without OSAS symptoms and history (Group 2).

Exclusion criteria: History of chronic obstructive pulmonary disease; hypertension; hepatic, renal, rheumatological, neoplastic, infectious and endocrine diseases; chronic alcohol use or substance abuse in the past 6 months; usage of antibiotics, antihistamines, antidepressants, anticonvulsants, and antineoplastic drugs; environmental toxins exposure; abnormal ear, nose and throat physical examination (congestion, infection, chronic rhinitis, septal deviation, nasal polyposis); history of nasal surgery; head trauma; malignance; head and neck radiation or chemotherapy; or upper respiratory infections in the past 2 weeks; presence of central nervous system diseases such as Parkinson or Alzheimer diseases; psychiatric diseases affecting mental status; pregnancy and breast-feeding.

Subjects with OSAS were divided into subgroups as smokers (Group 1a) and non-smokers (Group 1B). Smoking duration, daily smoking rate, cumulative smoking load and the Fagerström nicotine dependency test (FNDT) were questioned in smokers. The control group was divided into subgroups as smokers and non-smokers (Group 2a and 2b). Smoking duration, daily smoking, cumulative smoking load and the FNDT were recorded in smokers in Group 2. A saccharin test was applied to groups 1 and 2. The doctor performing the saccharin test did not know in which group and subgroup the patients were.

Complete ear, nose, and throat examinations were performed in order to exclude any sinonasal disease (septal deviation, acute or chronic rhinosinusitis, nasal polyps, etc).

The NMCT values of OSAS in control groups and subgroups; the NMCT and AHI values of smokers and non-smokers with OSAS; the severity of dependence according to the FNDT, and the NMCT values of smokers with OSAS were compared statistically.

Saccharin test

The saccharin test was discovered by Anderson *et al.* [5] in 1974 and modified by Rutland and Cole [6] in 1980. Subjects were tested early in the morning in a quiet, well-ventilated room. After the nasal secretions were cleaned, a saccharin particle with a diameter of about 1 mm was placed in the subject's nose about 1 cm behind the anterior end of the lower turbinate. The subject was in the sitting position and the head was flexed by 10 degrees. They were asked not to eat, drink or brush their teeth beforehand and try not to cough and sneeze. The moment when the subject first felt the taste in his mouth was noted as mucociliary clearance time [5–7].

Polysomnography

Polysomnography recording simultaneously multiple physiological parameters related to sleep is the gold standard for diagnosis of OSAS [17]. Polysomnography (PSG) was performed using a 58-channel polysomnography device (Compumedics E-Series) on all patients with 4-channel EEG (Electroencephalography), Chin EMG (electromyography), Leg EMG, ECG (electrocardiography), EOG (electrooculography), pulse oximetry, air flow, the combination of thoracic and abdominal respiratory inductance plethys-

Table 1. Smoking data, Polysomnography and NMCT values of Groups 1a and 2a

	Group 1a (n = 58)	Group 2a (n = 37)	p value
Daily cigarettes, median (IQR)	20 (13)	12 (10)	0.99[a]
Cumulative smoking story (package/year), median (IQR)	19 (25.25)	12 (10)	0.83[a]
Fagerström Testi, median (IQR)	6.5 (5)	5 (3)	0.65[a]
Dependence, n (%)			0.24[b]
Mild	18 (31)	15 (40.5)	
Moderate	21 (36.2)	16 (43.2)	
Severe	19 (32.8)	6 (16.2)	
NMCT [s] median (IQR)	728.5 (622)	660 (240)	0.042[a]*
Mean oxygen saturation [%] median (IQR)	93 (2.25)	94 (2)	0.004[a]*
Oxygen desaturation index [%] ≥ 4 (ODI) median (IQR)	16 (24)	17.5	0.27[a]

[a] Mann Whitney-U test; [b] Chi-Square test; * $p < 0.05$. IQR — interquartile rang; NMCT — nasal mucociliary clearance time;

mography (RIP) to provide an accurate representation of the respiratory effort, and snore detecting microphones. The PSG records were evaluated by the specialist doctors certified by the Health Ministry of Turkey, according to the AASM Manual for the Scoring of Sleep and Associated Events Version 2.0. The mean oxygen saturation, Apnea-Hypopnea Index (AHI) and oxygen desaturation index % ≥ 4 (ODI) were evaluated while sleeping.

Apnea-Hypopnea Index

The APNEA-HYPOPNEA INDEX (AHI) is the combined average number of apneas and hypopneas that occur per hour of sleep. If the AHI was 5 or more per hour, OSAS was diagnosed. AHI was categorized as mild OSAS with 5–15/hour, moderate with 15–30/hour and severe with > 30/hour [18, 19].

Fagerström test

The Fagerström test (FNDT) is used to measure nicotine dependence and consists of 6 questions [19, 17]. Physical nicotine dependence is scored on a scale of 1-10 based on the responses of the patient. Subjects were divided into 3 subgroups according to their FNDT scores: mildly dependent (1–4 points), moderately dependent (5–7 points) and severely dependent (8–10 points).

Cumulative smoking load (pack/year)

A pack year is the quantification of cigarette smoking [20, 18]. It is calculated by multiplying the number of cigarette packs smoked per day by the number of years the person has smoked.

Statistical analysis

Statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 23 program. The Kolmogorov-Smirnov test analysed the distribution of the groups. Normally distributed numerical values mean ± standard deviation, distribution of non-normal numerical values median (interquartile range, IQR); categories were evaluated with percentage ratios. A Student t-test and Mann-Whitney-U test were used for comparison of means. An Anova and Kruskal-Wallis test were used in comparison of means of multiple groups. A Chi-Square test was used to compare the ratios. A Spearman correlation test was used for the evaluation of the correlations. $P < 0.05$ values were considered statistically significant.

Results

The study included 123 subjects with OSAS (Group 1) and 92 healthy controls (Group 2). There were no statistically significant differences between the two groups in terms of age, sex and smoking rates ($p = 0.69$, $p = 0.74$, $p = 0.31$, respectively). Subjects with OSAS had a significantly higher NMCT than the control group ($p = 0.005$). The number of cigarettes smoked per day, cumulative smoking history, FNDT and NMCT values in Group 1a and Group 2a were shown in Table 1.

There was no statistically significant difference between the groups in terms of daily smoking, cumulative smoking history, the Fager-

Table 2. Values according to OSAS severity in Group 1

	Mild OSAS n = 27	Moderate OSAS n = 51	Severe OSAS n = 45	P-value
Age [years], mean ± standard deviation	49.1 ± 11.6	49.5 ± 9.2	45.9 ± 9.4	0.2[a]
NMCT [s] median (IQR)	670 (347)	600 (457)	597 (454)	0.55[b]
Number of cigarettes per day, median (IQR)	10 (12.5)	20 (21.25)	10 (16)	0.31[b]
Cumulative smoking history [pack/year], median (IQR)	18 (20.5)	23.5 (31.75)	10 (22)	0.55[b]
Fagerström test, median (IQR)	6 (3.5)	7 (5.5)	5 (6.5)	0.66[b]
Gender (male), n (%)	20 (76.9)	36 (70.6)	36 (80)	0.55[c]
Smoking rate, n (%)	17 (65.4)	20 (9.2)	21 (46.7)	0.09[c]

[a]Anova test; [b]Kruskall-Wallis test; [c]Chi-square test. OSAS — obstructive sleep apnea syndrome; NMCT — nasal mucociliary clearance time; IQR — interquartile range

ström test and addiction severity. The NMCT of smokers with OSAS was statistically significantly longer than the smokers without OSAS. The data of the patients with OSAS who were smokers and non-smokers were compared (Group 1a and Group 1b). The duration of NMCT in smokers was statistically significantly longer than non-smokers ($p = 0.002$). The mean age was significantly higher in Group 1b ($p < 0.001$). There was no significant difference between patients with OSAS who smokers and those were who were non-smokers in terms of gender, AHI and ODI ($p = 0.13$, $p = 0.97$ and $p = 0.27$ respectively).

Gender, age and NMCT were significantly different among subgroups of smokers OSAS according to the severity of nicotine dependence ($p = 0.08$, 0.08 and 0.004, respectively). AHI did not make a statistical difference ($p = 0.49$). When NMCT was found to be significantly different between the three subgroups, the subgroups were also compared in pairs. NMCT of patients with mild dependence was significantly shorter than those with moderate or severe dependence. When the NMCT was found to be significantly different between the three subgroups, the subgroups were also compared in pairs. The NMCT of the patients with mild dependency was significantly shorter than those with moderate or severe dependence ($p = 0.032$, $p < 0.001$, respectively). There was no significant difference between moderate and severe dependent groups ($p = 0.29$).

There was no statistically significant difference in the NMCT between patients who were divided into three groups according to OSAS severity (AHI value) as mild, moderate and severe (Table 2). The NMCT in patients with mild OSAS was statistically significantly longer than the control group ($p = 0.048$). However, no significant difference was found between moderate and severe OSAS and the control group ($p = 0.27$ and $p = 0.44$, respectively).

In Table 3, we compared the NMCT of smokers and non-smokers in mild OSAS, moderate OSAS and severe OSAS subgroups. The NMCT values of smokers in mild and moderate OSAS were significantly longer than non-smokers. There was no significant difference in severe OSAS.

Again, no significant difference was found between these subgroups in terms of age, daily smoking, cumulative smoking load and the FNDT ($p = 0.65$, $p = 0.35$, $p = 0.68$, $p = 0.71$, respectively).

Patients' smoking status, daily smoking, cumulative smoking history, and the FNDT were correlated with NMCT (*sırasıyla*, $p = 0.001$, 0.007, 0.014, 0.001 *vs* $p = 0.287$, 0.351, 0.321, 0.405). There was no correlation between NMCT and OSAS severity ($p = 0.33$).

Discussion

There are studies investigating the relationship between smoking and mucociliary clearance in literature. Studies have shown that long-term smoking causes structural and functional changes in the respiratory system. In addition, epithelial remodelling develops as a result of smoking.

Table 3. Nasal mucociliary clearance time values [s] of smokers and nonsmokers in obstructive sleep apnea syndrome (OSAS) subgroups

	Smokers	Nonsmokers	P-value
Mild OSAS (n = 27)	713 (402)	543 (298)	0.02*
Moderate OSAS (n = 51)	891 (598)	569 (310)	0.01*
Severe OSAS (n = 45)	608 (557)	584 (407)	0.43

*P < 0.05; IQR — interquartile rang

The number of goblet cells increases, and hypertrophy occurs. Silia structure and function of respiratory tract is impaired. An increase in mucus production and a decrease in mucociliary activity result in impaired mucociliary clearance [4, 21].

In various studies, the relationship between smoking and OSAS has been mentioned. Kim et al reported that AHI values of male smokers with OSAS were higher than patients with non-smoker OSAS [13]. In a cohort study, the rate of moderate and severe OSAS was found to be higher in smokers than non-smokers [14]. In our study, there was no statistically significant difference between AHI values of smoking and non-smoking OSAS patients. Cigarette smoke contains a wide range of compounds such as chemicals, heavy metals, free radicals and nicotine and has been proposed as a risk factor in OSAS [22]. Mechanisms that explain how smoking may cause OSAS include: (A) Changes in sleep architecture, (B) Neural reflexes caused by nicotine relaxation, (C) Upper respiratory tract muscles relaxation, and (D) Increased awakening threshold in sleep induced by nicotine[8], increased upper airway inflammation due to inhalation [15].

The most important finding of our study is that there is a significant difference in the NMCT values of the smokers with OSAS according to the subgroup of smoking dependency severity. NMCT values of patients with mild cigarette addiction were shorter than those with moderate and heavy cigarette addicts. No significant difference was found between AHI and subgroup of addictive severity. We believe that our study is important in terms of comparing NMCT and AHI values in OSAS patients according to the severity of smoking dependence. Deniz *et al.* found no significant difference in NMCT in patients with mild to moderate OSAS compared to the control group, but reported that NMCT was significantly prolonged in patients with severe OSAS [16]. In our study, there was no difference in NMCT between subgroups according to OSAS severity. There are not many studies on the effect of OSAS on the upper respiratory tract. Schrodter *et al.* [23] reported that atrophic epithelium is common in untreated OSAS and ciliary epithelial types are rare. Oxygen desaturation occurs in the nasal mucosal tissue as a result of obstruction in the upper airways. As a result, ultrastructural changes occur and mucociliary clearance is impaired [16]. The NMCT of smokers with OSAS was statistically significantly longer than that of the smokers without OSAS. This finding supported the negative impact of not only smoking but also OSAS on NMCT.

Deniz *et al.* [16] found a statistically significant difference between smokers and non-smokers in terms of mucociliary clearance times in all OSAS groups. In our study, NMCT was significantly longer in smokers with mild and moderate OSAS than in non-smokers. However, no significant difference was found in severe OSAS. Although there was no significant difference in age, cumulative cigarette load, severity of smoking dependency and daily smoking among the subgroups, it was surprising that there was no significant difference between smokers and non-smokers in severe OSAS.

Patients with OSAS were correlated with non-smoking status, daily number of cigarettes, cumulative smoking history and Fagerström dependency test. In this respect, our study supported previous studies [24–26].

Limitations of our study can be listed as follows: Saccharin test was a subjective test due to the patient feeling the taste of saccharin and was considered as a test result when they said that they received the sensation of taste. In addition, since we divided the patient group into three subgroups according to the severity of OSAS and smoking dependence, the sample size of these subgroups was relatively small.

Conclusion

In our study, mucociliary clearance time was significantly higher in smokers with OSAS compared to non-smokers. While OSAS is al-

ready adversely affecting mucociliary clearance, smoking also exacerbates the situation. Smoking in OSAS patients eliminates a very important defence mechanism such as mucociliary clearance of the lungs.

Conflict of interest

None declared.

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Risk factors for complicated community-acquired pneumonia course in patients treated with β-lactam monotherapy

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ABSTRACT

Introduction: We aimed to investigate community-acquired pneumonia (CAP) requiring hospitalisation, empirically treated with β-lactam monotherapy, with 30-day mortality and risk factors predicting its complicated course.

Material and methods: A prospective observational study was conducted at the Pulmonology and Allergology Department in a tertiary care university hospital. 253 consecutive patients diagnosed with CAP requiring hospitalisation were enrolled. Hospital admission was based on PSI or CRB-65 scores, severe comorbidities, signs of intoxication, aspiration risk, social risk considerations, ineffective prior antibiotic treatment.

Results: Fourty seven percent of the subjects had complications on admission, 13% developed new CAP complications during inpatient treatment. Overall, 53% of individuals had a complicated CAP course. 30-day mortality rate was 5.9%. The factors predicting a complicated CAP course were as follows: neuromuscular disease, multilobar opacities on chest X-ray (or computed tomography), and clinically unstable condition as evaluated using Halm's criteria.

Conclusions: The mortality rate in CAP patients treated with β-lactam monotherapy is low. Neuromuscular disease, multilobar opacities, and clinically unstable condition as evaluated using Halm's criteria predict a complicated CAP course.

Key words: community-acquired pneumonia, β-lactam monotherapy, mortality, complications

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Introduction

Community-acquired pneumonia (CAP) is the deadliest communicable disease and a major cause of morbidity and mortality worldwide [1]. Regardless of the progress in medical science, better health-care access, including specialised units, CAP prevention, pneumonia mortality still accounts for over 30% of all respiratory disease mortality rates [2]. In most cases of CAP, the patients recover completely, however, a part of them develop a complicated disease course which is linked to increased mortality from 11% to 24% [3]. Parapneumonic effusion is the most common pulmonary CAP complication affecting 20–40% of hospitalised patients [4]. Other frequent complications include empyema, lung abscess, acute respiratory failure and sepsis. There is an established link between complicated CAP course and an increased risk of prolonged hospitalisation, and 30-day mortality [5].

Streptococcus pneumoniae is the leading cause of death in severe CAP [6]. Therefore, timely and appropriate antibiotic management is the foundation for CAP treatment. It should be started empirically and guided by regional treatment recommendations and local microbial antibiotic resistance patterns. Based on the 2019 World Health Organization (WHO) Model List of Essential Medicines, amoxicillin is recommended as the first-choice therapy for CAP [7]. Reported *Streptococcus pneumoniae* penicillin resistance is relatively low in several countries, including Lithuania, where the rates are up to 2% in 2015–2018 [8]. Taking CAP aetiology and local antimicrobial resistance patterns as well as the long-term CAP treatment outcomes data into account, the Lithuanian guidelines for adults' pneumonia

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diagnostics and treatment were published first in 2006 and later updated in 2016. In both versions β-lactam monotherapy is still recommended as the first-choice inpatient CAP treatment [9, 10].

In agreement with the WHO model, several national CAP guidelines outside the USA also list amoxicillin as first-choice antibacterial treatment [11, 12]. In 2019, ATS and IDSA published the updated CAP guidelines [13] where broad-spectrum and combination antibiotic therapy remains to be recommended for CAP inpatient treatment.

The objective of the study is to investigate CAP treated with β-lactam monotherapy, 30-day mortality and risk factors predicting complicated CAP course.

Material and methods

Study design and population

We have conducted a prospective observational study at the Pulmonology and Allergology Department of Vilnius University Hospital Santaros Klinikos in Vilnius, Lithuania from July 2015 until May 2018. 253 consecutive patients diagnosed with CAP requiring hospitalisation were enrolled. We included all adults with clinical symptoms compatible with pneumonia (fever, cough, dyspnoea, chest pain, sputum production) and the presence of new opacities on chest X-ray or computed tomography. Patients with inherited or acquired immunodeficiency or drug-induced neutropenia were not included in the study. Figure 1 depicts the study design.

Data collection, evaluation and outcomes

The data included clinical symptoms, pre-existing conditions, pneumonia complications on admission, clinical stability evaluation using Halm's criteria [14], and initial antibacterial treatment (Table 1).

A chest X-ray or computed tomography was performed on admission and repeated at least once during the course of the treatment to evaluate the resolution or deterioration of pneumonia or pleural effusion. Inflammatory markers (C-reactive protein levels and white blood cell count) were repeatedly tested during the course of treatment. Pneumonia severity was quantified using PSI/PORT (Pneumonia Severity Index) [15] and CRB-65 (confusion, respiratory rate, blood pressure and age ≥ 65 years) [16] scores.

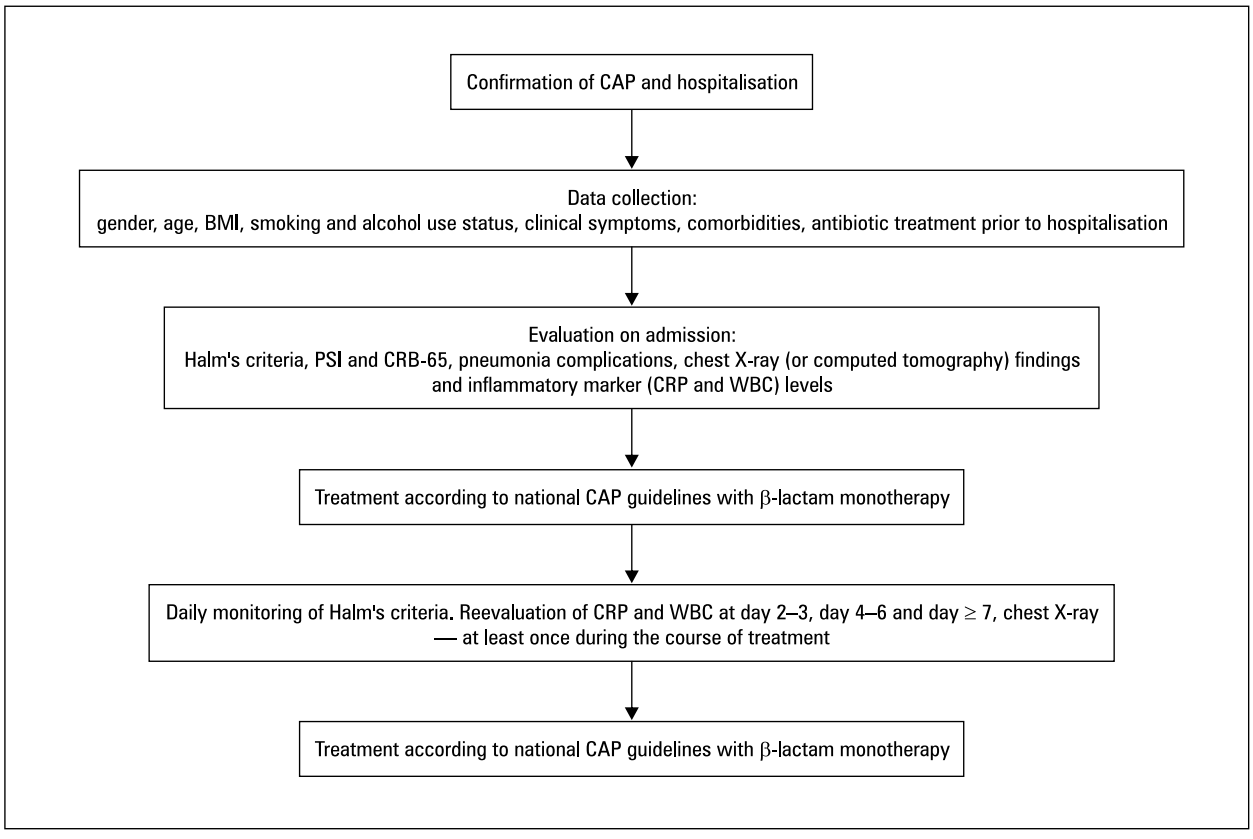

Figure 1. Study design. BMI — body mass index; CAP — community-acquired pneumonia; CRB-65 (confusion, respiratory rate, blood pressure, age ≥ 65 years); CRP — C-reactive protein; PSI — Pneumonia Severity Index; WBC — white blood cell count

Table 1. Baseline characteristics

Characteristics	N = 253
Gender, male	159 (63)
Gender, female	94 (37)
Age, years	57 (± 19)
PSI class	
PSI — I*	47 (19)
PSI — II*	80 (31)
PSI — III	63 (25)
PSI — IV	53 (21)
PSI — V	10 (4)
CRB-65 score	
CRB-65 — 0*	132 (52)
CRB-65 — 1	82 (33)
CRB-65 — 2	36 (14)
CRB-65 — 3	3 (1)
Smoking	144 (57)
Alcohol abuse	25 (10)
Obesity (BMI > 30 kg/m2)	33 (13)
Malnutrition (BMI < 18.5 kg/m2)	13 (5)
Prior antibiotic treatment	91 (36)
Multilobar opacities (on chest X-ray or CT)	95 (37)
Comorbidities	126 (50)
Diabetes mellitus	16 (6)
COPD	27 (11)
Asthma	10 (4)
Bronchiectasis	13 (5)
CHD	76 (30)
Neuromuscular disease	19 (8)
Malignancies	32 (13)
Polymorbidity	50 (20)
Symptoms	
Dyspnoea at rest	98 (39)
Dyspnoea at exertion	153 (61)
Pleuritic chest pain	123 (49)
Cough	193 (76)
Sputum production	108 (43)
Malaise	230 (91)
Confusion	46 (18)
Haemoptysis	36 (14)
Complications on admission	120 (47)
Respiratory failure**	77 (30)
Parapneumonic effusion	49 (19)
Lung abscess	9 (4)
Sepsis	6 (2)
Empyema	5 (2)
Septic shock	3 (1)
Halm's criteria	
Temperature ≤ 37.2°C	121 (48)
Respiratory rate ≤ 24 times/minute	223 (88)
Heart rate ≤ 100 beats/minute	207 (82)
Systolic blood pressure ≥ 90 mm Hg	232 (92)
Arterial oxygen tension ≥ 60 mm Hg or oxygen saturation ≥ 90%	180 (71)
Initial antibacterial treatment	
β-lactam monotherapy	244 (96)
Fluoroquinolone monotherapy***	7 (3)
Antibiotic combinations****	2 (1)

Data are presented as n (%) or mean (SD), unless otherwise stated. *Hospital admission was based on severe comorbidities, advanced age, initial treatment failure. **Confirmed with arterial blood gas test showing PaO_2 of < 60 mm Hg on room air with/without $PaCO_2$ of > 50 mm Hg. Fluoroquinolone monotherapy was used in cases of confirmed or suspected allergic reactions to β-lactams or suspected *Legionella pneumophila* aetiology. Combination therapy with vancomycin was only used in 2 cases where *Staphylococcus aureus* aetiology was suspected.
BMI — body mass index; CAP — community-acquired pneumonia; CHD — coronary heart disease; COPD — chronic obstructive pulmonary disease; CT — computed tomography

All subjects were treated according to the national guidelines for CAP. 96% of patients received β-lactam monotherapy for initial empiric CAP treatment. To investigate the implications for 30-day mortality and factors predicting a complicated CAP course, the group of patients who developed CAP complications over the course of inpatient treatment (n = 32) were compared to individuals who had complication-free course of CAP (n = 118) (Figure 2).

Statistical analysis

We performed the analysis using SPSS software. Categorical variables were expressed as numerical values (percentage) and continuous variables as median (standard deviation (SD)). Data was checked for normality of distribution with the Shapiro-Wilk test. Student's t-test and Mann-Whitney U test were used for comparisons of continuous data. Categorical variables were analysed with the chi-square test. A bivariate analysis was made to identify risk factors significantly associated with CAP complications. Covariates reaching significance from the bivariate analysis were included in the multivariate model. Multivariate logistic regression was performed with CAP complications as the dependent variable and the results reported as odds ratios (ORs) and 95% confidence intervals (95% CIs). A p-value of < 0.05 was considered statistically significant.

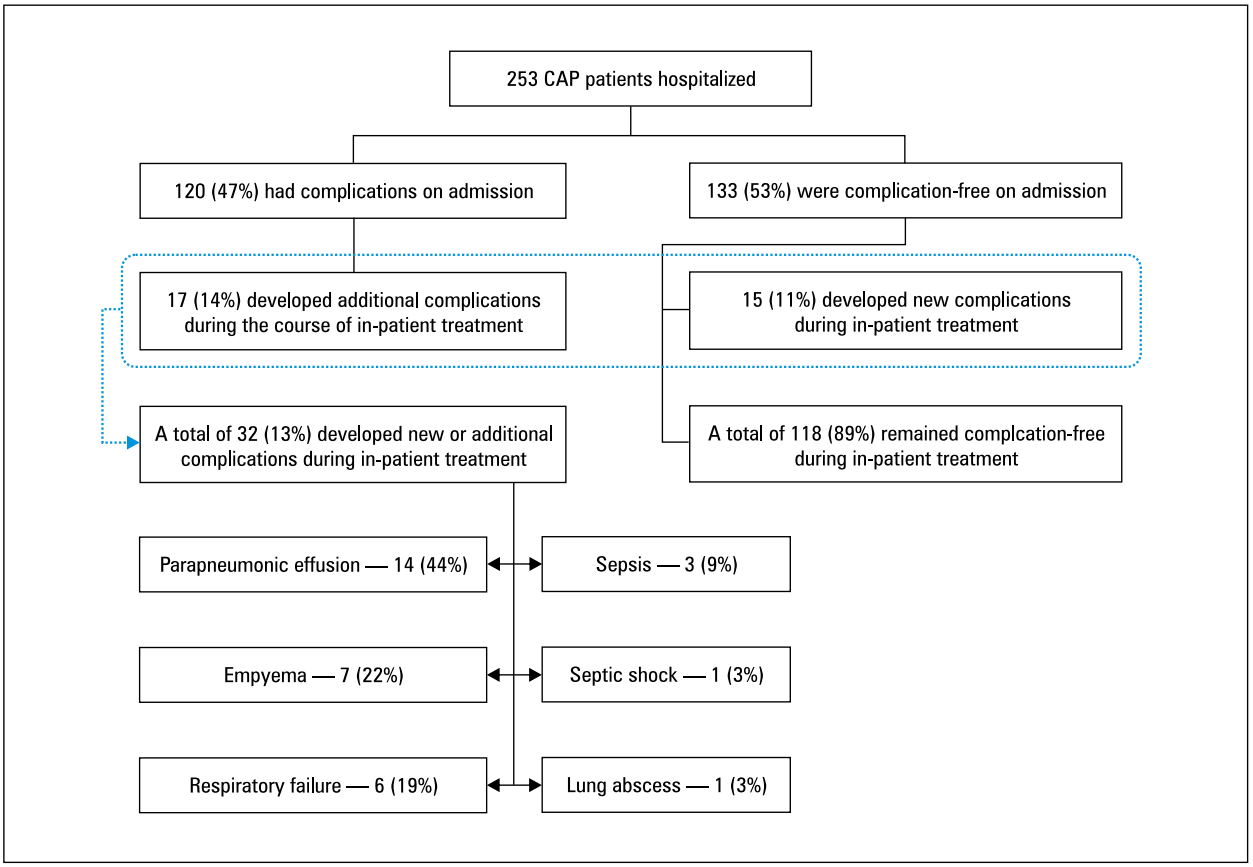

Figure 2. Patient selection for comparison groups and CAP complications developed during the course of inpatient treatment. CAP — community acquired pneumonia

RESULTS

Thirty two (13%) patients developed new CAP complications during inpatient treatment (Figure 2). Over a half of the new complications were parapneumonic effusion or empyema.

Detailed comparison between the patients who developed CAP complications over the course of treatment and those who had complication-free course of CAP is displayed in Table 2.

We found the following clinical symptoms to be significantly associated with complicated CAP course: dyspnoea at rest (50% vs 29%) and exertion (72% vs 52%), and pleuritic chest pain (66% vs 42%). Clinical stability as evaluated using Halm's criteria also proved to be associated with CAP complications. In the complicated CAP group, there was significantly higher percentage of clinically unstable patients than in complication-free CAP group (91% vs 58%).

Radiological evaluation contributes to CAP complications. Only 20% of those with complication-free CAP course had multilobar involvement in contrast with 66% of those with CAP complications. Furthermore, the rate of COPD, neuromuscular diseases, and polymorbidity were all significantly different at 5% level in the comparison groups.

Therefore, we conclude that dyspnoea and pleuritic chest pain, clinically unstable condition as evaluated using Halm's criteria, multilobar opacities and comorbidities are associated with (or contribute to) CAP complication development. However, after multivariate analysis only neuromuscular diseases, multilobar opacities on chest X-ray (or computed tomography) and clinically unstable condition as evaluated using Halm's criteria were identified as independent CAP complication risk factors (Table 3).

Time to radiological resolution was significantly longer for patients who developed CAP complications during in-hospital treatment. The average in-hospital stay was 9 (± 5) days. Patients with CAP complications required longer inpatient treatment (13 [± 8] vs 8 [± 3] days).

There was no significant difference regarding median CRP or WBC levels on admission or at day 2–3 between the patient groups who developed and did not develop CAP complications. However, CRP and WBC values were detected significantly

Table 2. Comparison between the patients who developed community-acquired pneumonia (CAP) complications over the course of treatment and those who had complication-free course of CAP

Characteristics	Complicated CAP course (n = 32)	Complication-free CAP course (n = 118)	P-value
Gender, male	21 (66)	66 (56)	0.420
Gender, female	11 (34)	52 (44)	0.420
Age, years	57 (±18)	55 (±20)	0.619
PSI class IV–V	9 (28)	26 (22)	0.485
CRB-65 score 2–4	4 (13)	14 (12)	1.000
Smoking	18 (56)	60 (51)	0.691
Alcohol abuse	5 (16)	6 (5)	0.057
Obesity	2 (8)	15 (16)	0.518
Malnutrition	2 (8)	6 (7)	0.679
Symptoms			
Dyspnoea at rest	16 (50)	34 (29)	0.034
Dyspnoea at exertion	23 (72)	61 (52)	0.046
Pleuritic chest pain	21 (66)	49 (42)	0.017
Cough	27 (84)	88 (75)	0.346
Sputum production	15 (47)	44 (37)	0.415
Malaise	31 (97)	106 (90)	0.301
Confusion	8 (25)	15 (13)	0.101
Haemoptysis	4 (13)	14 (12)	1.000
Comorbid conditions			
Diabetes mellitus	2 (6)	5 (4)	0.641
Bronchiectasis	4 (13)	5 (4)	0.098
Asthma	3 (9)	3 (3)	0.112
COPD	7 (22)	7 (6)	0.012
CHD	11 (34)	31 (26)	0.381
Malignancies	4 (13)	19 (16)	0.785
Neuromuscular disease	9 (28)	4 (3)	0.000
Polymorbidity	12 (38)	15 (13)	0.003
Unstable as evaluated using Halm's criteria			
Temperature > 37.2°C	20 (63)	65 (55)	0.548
Respiratory rate > 24 times/minute	3 (9)	4 (3)	0.167
Heart rate > 100 beats/minute	3 (9)	15 (13)	0.765
Systolic blood pressure < 90 mm Hg	1 (3)	9 (8)	0.690
Arterial oxygen tension < 60 mm Hg or oxygen saturation < 90%	15 (47)	0 (0)	NA
Clinically stable (as evaluated using all Halm's criteria)	3 (9)	50 (42)	0.000
Multilobar opacities (on chest X-ray or CT)	21 (66)	23 (20)	0.000
Complete radiological resolution	10 (31)	64 (54)	0.028
Time to radiological resolution, days	9 (± 4)	6 (± 3)	0.030
Inpatient stay, days	13 (± 8)	8 (± 3)	0.000
30-day mortality	5 (15.6)	3 (2.5)	0.011

Data are presented as n (%) or mean (SD), unless otherwise stated; CCHD — coronary heart disease; COPD — chronic obstructive pulmonary disease, CT — computed tomography

Table 3. Adjusted odds ratio (OR) and 95% onfidence intervals (CI) for independent predictors of community-acquired pneumonia (CAP) complication risk

Predictors of CAP complication risk	OR	95% CI	P-value
Neuromuscular disease	20.440	3.026–138.083	0.002
Multilobar opacities (on chest X-ray or CT)	7.028	2.068–23.888	0.002
Clinically unstable (as evaluated using Halm's criteria)	5.422	1.082–27.174	0.040

CAP — community-acquired pneumonia; CT — computed tomography

Table 4. C-reactive protein (CRP) and white blood cell (WBC) count levels during the course of treatment in the comparison groups

CRP and WBC levels	Complicated CAP (n = 32)	Complication-free CAP course (n = 118)	P-value
CRP on admission, mg/L	196.1 (± 83.8)	190.4 (± 106.2)	0.785
CRP at day 2–3, mg/L	160.6 (± 100.7)	142.3 (± 90.4)	0.451
CRP at day 4–6, mg/L	128.8 (± 75.3)	71.6 (± 66.9)	0.001
CRP at day ≥ 7, mg/L	90.7 (± 69.8)	38.0 (± 37.0)	0.000
WBC on admission, x 10^9/L	12.0 (± 4.8)	11.6 (± 5.9)	0.725
WBC at day 2–3, x 10^9/L	9.1 (± 2.5)	8.8 (± 4.2)	0.764
WBC at day 4–6, x 10^9/L	9.2 (± 3.1)	8.3 (± 3.1)	0.262
WBC at day ≥ 7, x 10^9/L	9.9 (± 3.4)	7.7 (± 3.1)	0.020

Data are presented as mean (SD); CAP — community-acquired pneumonia; CRP — C-reactive protein; WBC — white blood cell count

higher in patients with CAP complications later during the treatment course (day 4–6, day ≥ 7) (Table 4). ROC curves were constructed to assess the discriminatory power of CRP and WBC levels over the course of treatment in identifying people with CAP complications. The areas under the curve (AUC) for CRP at day 4–6, CRP at day ≥ 7 and WBC at day ≥ 7 were respectively: 0.726; 0.732 and 0.703.

Overall, 30-day mortality rate was 5.9% (n = 15). Patients who developed new CAP complications during in-hospital treatment had 15.6% mortality rate, whereas those who had complication-free course of CAP — 2.5%. CAP complications substantially increased mortality risk (RR = 7.099; 95% CI, 1.598–31.544).

For each patient who died all the medical records were thoroughly reviewed to establish the contributions of CAP to death. High comorbidity burden, poor functional reserve and advanced age were major contributors to mortality in two-thirds of the patients. CAP was judged to be the direct cause of death in one-third (progressing respiratory failure, cardiopulmonary arrest prior to stabilisation of CAP, etc.).

Discussion

The main findings of the study are as follows. First, the mortality rate in CAP patients treated according to national guidelines with β-lactam monotherapy is relatively low. Second, CAP complications significantly increase mortality risk. Third, multilobar radiological involvement, concomitant neuromuscular disorder and altered vital signs as characterised using Halm's criteria were independent risk factors for CAP complications. Below, we discuss our findings regarding CAP complications, specifically, the implications of multilobar involvement and comorbidities, as well as our CAP mortality outcomes with considerations for antibacterial treatment choices.

We have identified multilobar opacities as a significant independent risk factor for CAP complications. There have been studies demonstrating a link between bilateral radiographic CAP infiltrates and unfavourable disease outcomes [17]. The predictive value of multilobar radiographic involvement is well recognised and therefore has been incorporated both into SMART-COP pneumonia scoring system [18]

and the 2007 IDSA/ATS criteria for defining severe CAP [13]. In their systemic review for the prognosis of multilobar pneumonia, Mannu *et al*. have concluded that multilobar radiographic involvement is an independent risk factor for CAP mortality and there also might be an association between multilobar opacities and complicated disease recovery or need for intensive care [19]. Our findings coincide with earlier research — we have demonstrated a sevenfold increase in CAP complication risk in patients with multilobar opacities. While the exact mechanism is unknown, multilobar infiltration is thought to be influenced by both the invasive features of the causative microbe and the host's inflammatory response to the infection. In a study by Cillóniz *et al*., multilobar opacities are regarded as a separate pulmonary CAP complication [20].

An elevated respiratory failure risk in individuals with neuromuscular comorbidities is quite well established — the patients with neuromuscular disorders develop respiratory muscle weakness, which in turn causes hypoventilation, steadily progressing and causing respiratory failure [21]. A study in Hong Kong analysing a group of patients with motor neuron disease revealed pneumonia as the major cause of death in 54.8% and respiratory failure in 40.5% of the subjects [22]. Interestingly, patients with neuromuscular disorders in our study had an elevated risk not only for respiratory failure but also for other CAP complications, mechanisms for which are most likely multifaceted and overlapping.

Our study demonstrated a relatively low 30-day mortality rate (5.9%). Even lower mortality rate was recorded in the complication-free patient group (2.5%). Numerous previous studies have shown varying mortality results ranging from 3.4% to 26.8% [23]. Waterer *et al*. (2018) were investigating CAP in-hospital deaths and have found CAP to be the direct cause of death in about half (51.9%) of their patients [24]. In our study, after conducting a manual case-by-case analysis of each CAP death, we found that only one-third was caused directly by CAP. Whereas, in two-thirds of cases, death was linked to older age, severe comorbidities and frailty. Host factors contribute decisively to outcomes of infectious diseases, and CAP is no exception. The population of older adults is growing by 2% each year [25] and, in part because of ageing population, the prevalence of chronic non-communicable diseases and disability increases. People at the advanced age and with severe or multiple long-term conditions have a higher general vulnerability to acute health threats such as CAP [26]. Higher Charlson Comorbidity Index scores are associated with higher risk of in-hospital mortality and aid in predicting pneumonia outcomes [27].

Risk factors such as age and long-standing severe chronic illnesses have long been associated with increased CAP mortality, and it appears that in some cases, these non-modifiable risk factors determine the course of CAP while antibiotic choice has a minor role in overall disease outcomes, meaning that some of CAP deaths realistically may not be preventable [24]. This might also partly explain the high variability in reported CAP mortality rates [23].

CAP mortality risk has mostly been investigated in clinical studies analysing different antimicrobial treatment regimens. Overall, pneumococcal CAP mortality rates seem to not have changed significantly over the past 20 years. Consequently, the lack of decreased mortality with increasing widespread use of broad-spectrum antibiotic regimens might support the notion that most culture-negative CAP is not caused by drug-resistant pathogens [28]. In their cohort study, Webb *et al*. (2019) have shown that 39.7% of patients received broad-spectrum antibiotics, but drug-resistant pathogens have been recovered in only 3%. Moreover, a broad-spectrum antibiotic use for CAP may be associated with poor clinical outcomes – higher mortality, longer hospital stay, higher cost and increased risk of *Clostridioides difficile* infection [29]. A fairly recent large multi-centre cluster-randomised trial in the Netherlands supports β-lactam monotherapy as an equivalent to β-lactam-macrolide combination or fluoroquinolone monotherapy with regard to 90-day mortality [30]. Given concerns over increasing drug resistance (macrolides) and safety issues (macrolides, fluoroquinolones), there is a need for measured decision choosing CAP treatment. In the 2017 Essential Medicines List (EML), WHO classifies antibiotics into Access, Watch, and Reserve (AWaR) groups, to improve prescribing decisions and guide antibiotic use for common clinical infections [31]. Recognising the need to stop the inappropriate use of antibiotics, the EML Committee recommends an extension of the AWaRe classification and assumes that most respiratory tract infections can be treated with Access antibiotics [7].

The current IDSA/ATS CAP guidelines have been updated in 2019 [13], and in this revision, the recommended antibiotic choices do not differ significantly from those listed in previous versions. CAP guidelines have also been developed

in other countries outside the USA [11, 12, 32, 33]. Most of CAP guidelines can be divided into two groups according to the recommended first-line antibiotics for hospitalised patients: those in line with the IDSA/ATS (macrolide combination with β-lactams) or those in line with the Northern European (β-lactam monotherapy) CAP guidelines [35]. The principal justification for recommending macrolide and β-lactam combination is coverage of atypical pathogens (*Mycoplasma*, *Chlamydophila*, and *Legionella*). However, there is a worrying lack of epidemiological data regarding atypical CAP pathogens and an unsatisfactory standardisation of testing techniques [36]. On the contrary, the β-lactam monotherapy recommendation is generally based on the substantial prevalence of CAP caused by *Streptococcus pneumoniae*, where atypical pathogen coverage is only used for patients with specific risk factors or failure to achieve clinical stability with β-lactam therapy. There is a growing concern that a lot of guidelines developed by scientific societies and professional associations recommending empirical antibiotic use (including IDSA/ATS CAP guidelines) do not routinely consider antimicrobial resistance in their choices [37].

The Lithuanian national guidelines propose initiating the treatment with β-lactam monotherapy for CAP hospitalised patients and using macrolides or fluoroquinolones only in cases of suspected *Legionella pneumophila* aetiology or whenever a patient has contraindications to β-lactams [10]. In our study, in-hospital treatment was started with β-lactams for 96% of patients. Ongoing national as well as local (our hospital's) antibiotic resistance monitoring programmes demonstrate that β-lactam monotherapy remains an effective first-choice therapy option for inpatient CAP treatment in our population. By demonstrating relatively low mortality rates, our study lends additional support for continued use of β-lactam monotherapy.

Universally used CRP and WBC have a well-documented history of usefulness in assessing the diagnosis and clinical course of CAP [38]. However, our study has showed that the predictive value of these biomarkers is limited. Other authors find some advantages adding CRB to Halm's criteria, i.e. improved predicting adverse outcomes, including 30-day mortality, a need for mechanical ventilation or vasopressor support (MV/VS), the development of a complicated pneumonia, and a combined outcome of the above [39]. In meta-analysis by Viasus *et al.*, CRP shows limited use in determining CAP prognosis [40]. We found that CRB and WBC levels on admission or at day 2–3 do not provide additional information for the prediction of a complicated CAP course. However, CRP values measured at day 4 and later were significantly higher in patients with CAP complications. Our findings may suggest that the early antibiotic treatment escalation should not be based exclusively on CRP response because only later measurements have some predictive power for a complicated disease course.

Strengths and limitations

The strengths of the study include its prospective design and 'real-life' management of CAP. We were able to identify factors statistically significantly associated with CAP outcomes. The data on complicated CAP course in an adult population are limited and therefore, our study adds valuable insights into this matter.

Nevertheless, the study has several potential limitations. First, it was conducted in a single centre. Larger multi-centre studies are necessary to define the potential risk factors for CAP complications more accurately. On the other hand, this is the largest specialised pulmonology unit in the region where CAP patients are treated from all over the country, and it likely represents the whole Lithuanian population rather well. Second, there was no control group receiving alternative antibiotic treatment regimen, e.g., macrolide–β-lactam combination. In our study, the vast majority of patients received β-lactam monotherapy because this was a 'real-life' study representing our actual national clinical practice regarding CAP. The third potential limitation of the paper is a lack of information about CAP microbiological aetiology. However, initial CAP treatment is generally empiric, and in our low *Streptococcus pneumoniae* resistance population, β-lactam monotherapy remains first-choice therapy. Whereas invasive diagnostic testing (cultures obtained from bronchial aspirate or bronchoalveolar lavage samples) is only indicated in cases of initial antibiotic treatment failure.

Conclusions

To sum up, the study has demonstrated that in the low *Streptococcus pneumoniae* resistance population, CAP treatment with β-lactam monotherapy results in relatively low mortality rate. The results provide additional evidence that comorbidities, especially neuromuscular diseas-

es, multilobar opacities and clinically unstable condition as evaluated using Halm's criteria have implications for poor CAP outcomes, whereas the predictive value of early CRP and WBC measurements is limited.

Conflict of interest

None declared.

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Clinical outcomes of chronic obstructive pulmonary disease phenotypes. One center prospective study

Abstract

Introduction: The clinical outcome of different chronic obstructive pulmonary disease (COPD) phenotypes is still unclear.
Objectives: This study was designed to detect the effect of different COPD phenotypes on disease outcomes.
Material and methods: One hundred stable COPD patients were included. They were divided into 3 phenotypes; 45 patients in exacerbator phenotype, 37 patients in non-exacerbator, and 18 patients in asthma COPD overlap (ACO) phenotype. Patient demographics, respiratory symptoms, grading of COPD, co-morbidities, spirometry, six minute walk test, and systemic inflammatory markers were measured. Also, exacerbation frequency and severity were assessed throughout the study period.
Results: COPD Assessment Test (CAT) score was significantly higher in exacerbator phenotype versus the other phenotypes(14.7 $\pm$ 1.5; $p = 0.04$).In addition, about 60% and 42% of exacerbator phenotype were in Global Initiative for Chronic Obstructive Lung Disease (GOLD) class D and C respectively which were significantly higher than the other phenotypes($p = 0.001$), while 58% and 50% of non-exacerbator and ACO patients respectively were in class B of GOLD. Twenty eight percent of patients of ACO had no comorbidity and this was significantly higher versus the other phenotypes ($p = 0.03$), while 40% of non-exacerbator had one comorbidity ($p = 0.003$) and 86% of exacerbator had ≥ 2 comorbidities ($p = 0.002$). COPD comorbidity index was significantly higher in exacerbator phenotype (2.5 $\pm$ 0.8; $p = 0.01$). Although patients of exacerbator phenotype had more and severe form of exacerbations than the other phenotypes, no significant difference in in-hospital outcome was found ($p = 0.3$).
Conclusions: Exacerbator phenotype has worse disease outcome than those of non-exacerbator and ACO phenotypes. These results support the need for more treatment options to alleviate the morbidity of COPD especially among exacerbator phenotype.

Key words: COPD severity, co-morbidity, exacerbation

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Introduction

Chronic obstructive pulmonary disease (COPD) is a prevalent, preventable and treatable disorder that is specified by constant respiratory features and limitation of airflow that is owing to airway and or alveolar flaws that is created by notable exposure to toxic particles or gases [1]. It is a complex disease and heterogeneous and has multicomponent elements so the concept of phenotype-emerged, and the traditional concept of pink puffers and blue bloaters, is now being replaced by a variety of different phenotypes [2].

The phenotyping phase occurs as a result of clinical necessity to group patients with similar presentation and/or behavior to provide them for the best quality health treatment, customize the therapeutic plan for the patient in terms of symptoms control, disease progression, the state of health, and the quality of life [3].

Some research studies have examined specific phenotype frequencies and features, but limited ones are available to address the effect of these phenotypes on clinical outcomes [4–6]. So, this study was carried out to highlight on outcomes of these phenotypes purposing to intensify the lines of treatment available for those with the worst outcomes.

Aim of the work

To appraise the impact of different COPD phenotypes on disease outcomes as regard disease severity, inflammatory burden, comorbidity, and exacerbation.

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Material and methods

This study was a prospective study that included 100 patients with stable COPD who attending the out-patients chest clinic at Cardiothoracic Minia University hospital during the period between October 2018 to December 2019. All patients were diagnosed according to the GOLD definition of COPD with a post-bronchodilator forced vital capacity/volume in the first second (FVC/FEV_1) ratio of < 0.7 [1]. Stable COPD was identified by a failure of hospitalization, urgent care visits, and changes in medications within 4 weeks before the study. Exclusion criteria included patients suffering acute COPD exacerbation within 1 month before the study, combined COPD and interstitial lung disease, patients with a history of pulmonary tuberculosis, and COPD patients on domiciliary long-term oxygen therapy. The Protocol to the study was accepted by the research ethics committee of Minia faculty of medicine. The research character was explained to all patients. In all patients, a verbal consent was obtained.

A full detailed history was taken from all patients included chest symptoms, dyspnea scale using the modified Medical Research Council (mMRC) scale [7], COPD Assessment Test (CAT) score [8]. Besides, assessment of the presence of comorbidities as diabetes mellitus (DM), arterial hypertension, ischemic heart disease were based on physician-based diagnosis and medications used for them. Evaluation of anxiety and or depression using the Hamilton Anxiety Rating scale [9] and Patient Health Questionnaire [10]. COPD cO-morbidity TEst (COTE) was also calculated. It is a score which include 5 categories of diseases which are cardiovascular diseases, metabolic diseases, musculoskeletal diseases, psychological diseases and oncologic diseases. The patient is scored 1 if at least one of the diseases belonging to that category is present, the total score is the sum of scores accounted to each category with the range from 0 to 5 [11].

Spirometry was performed using a 2130 spirometer (V_{max}, Sensormedia, USA), which was calibrated daily. Results were obtained for FVC, FEV_1, and FEV_1/FVC ratio. Post bronchodilation test was done following 400 mcg of salbutamol inhalation.

Body mass index, 6-minute walk test, and BODE index (Body mass index, Obstruction, Dyspnea, Exercise capacity) [12] were calculated. Peripheral capillary oxygen saturation (Sp_{O2}) on room air was also detected.

A chest X-ray was done to each patient and a high-resolution computed tomography chest was done to detect the type and distribution of emphysema in some cases.

Complete blood count in addition to inflammatory markers in the form of, C-reactive protein (CRP), and serum fibrinogen were assayed. Blood samples were collected, centrifuged within 2 hours of sampling and the serum was frozen and stored at –20°C until analyzed for measurement of CRP by enzyme immunoassay kits supplied by European Authorized Representative (normal value: 1–6 mg/L) [13].

Serum fibrinogen was assayed using Human Fibrinogen ELIZA kits, the United States of America (normal value: 1.25–100 ng/mL) [14].

All patients offered telephone follow up and or on outpatient visit clinic till the end of the study period for assessment the following; frequency and severity of exacerbation, hospitalization for exacerbation, and outcome of hospital stay. The follow up period is determined from the point of inclusion of the patient till the end of the study (December 2019) and this period is ranged from 6–12 months.

Under Spanish guidelines for COPD [15], the studied patients were classified into the following 3 phenotypes; exacerbator group (I): 45 patients with frequent exacerbations (34 with chronic bronchitis and 11 with emphysema predominant). Those with two exacerbations or one exacerbation that needs hospitalization in a year, 3 months/year with cough and expectoration for 2 successive years were that of frequent exacerbator with chronic bronchitis predominant, while those with frequent exacerbation without chronic bronchitis and with radiological (chest x-ray or computed tomography) diagnosis of emphysema were those of frequent exacerbator with emphysema. Non-exacerbator group (II); 37 patients (32 with chronic bronchitis and 5 with emphysema), these patients had < 2 exacerbations per year. Finally, 18 patients with asthma COPD overlap (ACO) group (III). ACO patients were diagnosed based on the presence of 2 major criteria or 1 major 2 minor criteria.

Major criteria were as the following: a) A personal asthma history. b) Positive bronchodilator test with increase FEV_1 > 15% and > 400 mL. c) Fractional exhaled nitric oxide > 40 in parts per billion (ppb). Minor criteria were: a) Elevated IgE in blood; b) Personal history of atopy; c) Positive post-bronchodilator test with an increase of FEV_1 > 12% and > 200 m/l in at least 2 different occasions. All patients were diagnosed according to the presence of 2 major criteria.

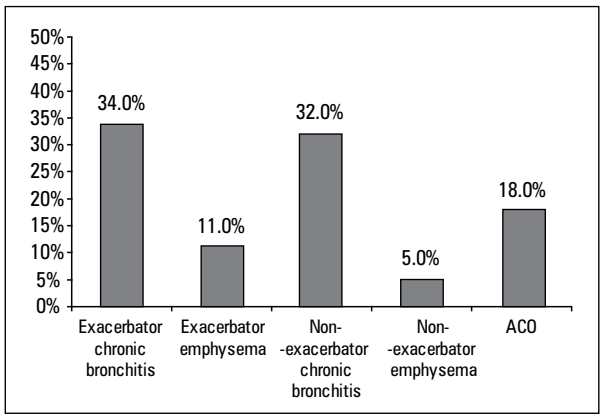

Figure 1. Distribution of each phenotypes of chronic obstructive pulmonary disease. ACO — asthma COPD overlap

Statistical analysis

Data were collected and entered using Statistical Package of Social Science, version 22. Parametric quantitative data were presented by mean and standard deviation, while qualitative data were presented by numbers and percentages. Chi-square and Fischer exact tests were used to compare qualitative data. One Way Analysis of Variance (ANOVA) was used to compare more than two means followed by post Hoc analysis when the results were found significant using Least Significant Difference (LSD) test. The confidence interval was set to 95% and the margin of error accepted was set to 5%; $p < 0.05$ was considered statistically significant.

Results

One hundred COPD patients were involved in the study. A summary of the distribution of each phenotype is presented in Figure 1. Patients with chronic bronchitis were more than those of emphysema in both exacerbator and non-exacerbator phenotypes (34 *vs* 11 patients and 32 *vs* 5 patients respectively).

It was found that patients of the exacerbator and nob-exacerbator phenotypes were significantly older than ACO patients, male gender was more in exacerbator and non-exacerbator phenotypes. Regarding respiratory symptoms, wheezes are the only symptom that was significantly predominant in ACO ($p = 0.01$) with a nearby similar distribution of other chest symptoms in all phenotypes. On analysis of spirogram results, both of FEV_1/FVC and FEV_1% predicted were lower in exacerbator phenotype ($p = 0.001$ for both), while 6-minute walk distance (6MWD) and SpO_2 showed similar values in all phenotypes. On analysis of post hoc test results, it was found that ACO patients were younger and most of them were females, while their spirometry readings were less affected than the other phenotypes (Table 1).

CAT was significantly higher in exacerbator than ACO. Interpretation of post hoc findings reveal that CAT score was lower in ACO patients by a significant degree than the other phenotypes. In addition, more than half of the exacerbator group of GOLD class D while non-exacerbator and ACO cases were more in class B ($p = 0.001$) (Table 2).

Although higher values of all inflammatory indices in the exacerbator phenotype than the others, no significant difference was found ($p > 0.05$; Table 3).

As regard comorbidities encountered in different COPD phenotypes, about one third and one forth of ACO and non- exacerbator phenotypes respectively had no comorbidity with a significant value for ACO patients in comparison to exacerbator phenotype only (Table 4). On the other hand, 40% of non-exacerbator suffered from one comorbidity only ($p = 0.003$), while 85% of the exacerbator phenotype had 2 or more comorbidities which was highly significant among them than the other 2 phenotypes ($p = 0.002$). Referring to psychological comorbidities, anxiety and depression were the predominant ones in all phenotypes with a higher significance only for anxiety among exacerbator phenotype (62.2%, $p = 0.02$).

Traditionally, comorbidity found in COPD patients has been evaluated using a non-disease specific score such as the Charlson comorbidity score [16]. Latest time, Divo et al. [11], elaborated an index unique to COPD, COPD Comorbidity Test, or COTE index that includes those comorbidities that impact survival in patients with COPD. We found a higher significant score for exacerbators in comparison with the other phenotypes (mean COTE score = 2.7 ± 0.8, with 95% CI: 2.35–3.04; 2.3 ± 0.9, 95% CI: 1.99–2.60 and 1.9 ± 0.9 with 95% CI: 1.68–2. 11, $p = 0.01$ for exacerbator, non-exacerbator and ACO respectively) (Figure 2).

Exacerbations are described as acute aggravation of respiratory symptoms that lead to further treatment and they are classified into mild, moderate and severe according to the treatment affordable for each type as issued by GOLD [1]. Referring to COPD exacerbations that the studied patients suffered (Table 5), ten patients missed to be followed, only 2 exacerbator patients out of 45 had no exacerbations thought the study period which was significant for them than the other phenotypes. Regarding the severity of exacerbations,

Table 1. Summary of demography, clinical and spirometry results among COPD phenotypes

Variable	Exacerbator (I)	Non-exacerbator (II)	ACO (III)	P-value		
	n = 45	n = 37	n = 18			
Age [years]	63.9 ± 8.2	62.1 ± 7	57.8 ± 7.3		0.019	
				I vs II	I vs III	II vs III
				0.294	0.008	0.039
Male, n (%)	40 (88.8%)	34 (91.8%)	6 (33.3%)		0.001	
Female, n (%)	5 (11.1%)	3 (8.1%)	12 (66.7%)			
				I vs II	I vs III	II vs III
				0.648	< 0.001	< 0.001
Dyspnea, n (%)	24 (54.5%)	28 (73.7%)	10 (55.6%)		0.160	
mMRC score						
Grade 1	1 (4.2%)	1 (3.6%)	1 (5.6%)		0.97	
Grade 2	13 (54.2%)	15 (53.6%)	5 (66.7%)			
Grade 3	10 (41.7%)	12 (42.9%)	4 (27.8%)			
Cough, n (%)	34 (77.3%)	32 (84.2%)	18 (100%)		0.08	
Wheezes, n (%)	21 (47.7%)	16 (42.1%)	15 (83.3%)		0.01	
				I vs II	I vs III	II vs III
				0.756	0.007	0.004
FEV_1/FVC (actual)	49.9 ± 11.1	54.8 ± 9.3	60.3 ± 8.4		0.001	
				I vs II	I vs III	II vs III
				0.036	0.001	0.039
FEV_1 (%, pred.)	37.2 ± 12.9	38.2 ± 9.6	49.2 ± 12.6		0.001	
				I vs II	I vs III	II vs III
				0.697	0.001	0.001
6MWD [m]	267.1 ± 50.5	272.4 ± 46.1	270.2 ± 49.2		0.86	
Resting SpO_2 [%]	92.2 ± 3.3	94.3 ± 3.5	93.3 ± 2.9		0.28	

Some data are presented as mean±SD.
Results were presented as numbers and percentages and compared using Chi-square test. If the results were significant multi-comparison were done between groups using Chi-square test.
Results were presented as mean ± SD and compared using one-way ANOVA test. If the results were significant the post hoc analysis was done using LSD test.
6MWD — 6-minute walk distance; FEV_1/FVC — forced expiratory volume in 1 second/forced vital capacity; mMRC — modified medical research council; SpO_2 — peripheral oxygen saturation

exacerbator and non-exacerbator phenotypes had a significant higher number of severe exacerbations that need hospitalization in comparison to ACO patients. While patients of ACO phenotype had a higher percent of moderate exacerbations than the other phenotypes.

Discussion

Some studies found that COPD patients with different phenotypes have variable disease characteristics [4], however, the fate of these phenotypes on morbidity and mortality is still elusive. So, this research was performed to assess the effect of different COPD phenotypes on disease outcome.

COPD severity indices that were measured in our study were (CAT score, GOLD categories, and BODE index).

We found that exacerbator and non-exacerbator groups had a higher CAT score than the ACO group (14.7 ± 1.5; 14.4 ± 1.4 *vs* 13.7 ± 1.7 respectively p = 0.04). Previous studies found that the exacerbator phenotype mainly exacerbator chronic bronchitis had the highest CAT score [5, 17]. A meta-analysis study found that in ten studies that included 4568 patients, the frequent exacerbator of chronic bronchitis phenotype was associated with a high CAT score than in the ACO phenotype [18].

Regarding COPD categories using A, B, C, D assessment, our study found that all exacerbators were in category class (C) and (D) (42.4% and 59% respectively) which represented the most severe categories, non-exacerbators and ACO patients had a lower degree of disease severity as more than 50% of the involved patients were in cate-

Table 2. Chronic obstructive pulmonary disease (COPD) severity classification among different phenotypes

Variable	Exacerbator (I)	Non- exacerbator (II)	ACO (III)	P-value		
	n = 45	n = 37	n = 18			
CAT score						
Range	12–20	10–18	12–18		0.040	
Mean ± SD	14.7 ± 1.5	14.4 ± 1.4	13.7 ± 1.7	I vs II	I vs III	II vs III
				0.356	0.024	0.111
BMI (kg/m^2)						
Range	20.4–44.9	20.6–37	20.4–50		0.267	
Mean ± SD	28.1 ± 6.9	25.6 ± 3.8	26.4 ± 6.1			
GOLD categories, n (%)						
A	0 (0%)	10 (26.3%)	1 (5.6%)		0.001	
B	0 (0%)	22 (57.8%)	9 (50%)			
C	19 (42.2%)	5 (13.5%)	7 (38.9%)	I vs II	I vs III	II vs III
D	26 (59%)	0 (0%)	1 (5.6%)	< 0.001	< 0.001	0.034
BODE index						
Range	4–9	4–7	4–8		0.11	
Mean ± SD	6.3 ± 1.2	5.9 ± 0.9	5.1 ± 0.8			

Results were presented as numbers and percentages and compared using Chi-square test. If the results were significant multi-comparison were done between groups using Chi-square test.
Results were presented as mean ± SD and compared using one- way ANOVA test. If the results were significant the post hoc analysis were done using LSD test.
CAT — COPD assessment test; BMI — body mass index; BODE = body mass index (B), degree of airflow obstruction by FEV_1% pred. (O) and functional dyspnea (D) measured by mMRC scale, and exercise capacity (E) as assessed by the 6-minute walk test

Table 3. Inflammatory biomarkers among different phenotypes

Variable	Exacerbator n = 45	Non- exacerbator n = 37	ACO n = 18	P-value
WBCs (/Cu.mm)				
Range	4000–13000	2000–12000	3000–11000	0.35
Mean ± SD	7947.7 ± 23.9	7221.1 ± 2328.7	7427.8 ± 2352	
CRP, n (%)				
Negative	22 (48.8%)	20 (54.1%)	11 (61.1%)	0.62
Positive	23 (51.1%)	17 (45.9%)	7 (38.9%)	
CRP titre [mg/L]				
Range	9–98	6–96	10–48	0.30
Mean ± SD	44.5 ± 35.1	33.3 ± 33.5	27.4 ± 19.5	
Serum fibrinogen [ng/mL]				
Range	15–735	25–685	30–670	0.66
Mean ± SD	155.3 ± 133.6	120.8 ± 139.3	130.6 ± 122.9	

ACO — asthma COPD overlap; CRP — C-reactive protein; WBCs — white blood cells

gory (B) (57.8% and 50% respectively). In another multicenter study, most of the COPD patients were in the GOLD (D) group (74.3%) and frequent exacerbators with chronic bronchitis were the higher prevalence than other phenotypes [6].

The BODE index is a multidimensional tool that integrate quantifications of nutritional position, airflow limitation, dyspnea, and functional status. It provides an integrated assessment of the respiratory and non- respiratory domains of the disease that better represent disease severity [12]. We figured out although the highest score of the BODE index was found in the exacerbator group, no substantial difference between phenotypes was found. In agreement with our findings, other study showed that there was no significant difference between the BODE index and different phenotypes [5]. In contrast to this finding, another study [19] found that frequent exacerbators have a significantly worse BODE scores and lung function than non-exacerbators and ACO patients.

Several studies have shown that COPD patients even in the stable state have higher levels of some inflammatory markers in the blood [20,

Table 4. Comorbidities among different phenotypes

Variable	Exacerbator (I)	Non- exacerbator(II)	ACO (III)	P-value		
	n = 45	n = 37	n = 18			
No comorbidity, n (%)	2 (4.4%)	6 (16.2%)	5 (27.7%)		0.038	
				I vs II	I vs III	II vs III
				0.073	0.007	0.314
1 comorbidity, n (%)	4 (8.8%)	15 (40.5%)	3 (16.6%)		0.003	
				I vs II	I vs III	II vs III
				0.001	0.374	0.076
≥ 2 comorbidities, n (%)	39 (86.6%)	16 (43.2%)	10 (55.5%)		0.002	
				I vs II	I vs III	II vs III
				0.000	0.007	0.390
Systemic HTN, n (%)	12 (27.3%)	13 (34.2%)	5 (27.8%)		0.800	
DM, n (%)	7 (15.9%)	4 (10.5%)	1 (5.6%)		0.460	
IHD, n (%)	5 (11.4%)	3 (7.9%)	3 (16.7%)		0.580	
Anxiety, n (%)	28 (62.2%)	13 (35.1%)	7 (38.9%)		0.020	
				I vs II	I vs III	II vs III
				0.014	0.092	0.092
Depression, n (%)	29 (64.4%)	16 (43.2%)	8 (44.4%)		0.070	

DM — diabetes mellitus; HTN — hypertension; IHD — ischemic heart disease

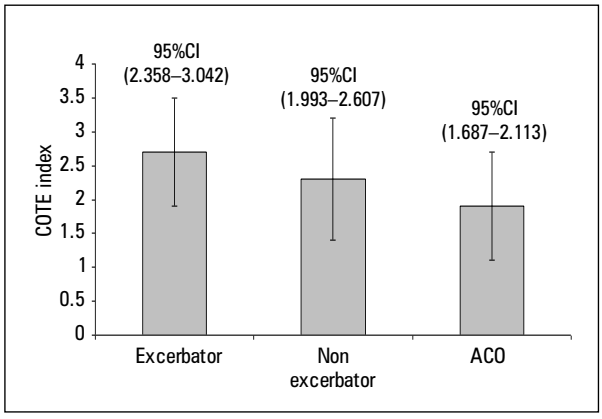

Figure 2. Mean value of COTE index among different phenotypes

21]. On assessment the systemic inflammatory markers in the current study, we found that in exacerbator phenotype, although, white cell counts, CRP, and serum fibrinogen were higher in that phenotype in comparison with the other phenotypes with no significant difference ($p > 0.05$). No previous studies have a comparison of systemic inflammatory markers to phenotype. Some studies showed that high plasma fibrinogen levels reflected severe symptomatic phenotypes and poor clinical outcomes [22], however, we did not perform a correlation analysis between it and outcomes.

The presence of significant comorbidities is one of the most important risk factors for severity in COPD, therefore, identifying and treating co-morbidities is an integral aspect of COPD's care plan.

Comorbidities can be related to any clinical phenotype [23] and should be included in a systemic therapy strategy. Some recent studies looked at the associations between comorbidities and unique COPD phenotypes [24] or identified novel phenotypes associated with comorbidities, but the findings of founding associations are still scarce or draw definite conclusions.

We found that 50% of the studied phenotypes had comorbidities with numbers of patients with anxiety and depression exceed those with the cardiovascular system affection and diabetes mellitus in all phenotypes. However, anxiety was the only significant one among the exacerbator phenotype ($p = 0.02$). Under these findings, a polish study [4], found also that depression and anxiety were significantly higher among exacerbator phonotype either chronic bronchitis or emphysema than other phenotypes ($p = 0.001$ and 0.04 respectively).

Table 5. Exacerbation characteristics among the studied phenotypes

Variable	Exacerbator (I)	Non- exacerbator (II)	ACO (III)	P -value		
	n = 45	n = 37	n = 18			
No exacerbation, n (%)	2 (5%)	14 (41.2%)	7 (43.7%)		0.0001	
				I vs II	I vs III	II vs III
				< 0.001	< 0.001	0.862
Frequency of exacerbation						
Range	3–4	0–1	0–3			
Mean ± SD	3.4 ± 0.5	0.8 ± 0.4	1.5 ± 1.2	I vs II	I vs III	II vs III
				< 0.001	< 0.001	0.003
Moderate exacerbation, n (%)	10 (25%)	4 (11.7%)	6 (66.6%)			
				I vs II	I vs III	II vs III
				0.147	0.349	0.033
Severe exacerbation, n, % Inward Admission	20 (71.4%)	14 (37.8%)	1 (11.1%)		0.02	
				I vs II	I vs III	II vs III
				0.447	0.002	0.011
ICU Admission	8 (28.6)	2 (5.4%)	2 (22.2%)		0.06	
Length of hospital stay [days], Mean ± SD	6.2 ± 1.8	6.1 ± 2.2	6.7 ± 1.4		0.78	
Hospitalization outcome						
Discharged alive, n (%)	26 (92.8%)	16 (100%)	2 66.7%)		0.30	
Death, n (%)	2 (7.2%)	0	1 (33.3%)		0.15	

COTE index that includes those comorbidities that impact on survival in COPD patients. It is the first specific COPD comorbidity index which predicts the risk of death associated with COPD accompanying co-morbidities [11], and more disease- specific than the Charlson comorbidity score, developed for patients with cancer. The scores range from 0–5. COTE Index was also described according to the mortality risk in < 4 points and ≥ 4 points. Our study is the first study to assess COTE index in different COPD phenotypes, we found that all COPD phenotypes had a mean index < 4 points with a higher significant score among exacerbators in comparison with the other phenotypes (COTE = 2.7; p = 0.01).

Exacerbations are an important occurrence, not just because they pose a major economic burden but more importantly because frequent exacerbations of COPD contribute to a worsening in health-related quality of life [25].

On follow up of the studied patients for assessment of frequency and severity of exacerbations, we found that frequency and severity of exacerbations were substantially more in exacerbator in comparison with other phenotypes. This is followed by ACO patients as 9 patients of ACO out of 16 (56%) had moderate to severe exacerbation during the time of the study with 2 patients (22 %) need intensive care unit (ICU) admission. Another study [26] found that the frequent exacerbator phenotype was closely associated with exacerbation-related hospitalizations, and exacerbation-related hospitalizations were associated with poorer survival. However, one study [27] suggested that the amount of exacerbation was similar in the three phenotypes, despite the evident differences in patient features.

In general, the frequency of exacerbation increases with the seriousness of the disease, as indicated by obstruction of the airflow [28], and some evidence suggests a possible role for extrapulmonary factors in exacerbation genesis like the BODE index, which is a better predictor of COPD hospitalization in a patient cohort than FEV_1 [29]. Based on these findings, we found that the frequent exacerbators with the higher frequency of exacerbation had also the lowest FEV_1 and a higher BODE index than other phenotypes. Another study [5] agreed with our findings as they found that the (frequent exacerbator chronic bronchitis) phenotype was the most symptomatic and had frequent exacerbation with higher BODE score and showed a trend to worse survival after one year.

This study has some shortcomings, one of them is the use of FEV1 and FVC in pulmonary function test in all phenotypes with no specification in specific phenotypes due to non-availability for measuring lung volumes as inspiratory capacity over total lung capacity ratio (IC/TLC) which is used as an index for assessing static lung hyperinflation which has a significant relationship to survival especially in COPD patients with emphysema phenotype [30].

Besides, 10% of the studied patients missed being followed which may influence the exacerbation history or the in hospital-mortality.

We have no data in this study on the timing of diagnosis of co-morbidities in different COPD phenotypes if they occur before or after diagnosis of COPD to understand if the pathophysiology of COPD and co-morbidity is common or they are considered as one of long term COPD complications.

Lastly, no follow up on 6MWD or BODE index was done during the follow- up visits to determine its change over time which may be one of the predictors for poor outcomes in some phenotypes.

Conclusion

Exacerbator phenotype is the most common phenotype encountered in this study followed by non- exacerbator then ACO patients. It is obvious that patients of exacerbator phenotype have a higher COPD severity index than the other phenotypes represented in CAT score and GOLD grade of categorization. COPD associated co-morbidities have a common denominator in all phenotypes with a predominance of psychological disorders than the other co-morbidities. Undoubtedly, exacerbators have a more frequency of exacerbations than the other phenotypes but also have a more severe exacerbations that require hospital admission.

Recommendations

Phenotypes classification should be done early in all COPD patients from the time of diagnosis as exacerbator phenotype has worse prognosis than other. More follow up visits to outpatients' clinics, educational training on diagnosis of exacerbations early and treatment options need to affordable especially for exacerbator phenotype.

Conflict of interest

None declared.

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Epidemiological characteristics and outcomes from 187 patients with COVID-19 admitted to 6 reference centers in Greece: an observational study during the first wave of the COVID-19 pandemic during the first wave of the COVID-19 pandemic

Abstract

Introduction: Epidemiological data from patients with COVID-19 has been recently published in several countries. Nationwide data of hospitalized patients with COVID-19 in Greece remain scarce.

Material and methods: This was an observational, retrospective study from 6 reference centers between February 26 and May 15, 2020.

Results: The patients were mostly males (65.7%) and never smokers (57.2%) of median age 60 (95% CI: 57.6–64) years. The majority of the subjects (98%) were treated with the standard-of-care therapeutic regimen at that time, including hydroxychloroquine and azithromycin. Median time of hospitalization was 10 days (95% CI: 10–12). Twenty-five (13.3%) individuals were intubated and 8 died (4.2%). The patients with high neutrophil-to-lymphocyte ratio (NLR) (> 3.58) exhibited more severe disease as indicated by significantly increased World Health Organization (WHO) R&D ordinal scale (4; 95% CI: 4–4 *vs* 3; 95% CI: 3–4, $p = 0.0001$) and MaxFiO_2% (50; 95% CI: 38.2–50 *vs* 29.5; 95% CI: 21–31, $p < 0.0001$). The patients with increased lactate dehydrogenase (LDH) levels (>270 IU/ml) also exhibited more advanced disease compared to the low LDH group (< 270 IU/ml) as indicated by both WHO R&D ordinal scale (4; 95% CI: 4–4 *vs* 4; 95% CI: 3–4, $p = 0.0001$) and MaxFiO_2% (50; 95% CI: 35–60 *vs* 28; 95% CI: 21–31, $p < 0.0001$).

Conclusion: We present the first epidemiological report from a low-incidence and mortality COVID-19 country. NLR and LDH may represent reliable disease prognosticators leading to timely treatment decisions.

Key words: COVID-19, severity, neutrophil-to-lymphocyte ratio, LDH, prognosticators

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Introduction

The emergence and spread of 2019 novel coronavirus disease (COVID-19) and the associated acute respiratory distress syndrome (ARDS) are causing a growing global public health crisis. The virus is presumed to have originated in Wuhan, Hubei province, China, in bats. On January 7th,

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2020, the virus was identified as a coronavirus with a > 70% similarity to the SARS-CoV and was named SARS-CoV-2 [1]. SARS-CoV-2 mainly affects the upper and lower respiratory tracts, entering into the respiratory mucosa through its receptor, the angiotensin-converting enzyme 2 (ACE2), and leading to a cascade of events, including pulmonary epithelial cell apoptosis, fibroblast proliferation, T-cell activation and a massive production of inflammatory cytokines [2]. The disease is mild in most people (80%) causing general symptoms such as cough, fever, general fatigue, and in some cases, dyspnea, anosmia and anorexia. In some patients, especially among the elderly and those with comorbidities, COVID-19 may progress to ARDS and multi-organ failure. There are no specific antiviral agents for COVID-19. All applied therapeutic regimens represent off-label use of non-COVID-19 drugs [3–6].

In Greece, the first case was reported on February 26th, and the peak incidence with 1.6 cases per 100,000 population per day (156 total cases) was reached on April 21st with regards to the first wave. Currently, we are facing the second wave of pandemic with far more cases (peak: 3,316 daily cases, November 12th, 2020). As of December 27th, 2020, which is the day that vaccination started in our country, 135,114 cases have been reported in Greece resulting in 4,553 deaths. Several epidemiological reports from all over the world have been recently published [7]. This article represents a summary of the 'success story' during the first wave in Greece. The aim of the study was to identify detailed baseline characteristics, outcomes and treatment approaches of a large (based on our national standards) cohort of hospitalized patients with COVID-19 during the first wave of the pandemic. Our paper represents the first epidemiological report from a low-incidence and mortality country during the first wave where the health-care system has not been overwhelmed by the pandemic. We provide scientific evidence that could potentially reflect the beneficial impact of early implementation of lockdown as well as other containment measures.

Material and methods

This was an observational, retrospective study. From February 26th, when the first case was documented, till May 15th, 2020, epidemiological data from patients hospitalized for COVID-19 in 6 reference centers in north, central and south Greece, including the Department of Internal and Respiratory Medicine, University Hospital of Patras, 1st and 2nd Academic Department of Respiratory Medicine, SOTIRIA and ATTIKON General Hospital, National and Kapodistrian University of Athens, 4th and 5th Department of Respiratory Medicine, SOTIRIA General Hospital, Athens and Department of COVID-19, Papanikolaou General Hospital, Thessaloniki, was retrospectively collected and analyzed. The study was approved by the Institutional Review Board and the Local Ethics Committee (Protocol Number: 8681/1-4-20). Diagnosis of COVID-19 was based on a positive real-time reverse transcriptase polymerase chain reaction (RT-PCR) of an upper respiratory nasopharyngeal (or oropharyngeal) swab. Subsequently, we collected demographics and laboratory tests, including parameters of complete blood count (CBC), lactate dehydrogenase (LDH), pro-calcitonin and D-dimers. Comorbid conditions were also recorded. Disease severity was evaluated through maximum fraction of inspired oxygen % (FiO_2%) during hospitalization, as well as through World Health Organization (WHO) R&D Blueprint ordinal scale on admission (minimum value: 1, maximum value: 7). Increased values of WHO R&D score were indicative of more severe disease.

Statistical analysis

Median values of all laboratory tests were recorded. Median values were preferred, as the Kolmogorov–Smirnov test for normal distribution rejected normality. The Mann–Whitney test was applied to assess differences in maximum FiO_2% during hospitalization and WHO R&D Blueprint ordinal scale on admission between the subgroups of patients split by the median value of the studied parameters (high and low subgroup). Follow-up assessment was performed from the date of admission till discharge or intubation or death (event). P-values < 0.05 were considered statistically significant. Results were illustrated in tables and figures.

Results

Clinical and radiological data

Baseline characteristics of the study population are presented in Table 1. A total number of 187 cases was enrolled and analyzed. The patients were mostly males (65.7%) of median age 60 (95% CI: 57.6–64) years and 16% had a recent travel history to a highly endemic country, including Italy, China and Israel. Strikingly, the majority of patients (57.2%) were non-smokers. Regarding clinical image, fever (85%), cough (51.3%) and general fatigue (50.3%) were the predominant features, while 1/10 experienced anosmia and only

34% of patients (64/187) suffered from dyspnea despite respiratory failure, as indicated by SaO_2 < 93% in 66% of cases. Radiological findings were strikingly homogeneous across the vast majority of patients and involved features of bilateral interstitial infiltrates in plain chest x-ray (95%) and features resembling organizing pneumonia and non-specific interstitial pneumonia with areas of consolidation and ground-glass opacities with no upper or lower zone predominance in chest computed tomography (71%) (Table 2). Arterial hypertension was the most commonly encountered comorbidity (32.6%), while 10% and 4.8% of our patients suffered from diabetes mellitus and hypothyroidism, respectively. Interestingly, the incidence of chronic obstructive lung diseases, including asthma and chronic obstructive pulmonary disease (COPD), was very low with an overall frequency of 1.1% (Table 3). The vast majority of patients were treated with the standard-of-care therapeutic regimen at that time, including hydroxychloroquine and azithromycin for 7 days. Lopinavir and ritonavir was administered in 17.6% of our patients, while only 1.7% and 5.1% received remdesivir and colchicine, respectively, within the context of a clinical trial. Low-molecular weight heparin on a prophylactic basis was administered in 85.6% of patients. Biological agents including anti-IL6 (tocilizumab) and anti-IL1r (anakinra) were administered in 7 patients (3.9%) (Table 4). Median time of hospitalization was 10 days (95% CI: 10–12). Twenty-five (13.3%) patients were intubated and 8 died (4.2%). Missing data on outcome analysis was reported in 11/187 (5.8%).

Laboratory data

Laboratory data of the study population are presented in Table 5. Lymphopenia (< 1200/μL) was the most commonly encountered laboratory finding (53.5%) of our study population with median values of 1112/μl (95% CI: 1000–1220). Subsequently, increased neutrophil-to-lymphocyte ratio (NLR) was also reported with a median value of 3.58 (95% CI: 2.98–3.91). Elevated ferritin levels (> 90ng/Ml) were present in most of our patients (67%) with a median value of 430.8 (95% CI: 363.1–483.9). Increased LDH levels (> 245 IU/ml) were present in the majority of the study population (58.8%) with a median value of 270 (95% CI: 250.7–291.2). Increased D-dimer levels (> 0.5 ng/ml) were present in 28/187 (15%) of the study population. Interestingly, the patients with high neutrophil-to-lymphocyte ratio (> 3.58) exhibited more severe disease as indicated

Table 1. Baseline characteristics of the patients enrolled in the study

Characteristics	
Total number of patients	187
Age median (95% CI)	60 (57.6–64)
Males/females, n (%)	123 (65.8)/64 (34.2)
Current/ex-smokers, n (%)	80 (42.8)
Never smokers, n (%)	107 (57.2)
History of recent travel abroad, n (%)	30 (16)

CI — confidence interval

Table 2. Clinical and radiological features on admission of the patients enrolled in the study

Disease	
Fever, n (%)	159/187 (85.0)
Anosmia, n (%)	19/187 (10.2)
Anorexia, n (%)	46/187 (24.6)
Cough, n (%)	96/187 (51.3)
Dyspnea, n (%)	64/187 (34.2)
Fatigue, n (%)	94/187 (50.3)
SaO_2 < 93%, n (%)	124/187 (66.3)
Bilateral infiltrates (chest X-ray) , n (%)	177/187 (94.7)
Bilateral infiltrates (chest computed tomography scan) , n (%)	131/187 (70.1)

Table 3. Comorbidities of the patients enrolled in the study

Comorbidity	
Hypertension, n (%)	61/187 (32.6)
Diabetes mellitus, n (%)	17/187 (9.09)
Cancer, n (%)	13/187 (7.0)
Atrial fibrillation, n (%)	8/187 (4.3)
Heart failure, n (%)	5/187 (2.7)
Hypothyroidism, n (%)	9/187 (4.8)
Asthma, n (%)	2/187 (1.1)
Chronic obstructive pulmonary disease, n (%)	4/187 (2.2)

Table 4. Therapeutic compounds administered to the study population

Compound	
Hydroxychloroquine, n (%)	170/187 (90.9)
Azithromycin, n (%)	169/187 (90.4)
Lopinavir/Ritonavir, n (%)	33/187 (17.6)
Remdesivir, n (%)	3/187 (1.6)
Tocilizumab, n (%)	5/187 (2.7)
Anakinra, n (%)	2/187 (1.1)
Colchicine, n (%)	9/187 (4.8)
Other antibiotics, n (%)	157/187 (84.0)
Low molecular weight heparin (prophylactic dose), n (%)	160/187 (85.5)

Table 5. Laboratory tests on admission of the patients enrolled in the study

Laboratory tests	
Neutrophils (/μL, median, 95% CI)	3864 (95% CI: 3492–4153)
Lymphocytes (/μL, median, 95% CI)	1112 (95% CI:1000–1220)
Neutrophils to lymphocytes ratio (median, 95% CI)	3.58 (95% CI: 2.98–3.91)
Monocytes (/μL,median, 95% CI)	461 (95% CI: 410–500)
Platelets (/μL, median, 95% CI)	201500 (95% CI: 185200–211500)
RDW (%, median, 95% CI)	12.8 (95% CI: 12.6–13.2)
MPV (fl, median, 95% CI)	9.3 (95% CI: 8.5–9.7)
LDH (IU/L, median, 95% CI)	270 (95% CI: 250.7–291.2)
Ferritin (ng/mL, median, 95% CI)	430.8 (95% CI: 363.1–483.9)
Procalcitonin (ng/mL, median, 95% CI)	0.07 (95%: 0.05–0.09)
D-dimers (μg/ml, median, 95% CI)	0.77 (95%: 0.68–0.95)

LDH — lactate dehydrogenase; MPV — mean platelet volume; RDW — red cell distribution width

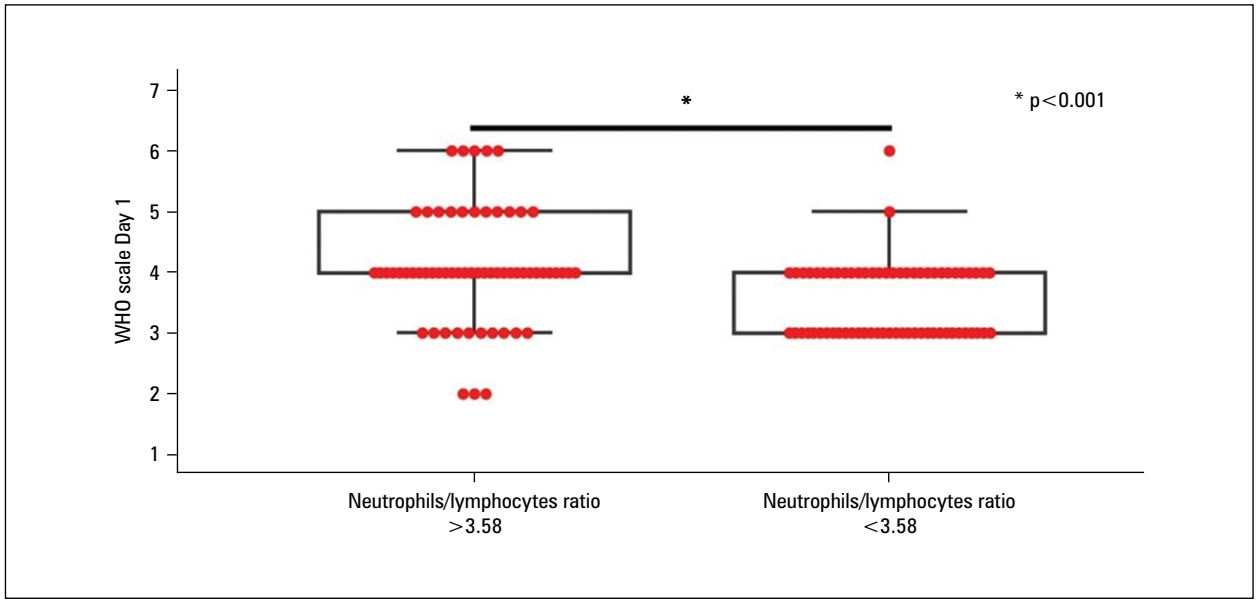

Figure 1. Median World Health Organization (WHO) R&D Blueprint score on admission was significantly higher for patients with baseline neutrophils-to-lymphocytes ratio > 3.58 (median value: 4; 95% CI: 4–4) compared to patients with baseline neutrophils-to-lymphocytes ratio < 3.58 (median value: 3; 95% CI: 3–4) (p < 0.001)

by significantly increased WHO R&D ordinal scale (4; 95% CI: 4–4 *vs* 3; 95% CI: 3–4, p = 0.0001, respectively) (Figure 1) and MaxFiO_2% (50; 95% CI: 38.2–50 *vs* 29.5; 95% CI: 21–31, p < 0.0001, respectively) (Figure 2). The patients with increased LDH levels (> 270 IU/ml) also exhibited more advanced disease compared to the low LDH group (< 270 IU/ml) as indicated by both WHO R&D ordinal scale (4; 95% CI: 4–4 *vs* 4; 95% CI: 3–4, p = 0.0001, respectively) (Figure 3) and MaxFiO_2% (50; 95% CI: 35–60 *vs* 28; 95% CI: 21–31, p < 0.0001, respectively) (Figure 4). Finally, the patients with elevated D-dimers (> 0.77 μg/ml) displayed advanced disease compared to the low D-dimers (< 0.77 μg/mL), as assessed by increased WHO ordinal scale (4; 95% CI: 4–4 *vs* 3; 95% CI: 3–4, p = 0.006, respectively, Supplementary Table 1). No other differences in terms of disease severity, were observed between high and low subgroups of patients split by parameters of general blood tests (Supplementary Tables 1, 2)

Discussion

This is the first epidemiological study reporting characteristics of patients with COVID-19 from 6 large reference centers in Greece during the first wave of the pandemic. This multicenter study further corroborated evidence from previous studies showing increased prevalence in middle-aged

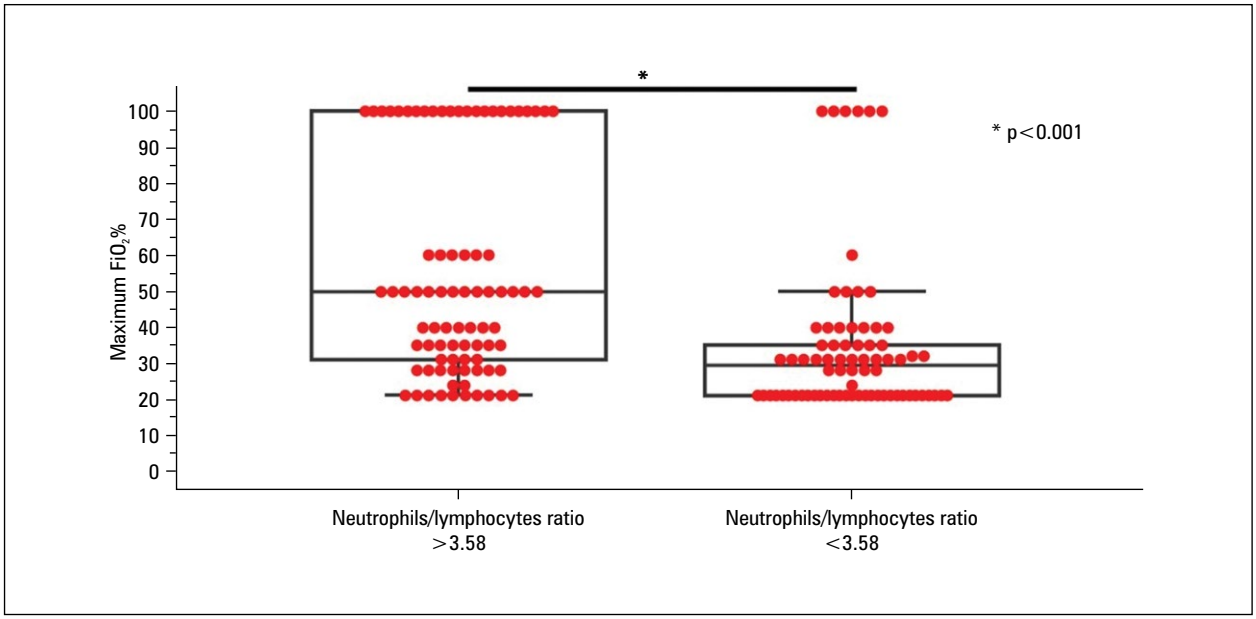

Figure 2. Maximum fraction of inspired oxygen (FiO_2) during hospitalization was significantly higher for patients with baseline neutrophils-to-lymphocytes ratio > 3.58 (median value: 50; 95% CI: 38.2–50) compared to patients with baseline neutrophils-to-lymphocytes ratio < 3.58 (median value: 29.5; 95% CI: 21–31) ($p < 0.001$)

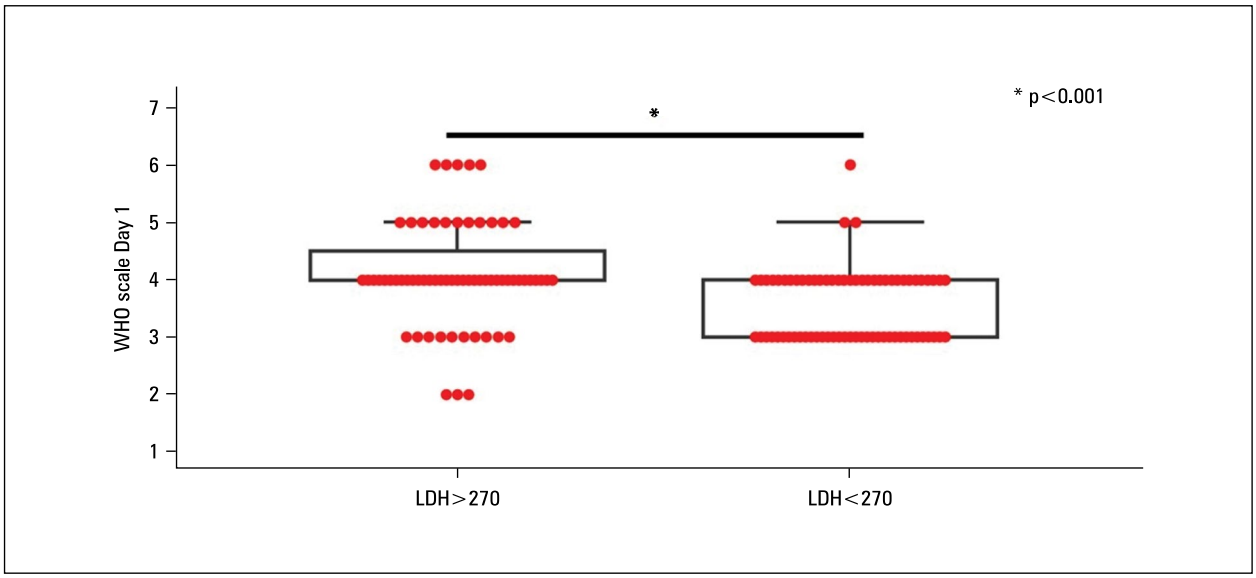

Figure 3. Median World Health Organization (WHO) R&D Blueprint score on admission was significantly higher for patients with baseline lactate dehydrogenase (LDH) > 270 IU/L (median value: 4; 95% CI: 4–4) compared to patients with baseline LDH < 270 IU/L (median value: 4; 95% CI: 3–4) ($p < 0.001$)

males, with cardiovascular risk profile (33% had arterial hypertension and 9% diabetes mellitus) and a case-fatality rate of 4%. Fever and cough were the most commonly encountered clinical features with only half of the patients experiencing dyspnea despite respiratory failure. Bilateral interstitial infiltrates with no lung zone predominance represented a strikingly homogeneous radiological picture in almost all patients. Combination of hydroxychloroquine and azithromycin were applied in the vast majority of the study subjects (90%) according to the WHO guidelines at the time of data collection and analysis.

Besides the above highly reproducible epidemiological data, our study revealed some interesting observations. First, our cohort predominantly consisted of non-smokers. This is in line with previous reports demonstrating increased prevalence of COVID-19 among non-smokers compared to smokers [8]. On the other hand,

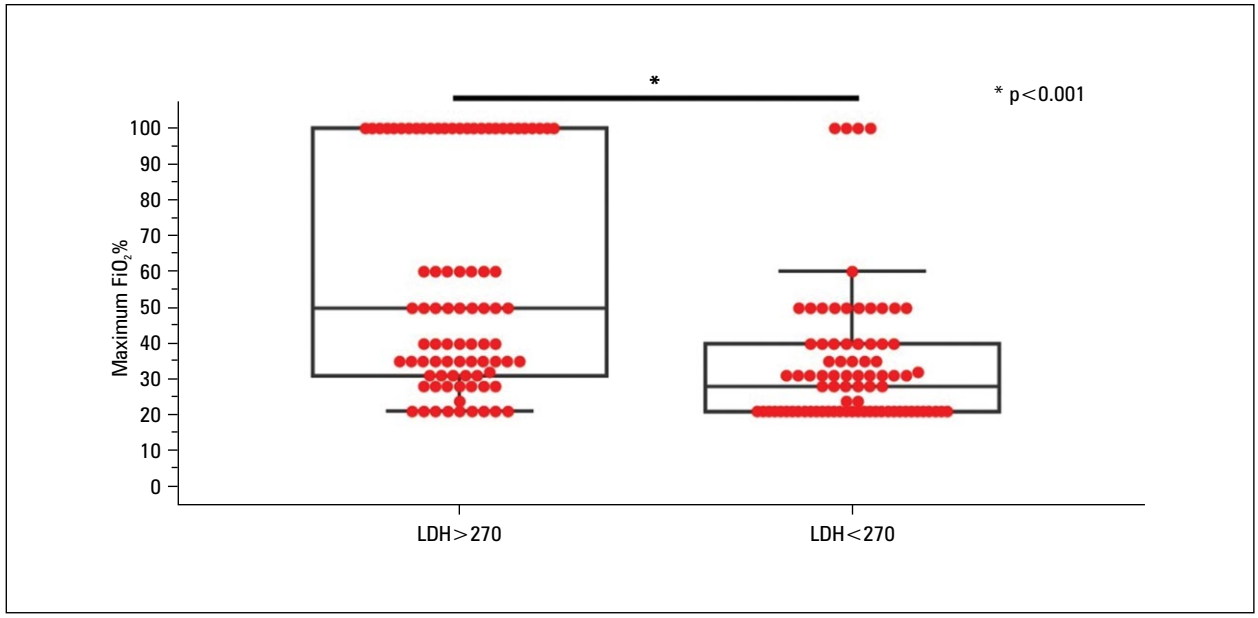

Figure 4. Maximum fraction of inspired oxygen (FiO_2) during hospitalization was significantly higher for patients with baseline lactate dehydrogenase (LDH) > 270 IU/L (median value: 50; 95% CI: 35–60) compared to patients with baseline LDH < 270 IU/L (median value: 28; 95% CI: 21–31) ($p < 0.001$)

the studies have shown worse clinical outcomes among smokers [9, 10], with mechanistic data supporting the premise of ACE2 upregulation in the airway epithelial cells mediated by nicotine exposure specifically through the $\alpha 7$ subtype of nicotine acetylcholine receptors ($\alpha 7$-nAChR) [11, 12]. At the time of this manuscript the available data suggests that smoking is associated with increased severity of disease and death in hospitalized COVID-19 patients. Nonetheless, there are currently no peer-reviewed studies that have evaluated the risk of SARS-CoV-2 infection and hospitalization among smokers. Population-based studies are needed to address these issues. Our study is severely underpowered and by no means provide any rigid epidemiological or mechanistic association between smoking and COVID-19 prevalence and severity.

Interestingly, our study population was characterized by low incidence of patients with asthma and COPD (3.3% combined). A decreased incidence of asthma and COPD has been previously reported in several countries affected by COVID-19 [13], indicating a relative protection. Experimental evidence to support this premise have suggested a role for inhaled corticosteroids (ICS) in the inhibition of coronavirus replication in infected epithelial cells. Investigation of gene expression of ACE2 and TMPRSS2 in the sputum of patients with asthma and COPD has shown reduced expression of these receptors in the presence of ICS [14] and attenuation of ACE2 receptors in human and murine *in vitro* and *in vivo* models [15]. More recently systemic use of dexamethasone significantly reduced mortality among patients with COVID-19 who were receiving mechanical ventilation and oxygen support but had no clear effect in less severe cases [16]. On the other hand, preliminary data from a recent epidemiological (OpenSAFELY) group has suggested that the use of ICS in patients with asthma and COPD is associated with worse clinical COVID-19 outcomes [17, 18]. The impact of corticosteroids on the COVID-19 disease course needs to be further explored.

Another interesting epidemiological observation is the null incidence of interstitial lung diseases (ILDs) and particularly idiopathic pulmonary fibrosis among our cohort of patients with COVID-19 [19]. Although mechanistic link is missing, this finding may be supported by previous experimental data demonstrating reduced expression of ACE2 in the lungs of patients with IPF indicating a relative protection against SARS-CoV-2 infection [20]. On the other hand, patients with ILDs appear to be at increased risk of death from COVID-19 as shown by a large multicenter epidemiological study reporting a 49% mortality rate in patients with COVID-19 and various forms of lung fibrosis [21].

The aforementioned epidemiological observations could also reflect the beneficial impact of timely implementation of lockdown and other self-protection measures such as masks,

social distancing and hand hygiene leading to containment of the pandemic in an era with no established treatment [22]. They may also result from a well-educated, disciplined and compliant group of patients who meticulously applied all prophylactic measures and thus limited further viral transmission. Regarding the relatively low number of hospitalizations, following extended discussions with primary cases' physicians and fully considering our experience in hospitals emergencies, these findings suggest that in Greece primary care likely played a key role in the management of suspected cases of SARS-CoV-2, plausibly relieving the referral hospitals from suspect and mild cases and contributing thus critically to our success story during the pandemic. On the other hand, our study was an observational study and was not designed to address the origin of these phenomena.

Finally, we performed analysis of laboratory parameters and revealed that increased NLR (> 3.58) could serve as a reproducible and clinician's friendly biomarker of disease severity as it reliably discriminated severe from non-severe cases based on WHO ordinal scale and MaxFiO_2% on admission. Our findings further corroborate previous evidence for the negative prognostic role of increased NLR in patients with COVID-19 [23–26]. In particular, a very similar cut-off threshold (NLR > 4) reliably predicted ICU admissions in a small cohort of patients with COVID-19 in Italy [27], while a threshold of 11.75 was significantly correlated with in-hospital mortality in a large cohort of 1004 COVID-19 patients [28]. Besides its prognostic significance, NLR provides interesting mechanistic links as SARS-CoV-2 initially infects and kills T-lymphocytes leading to profound lymphopenia while at the same time viral inflammatory response impairs lymphopoiesis and increases lymphocyte apoptosis. Similarly to NLR, increased LDH levels were associated with worse clinical outcomes, as assessed by WHO ordinal scale and MaxFiO_2% on admission. Although LDH has been traditionally used as a marker of cardiac damage, elevated levels can result from multiple organ injury and may reflect decreased oxygenation with upregulation of the glycolytic pathway [29]. Nonetheless, it represents a marker with low specificity and sensitivity, as it can be increased in a variety of conditions resulting in tissue hypoxia, including infections, renal, lung and cardiac diseases. An association between elevated LDH blood levels with poor prognosis in patients with COVID-19 has been recently reported [30].

Our study exhibits a number of limitations that need to be treated cautiously. First, this was an epidemiological report and was not designed to provide mechanistic data. Second, as it happens with all hospital-based epidemiological studies that report patient characteristics, data quality tends to be scarce, underpowered and limited by sampling bias. Finally, follow-up assessment was short and thus valid conclusions cannot be drawn.

In conclusion, we present the first epidemiological report from a low-incidence and mortality COVID-19 country during the first wave of the pandemic. Our data validates previous findings supporting a relatively homogeneous clinical, laboratory and radiological appearance of COVID-19 on a global scale. NLR may represent a reliable disease prognosticator with important mechanistic links leading to timely and optimal treatment decisions. Low prevalence rates of chronic lung diseases, including COPD, asthma and ILDs could reflect the beneficial impact of early implementation of containment measures. Larger epidemiological studies and longitudinal population-based analyses are sorely needed to prove these concepts.

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Conflicts of interest

AT received consultant honorarium and travel grants from Boehringer Ingelheim Hoffmann La Roche, Elpen Pharma and Chiesi Hellas outside the submitted work. DB received consultant honorarium and travel grants from Boehringer Ingelheim Hoffmann La Roche and Elpen Pharma outside the submitted work. EM received consultant honorarium and travel grants from Boehringer Ingelheim and Hoffmann La Roche

outside the submitted work. SP received consultant honorarium and travel grants from Boehringer Ingelheim and Hoffmann La Roche outside the submitted work. SL received consultant honorarium and travel grants from Boehringer Ingelheim, Chiesi Hellas, Astra Zeneca, Menarini and GlaxoSmithKline outside the submitted work. AB received honorarium and travel grants from Chiesi Hellas, Elpen Pharma and Menarini outside the submitted work. The rest authors have nothing to disclose.

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VIA MEDICA

RESEARCH PAPER

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"To do or not to do — that is the question". Transvascular needle aspiration during EBUS (EBUS-TVNA) with review of the literature

Abstract

Introduction: Large vessels are often encountered during endobronchial ultrasound (EBUS). Safety of traversing the vessels weighed against a more invasive procedure can be a dilemma.

Material and methods: We describe a case series of 8 patients who underwent transvascular needle aspiration during EBUS, to access a lesion in the absence of an alternate safe window. A 21 gauge EBUS needle was used to traverse either the main or a major branch of the pulmonary artery.

Results: Malignancy was suspected at ROSE in five cases. Granuloma and necrosis noted in 2 cases were confirmed as tuberculosis on culture. Diagnostic yield of EBUS-TVNA was 87.5% (7/8). No complications were noted in the immediate post-operative period as well as during 6 months of follow up.

Conclusion: EBUS-TVNA in carefully selected patients is a feasible alternative to more invasive procedures with excellent yield. Appropriate intraoperative, perioperative and postoperative monitoring and care must be available in the case of fatal bleeds.

Key words: EBUS, transvascular, TVNA, transvascular needle aspiration, transvascular biopsy

Adv Respir Med. 2021; 89: 386–391

Introduction

Invasive sampling of the mediastinal lymph nodes for lung cancer staging is recommended by American Thoracic Society (ATS), European Respiratory Society (ERS), and American College of Chest Physicians (ACCP) [1]. Minimal invasive procedure — endobronchial ultrasound (EBUS) [2] — guided needle biopsy is a well proven diagnostic tool for both malignant and nonmalignant diseases [3].

EBUS has, for the most part, made a greater portion of the mediastinum accessible to biopsies, especially when combined with EUS-B (transesophageal endobronchial ultrasound). Some stations in the mediastinum are inaccessible or, access to these locations is limited by the presence of one of the great vessels. These areas include stations 3a, 5 and 6 and elsewhere in the hilum when a branch of the pulmonary artery or more proximally, azygos, superior vena cava or brachiocephalic vessels, may be interposed between the airway and the pathology. These were once upon a time considered beyond the reaches of EBUS [4] because biopsy of these lesions needed traversing the great vessels. Life-threatening bleeding with EBUS-TBNA resulting probably from accidental pulmonary artery puncture have been reported [5, 6]. Multiple case series have now been published confirming relative safety of traversal of the great vessels in experienced hands [7–11].

Material and methods

We retrospectively analyzed patients who underwent endobronchial ultrasound with transvascular needle aspiration (EBUS-TVNA), during a 3-year period from January 2018 to December 2020. Procedural informed consent was obtained, and the study being a retrospective observation, was approved by the institutional ethics committee.

The protocol followed for the patients was not any different than for the other cases of

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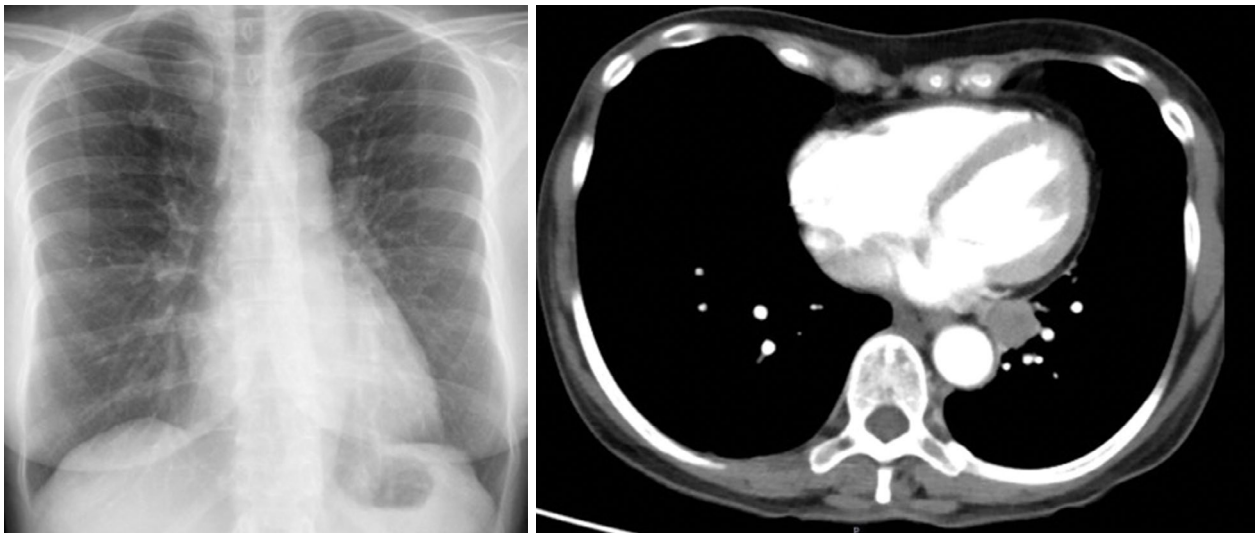

Figure 1. Retrocardiac lesion

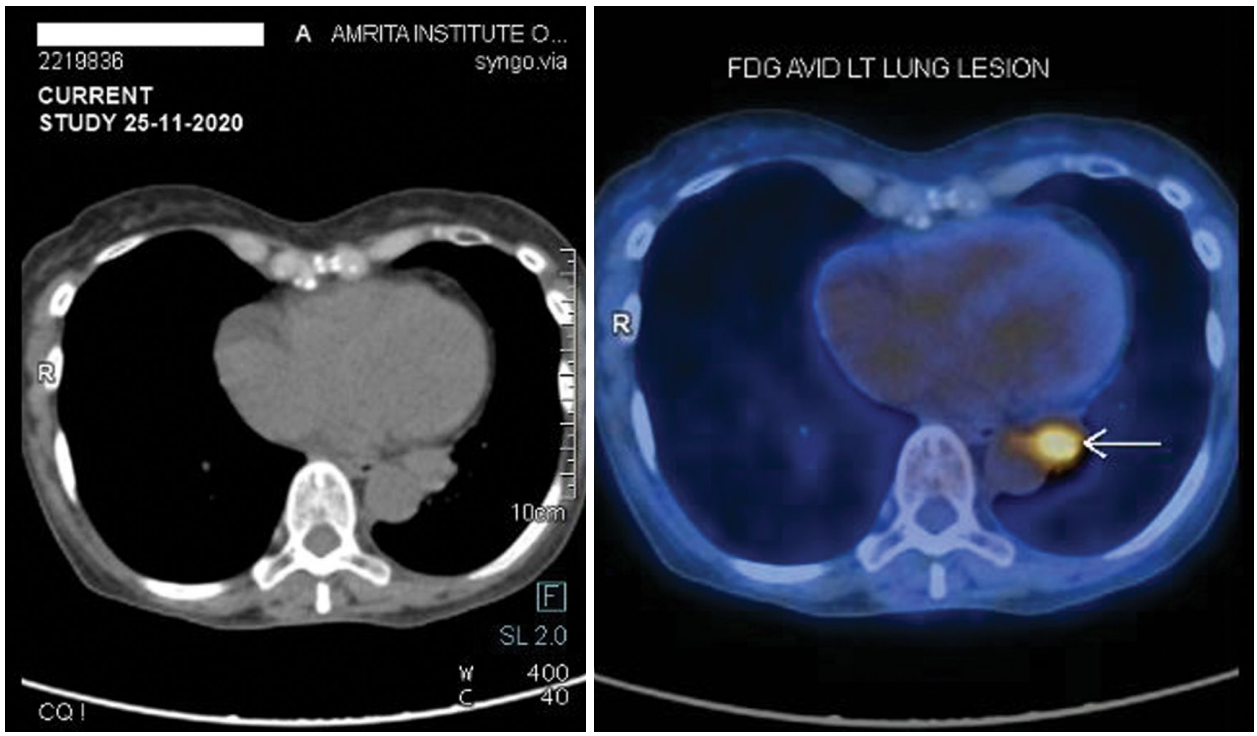

Figure 2. Fluorodeoxyglucose avid retrocardiac mass lesion

EBUS. Images available, including CT chest and/or PET-CT, were evaluated preoperatively (Figure 1 and 2). All patients posted for EBUS-TBNA at our unit are screened for cardiopulmonary and other systemic diseases and if necessary, further workup is done before the procedure. Basic blood work is always reviewed. Additionally, planning with the anesthesiology team included risks regarding major bleeding. Patients were type- and cross- matched. All the cases were consulted by experienced interventional pulmonologists. NSAIDs, anticoagulation and antiplatelet medications were discontinued. All the cases were planned as a day case with thoracic surgery backup.

A laryngeal airway was placed for general anesthesia and this allowed precision of biopsy and limited passes. Careful evaluation of the lesion and the vascular structures were done to find an appropriate window for biopsy. Every

Table 1. Details of the cases who underwent transvascular needle aspiration during EBUS TVNA at Amrita Institute Of Medical Sciences, Aims Ponekkara, Kochi, India

Case no. & patient details	Indication	Size of lesion	Vessel traversed	No of passes	EBUS rose diagnosis	Final diagnosis
1. 76/M	Mass encasing brachiocephalic with right hilar mass	26 × 29	Interlobar PA	2	Squamous cell carcinoma	Esophageal cancer
2. 74/M	LLL mass	23 × 22	LMPA	1	Metastatic nsclca	Adenocarcinoma lung
3. 65/M	Station 5 lymph node	25 × 30	LMPA	3	Necrotic tissue	Tuberculosis
4. 53/F	LUL mass	53 × 39	LMPA	3	Adenocarcinoma	Adenocarcinoma lung
5. 55/M	Right hilar mass	28 × 27	Interlobar PA	3	Granuloma	Tuberculosis
6. 64/F	LLL mass/lymph node	24 × 17	LMPA	3	Nsclca	Adenocarcinoma lung
7. 47/M	Right hilar mass	26 × 29	Interlobar PA	3	Suspicious for malignancy	Spindle Cell Neuroendocrine neoplasm
8. 49/F	Left hilar mass encasing the great vessels	25 × 28	LMPA	3	Nsclca	Adenocarcinoma

Interlobar PA — right main PA; NSCLCa — non small cell lung cancer; PA — pulmonary artery; LMPA — left main pulmonary artery; PA — pulmonary artery

attempt was made to avoid traversing the vasculature. Olympus scope BFUC180F was used for all the procedures. Twenty-one-gauge EBUS needle was used for the biopsy. Once the site of entry was finalized, the needle was advanced into the lesion traversing the vessel (Figure 3, 4). Ventilation was held during the puncture to avoid any shearing of the vessel wall. Once the needle was in the lesion, the stylet was removed, and the needle was moved back and forth 5 times with the application of suction. The sample was analyzed using rapid onsite evaluation (ROSE). Maximum of 3 needle passes were done for each of the lesions. After each pass, the airway was examined for bleeding and the vessel was checked for hematoma. Cold saline and epinephrine (1:10000) were used for hemostasis. Fogarty balloon catheter and a bronchial blocker was on standby for all the procedures.

Results

During the study period 2018–2020, 8 patients underwent EBUS-TVNA at our institution. The lesions were either at regular lymph node stations or had one of the vessels obstructing the needles direct access to the lesion.

Eight patients were identified out of which six had planned procedures done using general anesthesia, while in the other 2 cases, the decision was taken during the procedure done under procedural sedation. A transvascular approach was only adopted if a clear window to access the lesion for biopsy was absent.

Three cases involved station 5 where the left main pulmonary artery was traversed. Two cases needed traversing the common basal trunk of the left pulmonary artery. Three other cases needed traversing the interlobar pulmonary artery (right) for right hila masses. Six out of the eight cases were noted on ROSE to be suggestive of malignancy (75%) and confirmed on final diagnosis. ROSE identified necrosis in 1 and granuloma in another subject, both of which were finally diagnosed as tuberculosis.

Four of the cases developed mild bleeding needing cold saline and epinephrine. No major bleeding was noted. Three needle passes were done to ensure adequacy of material for immunohistochemistry (IHC).

Discussion

Prior to the advent of EBUS, TBNA typically of station no. 7 (subcarinal) was a blind procedure done based on pre-procedure CT scans. Blood aspiration meant pulmonary artery puncture in which case, the needle was withdrawn and re-inserted at a different site if the airway was clean. EBUS-TBNA has prevented such accidental punctures. Fatal bleedings [5] have been reported in the literature, but EBUS-TBNA is for the most part a safe procedure [12]. Complications of hemomediastenum, hemothorax and pneumomediastinum after EBUS-TBNA [6] have been reported. Despite these concerns, multiple case series have documented relative safety of transvascular needle aspiration as summarized in Table 2.

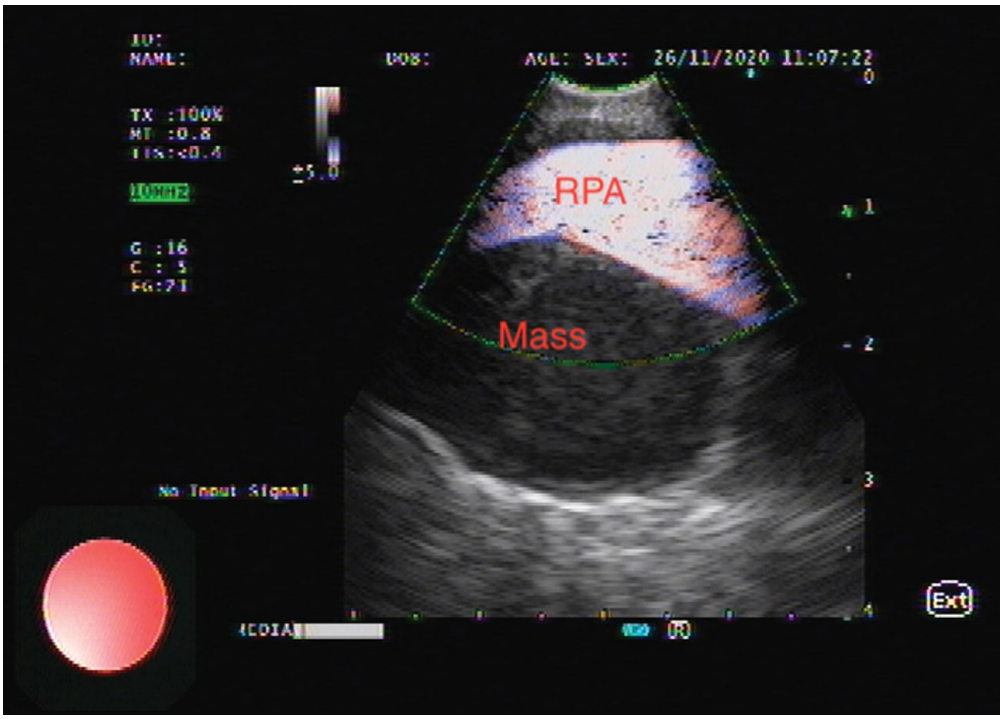

Figure 3. Lesion beyond the right main pulmonary artery with Doppler

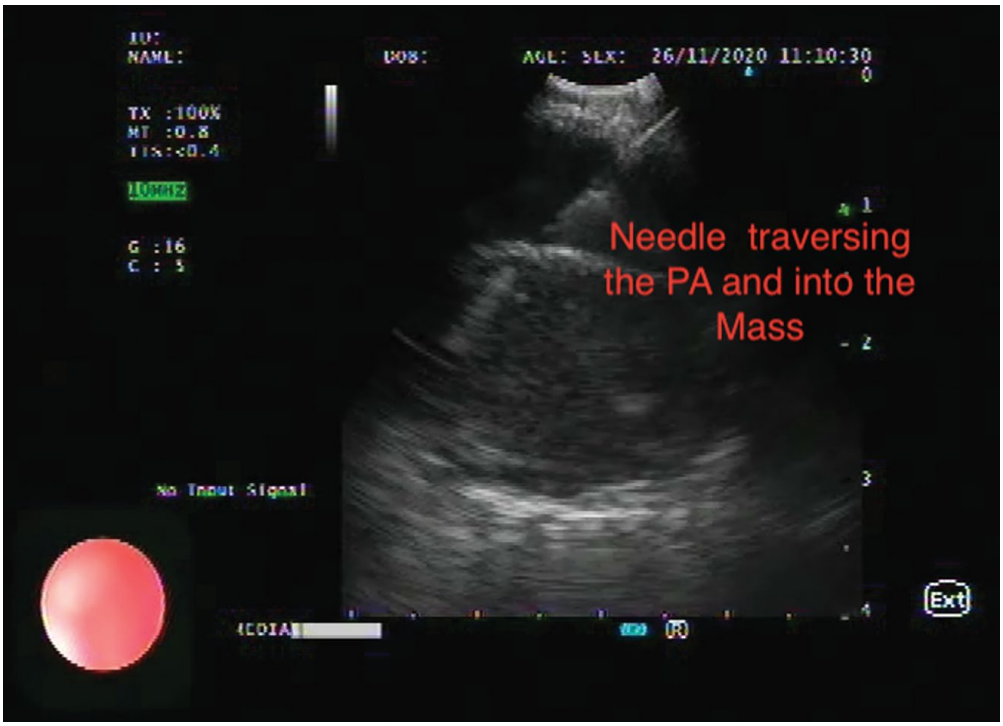

Figure 4. Transvascular needle aspiration during endobronchial ultrasound with transvascular needle aspiration. PA — pulmonary artery

Suprasternal puncture of the pulmonary artery has been recorded in the literature to be harmless dating back to the 1950's when the pulmonary arteries were inadvertently entered while aiming for the left atrium to do flow studies [13]. Physiologically, the pulmonary artery system is a low-pressure high-capacitance structure. Anatomically, the pulmonary veins have a thin wall. The low-pressure system contributes to less bleeding with a small-bore needle (21–22 gauge)

Table 2. Summary of cases series with endobronchial ultrasound with transvascular needle aspiration

Study	Year of publishing	No. of cases	Vessel traversed	Diagnostic yield [%]	Complications
Nuguru *et al.* [18]	2017	12	MPA/branch	100	None
Naaman *et al.* [16] (Abstract)	2020	35	MPA/branch, azygous, SVC, brachiocephalic artery	Unclear but 89% of the malignancies diagnosed correctly	1 each of: respiratory failure, atrial fibrillation-related tachycardia, moderate bleeding
Rachid MM [19] (abstract)	2018	24	MPA/branch	91.66	None
Folch [9]	2016	10	MPA	90	None
Kazakov [8]	2017	33	MPA/branch, aorta	73	None
Panchabhai [11]	2015	10	MPA/branch	90	None
Mehta *et al.* [12]	2018	10	MPA/intra PA	90	None
Our study	2020	8	MPA/branch	100	None
Boujaoude *et al.*[10]	2013	2	Branch MPA	100	None
Cetinkaya *et al.* [20]	2018	4	MPA/branch	100	None

LMPA — main pulmonary artery; PA — pulmonary artery

biopsy, but the thin wall makes the risk of tear higher. Longer needle dwell time in the vessel and respiratory excursions increase the risk of vessel injury. The pulmonary artery is in close proximity to a significant portion of the airway and need for EBUS-TVNA is not unusual.

Boujaoude *et al.* [9] reported safety of puncturing the main pulmonary artery in 2013 in 2 cases and since then most of the vessels seen during EBUS [7, 14] have been traversed. Intrapulmonary artery lesions have also been sampled with good yield and safety profile [11, 15].

General anesthesia is used in most of the case series except Folch *et al.* [18] and Kazakov *et al.* [7]. Just as in our series, they also noted that anesthesia did not influence the complications. In a large series of 35 patients, Naaman *et al.* [14] (published as an abstract) noted reversible mild to moderate complication in only 3 patients, and all of them recovered fairly well without any major sequalae. Similarly, puncturing other vessels, e.g. azygous, SVC and brachiocephalic artery did not change the risk [14]. Our study involved puncturing the main pulmonary artery at different sites, and we did not notice any increased complication rate just as noted by Panchabhai *et al.* [10]. Kazakov *et al.* [7] in their series traversed the aorta as well and did not notice any increase in complications.

The average diagnostic yield for the EBUS-TVNA in various case series seems to be well over 80%. We found a diagnosis in 87% in our study, though in 2 of the cases, the definitive diagnosis was not confirmed immediately but was ultimately established as tuberculosis. ROSE had identified granuloma and necrosis. As with the other case series, overall final diagnosis of malignancy was very high in our series as well, i.e., at 75% (6/8).

Findings from our study add to the growing literature of safety and excellent diagnostic yield of EBUS-TVNA in selective cases.

Our case series has several limitations. The sample size was small, and this was a retrospective chart review which carries its drawbacks. The small number is probably related to the relative low frequency of these types of cases seen in clinical practice, which was also noted in many other series in the literature. Ours was also a single-center experience. Being retrospective in nature, subtle postoperative complications could have been missed. We had an excellent diagnostic yield but did not have another procedure to compare with. Overinterpreting of our results, both diagnostic yield and the absence of complications, would be unwise and we would strongly advice against that.

The EBUS-TVNA should only be attempted as a last resort after weighing all options and is not a replacement for the more invasive procedure like mediastinoscopy. Adequate preoperative, intraoperative and perioperative planning, monitoring and taking precautions like cardiothoracic surgery on standby, are the keys to doing a successful procedure. The opinion as mentioned in the various case series is one of caution. Preferably, this procedure should be attempted in a high-volume center and only by skilled interventional pulmonologists. We strong-

ly recommend choosing the patient carefully and always attempting to avoid vessel, even though EBUS-TVNA appears to be a relatively safe procedure. While general anesthesia seems to be the common consensus for this high-risk procedure, cases done under conscious sedation seem to have similar results. But we would still advice taking a safer approach to this procedure.

Conclusion

In conclusion, using transvascular approach expands the stations and areas in the mediastinum amenable to biopsy during EBUS. EBUS--TVNA appears to be a relatively safe procedure in skilled hands, but safety precautions with strict adherence to protocols and patient selection are advised.

Conflict of interest

None declared.

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Potential benefits and hazards associated with the use of e-cigarettes — a guide for practitioners and current status in Poland

Abstract

The use of electronic cigarettes has dynamically increased over the last few years. Meanwhile, the knowledge regarding their safety has been rapidly changing, which could be a challenge for a medical practitioner. The purpose of this review is to summarize the latest reports and to verify statements on e-cigarettes' influence on health, including in the context of the ongoing SARS-CoV-2 pandemic. Awareness of the benefits of e-cigarettes can provide vital support for doctors caring for patients who smoke traditional cigarettes. Nevertheless, attention should be paid to the dangers of the medically unjustified use of electronic cigarettes. Despite the idea of releasing e-cigarettes into the market as a harmless alternative to traditional cigarettes, this product also has a negative impact on health. Replacing traditional cigarettes with e-cigarettes provides well-documented benefits to patients with certain indications such as hypertension and asthma, as well as to smokers who intend to minimize the negative effects of passive smoking on their environment. Moreover, it could be valuable for patients who are willing to permanently overcome a nicotine addiction, especially when previous attempts to quit smoking with nicotine replacement therapy (NRT) monotherapy were unsuccessful. Electronic cigarettes are a rapidly developing technology and an innovative form of a well-known addiction, so it is essential for practitioners to stay informed.

Key words: electronic cigarettes, smoking, nicotine addiction, SARS-CoV-2

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Introduction

The popularity of e-cigarettes worldwide has considerably increased over the last few years. It is claimed that they are consistently used by 1,4% of the Polish population aged 15 and older, and 4% declare having used them at least once [1]. From 2014 to 2017, a 20% increase in their use had been noted in the European Union. Currently, it involves almost 2% of Europeans [2]. Numerous myths have existed in the public sphere and the confidence in their authenticity is the reason why the popularity of the phenomenon called vaping is growing. In Poland, aside from being a fashionable trend, the primary reasons for using e-cigarettes include them being a healthier alternative to smoking, the belief in their efficacy in overcoming nicotine addiction, and saving money [3]. Foreign studies confirm that the most common excuse for the initiation of e-cigarette usage is a conviction about their beneficial effects on smoking cessation and on the environment [4]. The increase in e-cigarette use is particularly high among young people [5].

An electronic cigarette is a small, battery-powered device filled with liquid containing nicotine. It gives an impression of traditional cigarette smoking [6]. The Polish legal definition describes an e-cigarette as a "product which is used for the consumption of vapour containing nicotine by using a mouthpiece […]; e-cigarettes may be of

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single-use or can be refillable […]" [7]. Currently, an e-cigarette resembles a thick pen made of two parts: a source of power, hence a battery, and a vaporizer with a container filled with fluid, which contains nicotine in various concentrations and flavours, as well as typically glycerine and propylene glycol. Nicotine consumption is based on inhaling steam produced as a consequence of boiling this liquid. Such a process does not produce smoke, which is associated with traditional cigarettes.

In Poland, the addiction to e-cigarettes has expanded specifically among the younger generation — 60% of middle school students admit using both traditional and electronic cigarettes, another 10% declare the use of just e-cigarettes, and 11% of teenagers who have not tried traditional ones tried an electronic cigarette during the last thirty days (an increase of over 1/3 as compared to the time period between 2014–2017). Subsequently, the percentage of people who regularly vape has risen three times over — up to 6%. Generally, the proportion of people who have used e-cigarettes exclusively during the last thirty days has grown fivefold from 2% in 2010–2011 to 11% in 2015-2016. As high as 24% of interviewed individuals confirm the consumption of both electronic and traditional cigarettes over the last thirty days. It represents a sixfold increase from 4% in 2010–2011 to 24% in 2015–2016 ($p < 0.05$) [5].

Even though the percentage of smokers has slightly decreased in our country (from 24% in 2017 to 21% in 2019 with a statistically unvarying difference between men and women), the number of people reaching for e-cigarettes has grown, especially among young people living in cities with more than 100,000 inhabitants [1, 3]. Taking into account the expansion of e-cigarette use in Poland, the aim of this review is to elaborately summarize the current state of knowledge concerning their potential benefits and hazards.

Influence on the respiratory system

Traditional cigarettes have been proven to have significant adverse effects on the respiratory system and are the main cause of lung cancer and chronic obstructive pulmonary disease (COPD) [8]. This toxic influence is derived from the occurrence of many hazardous substances in tobacco smoke. Studies showed that they initiate the inflammatory process resulting in the development and progress of COPD and lung cancer [9, 10]. In recent years, the morbidity and mortality from COPD has notably increased [11]. According to the estimated data, 2 million people in Poland suffer from this disease and 15 thousand die from its complications every year. It is well-known that adults who smoke traditional cigarettes have a 61% greater risk of developing asthma, and a 1.71 times greater risk of exacerbations of asthma in comparison to non-smokers ($p < 0.05$) [12]. Furthermore, it is confirmed that children who are subject to passive smoking have a 20% greater risk of developing asthma. The need to utilize corticosteroids for asthma exacerbations increases by 1.71 ($p = 0.004$) [13, 14]. Additionally, the fetal exposure to tobacco smoke is associated with lung underdevelopment and other minor dysfunctions which lead to obstructed air flow later in life [15].

The presence of many toxic substances in tobacco smoke has not been confirmed in e-cigarette vapour. However, flavour extracts present in e-cigarettes may expose their users to health risks [16]. Moreover, according to research where the influence of 5-minute usage of an e-cigarette on lung function was tested among healthy adults, it was found that even short-term vaping causes immediate serious adverse effects similar to traditional cigarette smoking [17].

Multiple sweet aromas were tested with the aim of searching for toxic chemical substances. The findings have shown that most of the samples contained diacetyl, the presence of which was associated with the occurrence of bronchiolitis obliterans, also called "popcorn lung" [16, 18]. Importantly, the *in vivo* study has demonstrated the inefficiency of cellular protease as a consequence of aerosol exposure, which suggests the potential role that e-cigarettes may have in the pathogenesis of emphysema in COPD [19]. Additionally, there is a 39% greater risk of developing asthma among current e-cigarette users as compared to people who have never used them. At the same time, the increased intensity of usage was observed to be associated with an increased probability of asthma development [20]. On the other hand, the substitution of traditional cigarettes for electronic cigarettes among people already diagnosed with asthma was observed to be associated with long-term benefits such as alleviation of symptoms and better asthma control with a reduction in the number of exacerbations [21]. Nonetheless, one of the e-liquid ingredients, propylene glycol, may worsen both asthma and COPD despite being relatively non-toxic [22].

In July of 2019, the Wisconsin Department of Health Services and the Illinois Department of Public Health received many notifications about lung injuries associated with e-cigarette usage

(EVALI, e-cigarette, or vaping, product use-associated lung injury), which started coordinated research projects in order to establish the exact cause of this pathology. On the 6th of September 2019, there were 98 patients who matched the definition of probable or confirmed cases of EVALI. The average age was 21. The chief reported complaints involving the respiratory system (especially shortness of breath, cough, and chest pain) as well as the digestive system (particularly nausea, vomiting, diarrhea, and abdominal pain). Among 81 extensively interviewed patients, 73% of them admitted using nicotine products and 89% of the products contained tetrahydrocannabinol, the basic psychoactive substance in cannabis [23]. According to another study from 2019, e-cigarettes were associated with a wide variety of lung diseases, primarily acute eosinophilic pneumonia, diffuse alveolar damage, and lipoid pneumonia. Presumably, the contributory factor in these pathological entities is tocopheryl acetate, also known as vitamin E acetate, an additive in e-liquids with tetrahydrocannabinol [24]. The analysis of the bronchoalveolar lavage led to the detection of vitamin E acetate in the epithelial lining fluid (ELF) among 48 of 51 patients with EVALI and, concurrently, the absence of this ester was confirmed in the healthy controls [25]. By the 18th of February 2020, the CDC had 2,807 reported cases of hospitalized or dead EVALI patients [26]. It should be emphasized that, in European Union countries where THC is forbidden, EVALI was not observed [27].

Influence on the circulatory system

The detrimental effect of traditional cigarette smoking on the cardiovascular system has been thoroughly acknowledged and described. Its association with coronary artery disease, myocardial infarction, arteriosclerosis, aortic aneurysm formation, and peripheral arterial disease has been proven. The harmful outcome of passive smoking was also confirmed [28]. Furthermore, studies on monozygotic twins where only one of them was a traditional cigarette smoker revealed that a smoking sibling had the median plasma renin activity 99% greater at rest and 84% greater during a submaximal exercise as compared to the other twin ($p < 0.01$). Additionally, during exercise, the smokers had the median level of aldosterone generally 40% greater than their nonsmoking siblings ($p < 0.05$). This phenomenon partly explains the tendency for vasoconstriction to occur in traditional smokers [29].

Considering the above, adverse effects of traditional cigarettes may be divided into those resulting from nicotine toxicity and those associated with other chemical substances and physical factors. Nicotine acts as sympathomimetic, thus it increases myocardial oxygen demand [30]. Moreover, it was shown that in rats with primary hypertension, it augments a risk of developing hypertrophic cardiomyopathy [31]. Another toxic substance present in tobacco smoke is carbon monoxide (CO), which binds with hemoglobin thus reducing the oxygen-binding capacity of blood. As a consequence, it leads to hypoxemia, overproduction of erythrocytes, and hypercoagulability [32]. The correlation between smoking and raised inflammatory markers was also proven [33]. The chemical composition of classic cigarettes was examined providing information about the presence of heavy metals in tobacco smoke – particularly manganese, nickel, and chrome [34]. These metals catalyze protein oxidation in epithelial cells, resulting in their malfunction [35]. In addition, the cobalt concentration in the aorta correlates with the number of smoked pack-years, which increase the risk of aortic aneurysm formation [36]. As a consequence of the above factors, the risk of developing cardiovascular disease increases.

Hazardous substances discovered in e-cigarette vapour include nicotine and heavy metals. However, it seems that the concentration of heavy metals in aerosols does not appear to be at toxic levels and is certainly lower than the concentration in traditional cigarette smoke [37]. In turn, the amount of nicotine and its resulting toxicity varies due to different liquids used in different types of these devices ranging from nicotine-free cartridges to ones with a nicotine content higher than in classic cigarettes [38].

It was proven that e-cigarettes elevate both the systolic and diastolic blood pressure and increase the heart rate, but to a much lesser degree than traditional smoking. Furthermore, e-cigarettes have no influence on the amount of exhaled carbon monoxide, which is in marked contrast to classic cigarette smoke (eight times greater than the baseline) [39]. Among smokers treated for hypertension, e-cigarettes facilitated a reduction in systolic and diastolic blood pressure. This effect was long-lasting and was seen early in the course of treatment when smokers of classic cigarettes were beginning to use e-cigarettes, which at the same time limited the use of the classic ones [40]. A prospective study based on healthy young individuals aged 29.7 (± 6.1)

did not show a negative impact of e-cigarettes on the circulatory system (respiratory rate [RR], systolic and diastolic blood pressure [SBP and DBP]) after 3.5 years of vaping. Presumably, we should wait longer to observe the adverse effects of aerosol exposure. Currently, long-term studies of this kind are not available [41].

Influence on carcinogenesis

The inhaled smoke from classic cigarettes is recognized as the main cause of neoplasia [28]. Multiple substances present within this smoke qualify as carcinogenic substances according to the International Agency for Research on Cancer [42].

Considering the influence of e-cigarettes on the development of oncologic conditions, there are only a few studies which have been conducted in the recent past. Currently, there is no clear evidence-based affirmation of the carcinogenic role of vaporizers. The majority of conclusions are based on studies involving animal and cell cultures [43].

In its 2018 report, the American National Academy of Sciences, Engineering and Medicine (NASEM) informed about the influence of e-cigarettes on public health, including the risk of carcinogenesis. The report states that the only correct conclusion which can be made from the up-to-date research is the confirmation of the presence of mutagens in e-cigarettes' aerosol. Indeed, these substances are proven, both in vitro and in vivo, to damage DNA. In high concentrations, they have also been proven to damage human DNA, yet they cannot be explicitly connected with the possibility of carcinogenesis among active e-cigarette users. Further research is required to clarify if the concentration of these mutagens in the inhaled aerosol is sufficient enough to initiate mutagenesis. However, there is limited existing evidence showing increased carcinogenesis in animal models secondary to long-term exposure to e-cigarette vapour [44].

Since the publication of the NASEM report, new studies have suggested the possibility of the causal association between e-cigarettes and the development of cancer. For instance, mice which were exposed to this aerosol for 54 weeks had a significant tendency to develop lung adenocarcinoma (225% out of 40 animals involved in this experiment) as well as urothelial hyperplasia of the bladder (575% out of 40). In the control group, there was only one mouse out of 40 which developed lung cancer [45]. In another research article, experimental mice were implanted with breast cancer cells. The prevalence of tumor progression was horrific. 100% of the animals which had been previously exposed to e-cigarette aerosol developed cancer, compared to 33% in the control group which had only been breathing room air. Moreover, this progression was evidently faster in the exposure group than in the control group. Lung metastases were also significantly more frequent among rodents from the exposure group. The same research clearly confirmed the increase of the capability of pathologic cells to escape apoptosis after exposure to e-cigarette vapor [46]. Based on these observations, it was assumed that vaping was associated with tumor progression as well as with a limitation of the immune response, which enabled destruction of cancer cells shortly after their implantation. Another study validated an increase in the degree of DNA degradation and a decrease in its repair activity in mice lungs, hearts, and bladders, as well as in cell cultures obtained from human lung and bladder cells ($p < 0.05$) [47].

There are only a few studies on the subject of the health hazards of passive e-cigarette smoking. Even though there are significantly lower concentrations of toxic substances in the aerosol than in tobacco smoke, they can potentially lead to serious consequences, especially when vaping takes place in a limited, enclosed space, and when there is a high concentration of vapour. Passive smoking may possibly result in irritation of the airways from inhaling glycerol and propylene glycol vapour. The effects of nicotine aspiration (activation of the sympathetic nervous system, increased blood pressure, and tachycardia) as well as the potentially mutagenic activity of tobacco-specific nitrosamines should also be taken into consideration, although they are not currently included in most of the liquids [48]. It seems that the use of e-cigarettes in closed spaces does not endanger passive smokers by noxious concentrations of toxic substances. However, the influence of nicotine may be perceptible and potentially adverse [49]. No evidence has been found pointing to increased carcinogenic risk or the absence of such a risk in individuals exposed to passive electronic cigarette smoking. The only undeniable fact is that smokers are subject to a lower concentration of toxic substances while using electronic devices rather than traditional cigarettes [44].

Influence on the oral cavity

There are two main dental problems related to tobacco smoke exposure: tooth decay and

periodontitis. It has been proven that biological mechanisms associated with active smoking are part of the pathogenesis of both of these afflictions [28, 50]. Additionally, it should be taken into consideration that smokers tend to less frequently and less effectively take care of their oral hygiene [51]. Other medical oral cavity conditions associated with tobacco smoking include: teeth discoloration, smoker's melanosis, acute necrotizing ulcerative gingivitis, burns, leucoplakia, nicotine stomatitis, palatial erosions, gingival recession, epithelial dysplasia, and oral squamous cell carcinoma [52]. The most commonly reported symptoms in patients smoking traditional cigarettes are halitosis, taste disorders, or xerostomia.

The answer to the question of whether electronic cigarette use makes any contribution to the development of tooth decay or not has still not been found. However, the recent findings show that some of the liquid formulations used for vaping may be conducive to the progression of this disease, especially those of sweet flavour which attract young people [53]. According to a cross-sectional study from 2013–2014, the condition of an e-smoker's periodontium is worse in comparison to individuals who have never smoked [54]. Periodontal parameters were tested in three groups: 1) men smoking only classic cigarettes; 2) exclusive e-cigarette users; and 3) non-smokers. Naturally, participants in the first group had the highest plaque index (PI) (dental plaque within the whole oral cavity), whereas the lowest PI belonged to the third group. However, it should cautiously be noted that there is a disproportion between the duration of classic cigarette smoking and the duration of vaping [55]. Moreover, there is a pilot study which showed a gradual improvement of periodontal indicators among people who exchanged traditional cigarettes for electronic devices [56]. Vaping was also associated with transient oral and throat irritation and contributed to adhesion and biofilm formation on the surface of teeth as a result of the intense growth of Streptococcus mutans [57, 58]. According to a wide range of *in vitro* research, epithelial cells and fibroblasts may be injured by e-cigarette aerosols and may subsequently account for poor oral health [44].

Influence on reproductive function and the course of pregnancy

The findings obtained by studying the influence of tobacco smoking on reproductive function present a correlation between the intensity of exposure to tobacco smoke and erectile dysfunction among men [28]. Moreover, smokers' sperm count and motility were both reduced and there was a 22% average decrease in their concentration in comparison with non-smokers ($p < 0.001$). Changes affecting female smokers involve the impairment of reproductive capability as well as the occurrence of premature menopause on average from one to four years earlier in contrast to women who have never smoked [59].

Most of the data concerned with the influence of electronic cigarette use on reproductive function is derived from studies on animal models. It was shown that embryo implantation is delayed in mice subjected to aerosol from e-cigarettes [60]. It was also observed that, after similar exposure in rats, the number of sperm cells in the semen obtained from an epididymal tail was significantly lower. A decrease of 24% was noted in the group exposed to e-liquids without nicotine ($p < 0.05$), and of 10% in the group exposed to nicotine-containing aerosol ($p < 0.01$), when compared to a control group. The level of circulating testosterone was also below normal range — 50% for e-liquids without nicotine ($p < 0.001$) and 30% for e-liquids containing nicotine ($p < 0.01$) [61]. Data acquired from animal models concerned with the influence of vaping on reproductive organs serves as a warning as there is increasingly frequent e-cigarette usage among young people who could become potential future parents [46].

Smoking cigarettes during pregnancy increases the risk of the occurrence of low birth weight, miscarriage, eclampsia, sudden infant death syndrome, and perinatal mortality [28]. During the perinatal period, it may be the cause of neurocognitive disorders.

The consequences of e-cigarette use during pregnancy are not yet completely known [44]. There is a popular conviction among pregnant women about their safer profile in comparison with traditional cigarettes, as well as their apparent beneficial effect in patients with a smoking addiction during this period [62]. The occurrence of unfortunate side effects as a result of frequent e-cigarette usage among pregnant women in the USA is a consequence of such thinking [63]. The importance in critically examining existing findings cannot be overstated.

The correctness of this intuitive confidence in e-cigarettes' less harmful effect in comparison to traditional cigarettes has not been proven unambiguously. Due to ethical concerns in prospective research, attempts were made to verify this matter by using nicotine replacement therapy (NRT). The

influence of NRT on birth weight is still unclear. The findings are actually contradictory [64, 65].

Studies conducted on animal models appear to be more suggestive yet still insufficient. They showed normal birth weight of offspring of mothers who were exposed to e-cigarette vapour. However, their subsequent growth was stunted as opposed to individuals born in a control group [60]. Additionally, studies which were performed on rats reported that rodents previously exposed to vaping delivered low birth weight offspring in contrast to rats from the control group, and this difference was statistically significant. The same research also presented a distinct decrease of blood flow in the uterine artery (49.5%) and in the umbilical artery (65.3%) among the experimental group versus the comparison group ($p < 0.05$) [66].

Difference in the cost of smoking traditional cigarettes and vaping

It is still a challenge to formulate a precise analysis about costs borne by people smoking classic cigarettes as opposed to e-cigarette users. It comes from the fact that there is a great diversity between prices of traditional cigarettes and a wide range of prices of electronic cigarettes. The comparative analysis from 2017, performed on the basis of estimated data collected from 45 countries comparing the prices of equal units of nicotine, showed that traditional cigarettes are generally cheaper than single-use e-cigarettes. In the USA, this difference was $6.82 vs $7.99. In Poland, the difference was $4.18 vs $4.76. On the other hand, the price of liquid refills used in multiple-use e-cigarettes may be lower than classic cigarette packs, although the initial purchase of multiple-use electronic cigarettes poses a definitive financial burden on people exchanging smoking for vaping. After proper review of prices of traditional cigarettes and electronic multiple-use devices, the authors of this analysis estimated the time needed to recover the initial expense of switching to e-cigarettes for each country. In Poland, this amounted to a total of 6 days [67]. Nevertheless, it should also be emphasized that on the 1st of July 2020, a new tax was implemented on e-cigarettes in Poland which may result in a change in the calculations described above.

Dual use of nicotine products

The introduction of e-cigarettes is associated with a certain risk of simultaneous use with traditional cigarettes, which constitutes a phenomenon known as dual smoking (i.e. dual users). During three time periods (2010–2011, 2013–2014, and 2015–2016), with the aid of an anonymous questionnaire intended for upper-secondary students and technical school students in Poland (n = 5708), data was collected regarding current and previous tobacco smoking and e-cigarette use. 45% of the respondents confirmed a dual supply of nicotine in the past, and 17% of them admitted to dual use during the 30 days preceding the filling out of the questionnaire [5]. According to a Polish nationwide cross-sectional study from 2019, 28.6% of people declaring e-cigarette usage were also smoking traditional cigarettes on a daily basis [1]. It is a common phenomenon and every vape enthusiast is potentially subjected to a growing tendency towards additional exposure to tobacco smoke. Data from surveys gathered in the USA also confirmed this foregoing regularity — 31.8% of former users and 34.6% of current e-cigarette users (during the last 30 days) answered affirmatively to the question about any additional classic cigarette smoking [68]. Importantly, it was proven that teenagers using exclusively electronic cigarettes displayed a stronger tendency towards the new onset of traditional smoking during a 5 year follow-up in comparison to non-users of these devices [69]. However, it should be stressed that this statement is only correct with regards to e-cigarette users without a documented strong nicotine addiction. In fact, people with an advanced addiction to nicotine who have been consistently using e-cigarettes are less prone to simultaneous traditional cigarette smoking [70].

The addiction to classic cigarettes and e-cigarettes was analyzed in the research conducted in 2018 on a group of dual smokers using specially designed psychometric measures. On average, stronger addiction to traditional cigarettes was reported than to electronic ones. The differences in nicotine craving in both methods of delivery were documented, which suggests that dual users may actually differentiate the degree of addiction to each of these products [71]. Another study confirmed that e-cigarette users regarded vaping as causing less dependence to nicotine. Additionally, for people inhaling the aerosol, it was easier to restrain themselves from smoking in places where it was forbidden. The same group also reported a longer time from awakening to the first intake of nicotine in comparison to traditional smokers [72].

Combating nicotine addiction

A potential role of e-cigarettes in the process of overcoming smoking addiction is not suffi-

ciently known. In January 2018, The US Surgeon General reported various conclusions of differing potential. The authors of this report confirm the presence of limited evidence for e-cigarette use in the process of overcoming the addiction to smoking. Statements which suggested a greater efficacy of e-cigarettes containing nicotine were actually believed to be of moderate strength. Documentation regarding the advantage of e-cigarette use in combating addiction over other methods approved by the FDA (i.e. NRT, varenicline, bupropion) was found to be insufficient [44]. Nevertheless, new and high-quality research conducted shortly after the publication of the above report allowed for new light to be shone on previously provided conclusions. A British study which compared the effectiveness of NRT and e-cigarettes based on a sizable sample of patients (about 700 people in two corresponding size groups which finished an annual program) showed that e-cigarette use had an eminently better result for the purpose of overcoming nicotine dependency. The percentage of the annual success rate in a group which used traditional replacement therapy was 9.9%, whereas it was almost twice as high in a group using e-cigarettes (18%) ($p < 0.001$). Both types of therapy were assessed as less satisfying than smoking cigarettes by patients who took part in research studies. However, vaping was graded higher than NRT. E-cigarette users reported less irritability and fatigue, and a better ability to concentrate (relative to the initial stage) than patients receiving nicotine replacement therapy. Nausea was also less frequent in the vaping group. The NRT profile was more preferable in only two subgroups: those where there was a general degree of irritation of the oral cavity and throat (63.5% vs 51.2%), and in those where the irritation was severe (5.9% vs 3.9%) [73]. The helpful role of e-cigarettes was also noted in a study conducted on a group of over 1,100 smokers in which the efficacy of three therapies was compared. One of them was based on a monotherapy with the use of nicotine patches, whereas two others were combination therapies based on patches and e-cigarettes with nicotine-containing liquid or nicotine-free liquid. NRT without the addition of electronic cigarettes showed the lowest success rate. The group which received traditional replacement therapy had the highest percentage of patients who finished the participation in the program prematurely. NRT supplemented by e-cigarettes with nicotine was seen to provide the most effective method of quitting smoking, both during semi-annual and annual periods. Depending on the observation's time period, this method was from 3 to 7 percentage points more efficacious than the one with nicotine-free e-cigarettes. According to the authors, the optimal therapy was also 5 to 10 percentage points more successful than monotherapy with nicotine patches ($p < 0.05$) [74].

Yet another study showed that the use of e-cigarettes, which are similar in appearance to classic cigarettes, may be more successful in combating nicotine cravings than the use of devices which have a different appearance (i.e. most e-cigarettes of the newest generation). Despite a small sample size ($n = 63$), this study may present a valuable clue to people trying to overcome the addiction [75].

The association with COVID-19

The SARS-CoV-2 pandemic requires careful and new reflection in the context of the safety profile of e-cigarettes. The research conducted in July 2020 revealed that both smoking tobacco and vaping may increase vulnerability to COVID-19. However, it should be emphasized that e-cigarettes, which do not contain nicotine, contribute to the development of these changes to a much lesser degree than classic cigarettes. In contrast to the exposure to e-liquid aerosol, smoking tobacco was also proven to increase ACE2 expression — a cell receptor with a high-affinity for SARS-CoV-2 [76].

Patients who have a positive history of smoking tobacco show a much higher risk of having a severe course of the coronavirus disease (COVID-19) (RR: 1.31), in-hospital mortality (RR: 1.26), subsequent progression of disease (RR: 2.18), and need for mechanical ventilation (RR: 1.20) [77].

According to an online study conducted in May 2020 among teenagers and young adults ($n = 4351$) between the ages of 13 and 24, the diagnosis of COVID-19 is five times more probable for exclusive e-cigarette users and seven times more probable for dual smokers [78]. Moreover, considering the potential acute toxicity of electronic cigarettes involving the lungs and cardiovascular system, the use of these products may expose patients infected with SARS-CoV-2 to a greater risk of developing a severe course of COVID-19 [79].

Discussion

The period of over ten years where e-cigarettes have been present on the market have not been able to unequivocally prove and/or assess long term effects of their use. The intensification

of research associated with the increasing use of these devices provided strong information which should influence the perception of e-cigarette use by general practitioners. Based on the above review of literature, we would like to carefully investigate potential benefits as well as risks associated with e-cigarette use.

Vaping as a replacement for smoking classic cigarettes is beneficial to patients diagnosed with bronchial asthma. The exposure to aerosol rather than tobacco smoke contributes to a decrease in respiratory system symptom intensity and to an improvement in lung function. At the same time, it should be emphasized that e-cigarette use predisposes to the development of asthma, although the risk of its occurrence seems to be lower than that of traditional smokers. A possible decrease in the risk of developing COPD is the additional advantage — classic cigarettes are the major etiopathological factor here, whereas vaping was not unequivocally associated with it.

A particular benefit of exchanging traditional cigarettes for electronic ones is seen in patients suffering from hypertension. In this group, the positive change in blood pressure is statistically significant and permanent. However, it should be noted that any form of nicotine, a sympathomimetic, causes temporary elevation of the systolic blood pressure. It is particularly important in patients with poorly controlled hypertension. In this case, the use of liquids with low nicotine content might be preferable. Additionally, a significantly lower amount of toxic metals coupled with the lack of CO emission by electronic cigarettes decreases the cardiovascular risk.

In marked contrast to classic cigarette use, there were no documented cases of cancer development in humans due to e-cigarette use. However, animal-based research indicated possible carcinogenesis as a result of aerosol exposure. Therefore, a practitioner who treats patients with a positive history of using electronic cigarettes needs to remain oncologically alert. A convincing argument in favour of exchanging traditional cigarettes for vaporizers is their less harmful, if any, influence on "passive smokers" — aerosols do not contain confirmed carcinogens, whereas tobacco smoke is a rich source of them.

Currently, there is no sufficient evidence confirming the superiority of using e-cigarettes over traditional cigarettes in relation to oral cavity disease prevention. Serious afflictions caused by tobacco smoke, such as precancerous conditions or squamous cell carcinoma, were not convincingly associated with exposure to e-liquid aerosols. Patients undergoing dental care may potentially benefit from an exchange of classic cigarettes for electronic devices. However, it should be noted that the sweet aromas contained in liquids cause more frequently observed occurrences of decay, hence they should be avoided.

E-cigarettes are confirmed to be an effective way of fighting nicotine addiction. They have a greater efficacy in comparison with a single NRT according to studies from recent years published in prestigious periodicals (Nature, The Lancet). In the process of combating the addiction, electronic cigarette use also decreases the intensity of unpleasant feelings associated with withdrawal from nicotine, which results in fewer treated patients who terminate the therapy prematurely. Moreover, social functioning is relatively easier for those individuals who use vaporizers during their combat with nicotine addiction because of their lower irritability and fatigue, unlike among smokers.

A significant hazard associated with e-cigarette use is the phenomenon of dual smoking. Not only can it cause the summation of negative effects of both of these stimulants, but also increases the addiction to nicotine and can render the potential future fight with the addiction to be more difficult. This problem is fairly common, hence every practitioner in contact with a vaping person should evaluate it on an ongoing basis. It is absolutely vital for dual smokers to gradually limit smoking traditional cigarettes. It allows for early substantial benefits to the circulatory system and additionally facilitates reduction of nicotine intake.

The substitution of classic cigarettes for e-cigarettes also provides for practical financial benefit. The statistical analyses of average selling prices indicate that refillable e-cigarettes are less expensive than traditional cigarettes. Obviously, every person deciding to exchange classic cigarettes for electronic devices should be aware of the initial expense. However, in terms of Polish costs, an initial financial burden is recovered after only a few days and vaping becomes more affordable in the long-term.

Presently, it is crucial to investigate the connection between both methods of nicotine intake and the probability of the occurrence of coronavirus disease (COVID-19), as well as its severity. It was proven that both smoking and vaping increase the chance of infection, although traditional smokers may be at a higher risk. As of now, there is an insufficient number of comprehensive studies which would uniquely determine if exposure to e-liquid aerosol (versus exposure to tobacco smoke) increases the gravity of the

infectious process. The general awareness of the negative influence of e-cigarettes on the respiratory system seems to be sufficient to serve as an alert to individuals who vape and have a positive test result for SARS-CoV-2 infection.

Despite the advantages of e-cigarettes over classic cigarettes in many aspects, the e-cigarette phenomenon poses a whole new kind of threat to public health. As shown by large meta-analyses based on studies of young adults and adolescents aged 14-30, the odds ratio for the subsequent smoking of classic cigarettes between previous users of e-cigarettes and individuals who never used them is as high as 3.50 [80]. This indicates the high addictive potential of e-cigarettes among young people who, through these devices, begin their tobacco addiction at an early age and go on to use classic cigarettes.

It should be noted that more research is required in order to study the potential influence of e-cigarettes on human carcinogenesis as well as the potential association of vaping with the onset of COPD. Currently, no evidence exists regarding the safe use of e-cigarettes by pregnant women, therefore complete cessation of smoking and vaping is to be advised.

Conclusions

It should be acknowledged that despite the primary branding of e-cigarettes as a safer alternative to traditional cigarettes, this product is not devoid of negative impacts on the user's health. For this reason, they cannot be advised, especially to pregnant women. Despite present restrictions in their use, there is a range of indications for the replacement of classic cigarettes with electronic ones with well-documented benefits for the patient. The beneficiaries mainly include predominantly smoking individuals who are being treated for arterial hypertension or asthma, as well as smokers who want to minimize the harmful influence of passive smoking on their environment. The application of e-cigarettes is worth considering in patients who wish to permanently overcome their nicotine addiction. It may be especially recommended if previous trials of quitting smoking with a single NRT were ineffective. Due to the more frequently observed tobacco addiction among young people as a result of the use of e-cigarettes, this phenomenon should be perceived as a new threat to public health worldwide. In view of this threat, appropriate legal regulations limiting access to such devices and information campaigns on the harms of tobacco addiction should be implemented. Apart from the therapeutic implications mentioned above, electronic cigarettes may have other properties which should be a focus of study in the near future. In conclusion, a medical practitioner should track the progress of such research on an ongoing basis.

Conflict of interests

None declared.

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Endocrine paraneoplastic syndromes in lung cancer: a respiratory physician's perspective

Abstract

Lung malignancy is known to be one of the leading causes of cancer-related mortality. Endocrine paraneoplastic syndromes in lung cancer are common. These are due to secretion of various substances and not because of direct tumour invasion or metastasis. These syndromes have also been associated with lung cancer prognosis. This review describes the many endocrine paraneoplastic syndromes seen in lung cancer and narrates their incidence, biology, clinical features, diagnosis, and management.

Key words: endocrine paraneoplastic syndromes, lung cancer, hypercalcaemia, hyponatraemia, small-cell lung carcinoma

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Introduction

Lung cancer is considered to be the foremost cause of cancer-related mortality around the world [1]. Mortality rate in lung cancer is significant and is considered equal to that of prostrate and breast cancers combined. This is mainly because most of these patients present in advanced stages of cancer at the time of diagnosis [2]. The most important risk factor for lung cancer to date is tobacco smoke [3]. The late diagnosis of lung cancer in advanced stages is mainly due to the lack of clinical findings. Some patients may seek medical advice for symptoms not directly related to a malignancy because of the appearance of paraneoplastic syndromes, which in turn may lead to the diagnosis of cancer in the early stage, with early initiation of chemotherapy [4].

Paraneoplastic syndromes (PNS) are seen in malignant conditions with the clinical features caused by either production of hormones or functional peptides secreted by tumour itself. These should not be induced by direct infiltration and growth of the primary malignancy or metastases [5]. Improper immune cross-reaction of tumour cells with normal host cells can also be the rare cause of this syndrome [6]. Most common malignancy associated with PNS is lung cancer. Ten percentage of patients with lung cancer can have these syndromes [7]. In lung cancer, PNS are numerous (Table 1). Humoral hypercalcaemia of malignancy along with the syndrome of inappropriate antidiuretic hormone secretion seen respectively more in squamous-cell carcinoma and small-cell carcinoma are two of the most common endocrine PNS. The size of the primary tumour or stage of cancer has no relation with the symptom severity in these syndromes. These syndromes are diagnosed with specific criteria (Table 2), but all need not be fulfilled in clinical practice [5]. Especially, demonstration of hormones in tumour biopsy tissue is not practical in many cases.

The present review aims to present the incidence, tumour biology, clinical features, diagnos-

Table 1. Lung cancer-associated paraneoplastic endocrine syndromes

Humoral hypercalcaemia of malignancy
Syndrome of inappropriate antidiuretic hormone secretion
Ectopic Cushing's syndrome
Carcinoid syndrome
Rare: Acromegaly, hypoglycaemia, glucagonoma syndrome, Zollinger–Ellison syndrome

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Table 2. Diagnostic criteria for paraneoplastic endocrine syndromes

Endocrine function abnormality with absent physiologic feedback regulation
Respective endocrine lung should not have any metastasis
Worsening not explained by increasing tumour burden
Cancer treatment improves endocrine function
Tumour biopsy sample showing evidence of either hormone substance or its production

tic criteria, and treatment options for endocrine paraneoplastic syndromes in lung cancer.They are briefly described in Table 3.

Humoral hypercalcaemia of malignancy (HHM)

Incidence

Ten percent of all patients with advanced lung malignancy can have hypercalcaemia, and among

Table 3. Four most common endocrine paraneoplastic syndromes encountered in lung cancer and their features

	HHM	SIADH	Ectopic cushing syndrome	Carcinoid syndrome
Incidence	Baseline: 2–6% During course of cancer: 10% Most common histology: squamous-cell carcinoma	SCLC 70% of all PNS SIADH In SCLC 7–16% In NSCLC < 1%	10% of Cushing's syndrome are paraneoplastic 50–50% of PN-lung NET (SCLC, carcinoid)	1–5% in bronchopulmonary NET
Mechanism	PTHrP (most common): bind to PTH receptors in the bone, kidney and influence calcium, phosphorous regulation	Ectopic ADH secretion by cancer cells which inhibit free-water excretion in the distal tubule of the kidney	Cancer cells express POMC precursor gene which is translated into a prohormone later cleaved into ACTH	Serotonin release by cancer cells. Can be precipitated by certain food, exercise, or alcohol
Clinical features	Altered sensorium, polydipsia, polyuria, hypertonia, renal failure, vomiting	1) Depends on duration and degree of acuity 2) Frequent falls, weight gain, seizures, vomiting, depression and rarely come	1) Carcinoid: typical cushingoid features like centripetal fat distribution, systemic hypertension, proximal myopathy 2) SCLC: less cushingoid features, hyperglycaemia common, unexpected weight gain due to chronic water retention	1) Acute: prolonged flushing in upper torso anteriorly, bronchospasm, or diarrhea 2) Chronic: fibrosis of right heart valves, retroperitoneum 3) Rare: carcinoid crisis causing hypotension, cardiac arrest
Diagnosis	↑ Ca ↑ PTHrP ↓/N PTH	Clinically euvolemic: serum sodium < 125 mEq/l, urinary sodium > 40 mmol/L, urine osmolality > 100 mOsm/kg Rule out other causes of hyponatraemia — drug-induced, excess fluids, low intake due to cachexia	1) Demonstration of hypercortisolism by- increased 24 UFC or salivary cortisol 2) 1 mg dexamethasone suppression test 3) High serum ACTH level with the absence of a pituitary tumour by CT or MRI brain	1) 24-hour urine 5-HIAA 2) Radiolabeled octreotide
Treatment	1) Tumour excision 2) Restore intravascular volume 3) Bisphosphonates, calcitonin, hemodialysis	1) Treatment of the underlying tumour 2) Firstline — fluid restriction (< 1 L/day) 3) Demeclocycline, vaptans	1) Tumour excision 2) Ketoconazole, octreotide 3) Bilateral adrenalectomy in refractory cases	1) Surgical excision of tumour 2) During carcinoid crisis: octreotide 3) Pre lung surgery manipulation of tumour-octreotide not usually recommended

Conversion factors for SI: calcium values to mmol/L, multiply by 0.25; cortisol values to nmol/L, multiply by 27.588; osmolality values to mmol/kg, multiply by 1; PTH values to ng/L, multiply by 1; and sodium values to mmol/L, multiply by 1.

ACTH — adrenocorticotropic hormone; CT — computed tomography; HHM — humoral hypercalcaemia of malignancy; HIAA — 5-hydroxyindoleacetic acid; MRI — magnetic resonance imaging; NET — neuroendocrine tumour; POMC — proopiomelanocortin; PTH — parathyroid hormone; PTHrP — PTH-related protein; SIADH — syndrome of inappropriate antidiuretic hormone secretion

these, the thirty-day mortality rate can be as high as 50% [8, 9]. Bone metastases or altered parathyroid gland function are usually absent in these cases. In lung cancer, the incidence of hypercalcaemia is around 5% at baseline diagnosis and can be twice as this eventually (around 8–12%) during the disease course [5–7, 10]. Most common histology causing HHM is squamous-cell carcinoma, and in up to one-fourth of them, hypercalcaemia can be the presenting feature [5, 6, 10–12].

Biology and mechanism

In lung cancer patients, hypercalcaemia in the majority of the cases is usually caused by HHM [13, 14]. Four mechanisms have been observed in malignancy-related HHM (Table 4). The main and most important mechanism of action is the parathyroid hormone-related protein (PTHrP) secreted from cancer cells [12, 14, 15]. Ectopic parathyroid hormone production is one of the rare mechanisms [13]. Sometimes, post-chemotherapy, chronic G-CSF exposure can cause hypercalcaemia in few of them as it promotes osteoclastic bone resorption [16, 17].

Parathyroid hormone molecule has eighty-four amino acids while the PTHrP molecule has 139 to 173 amino acids. These two substances manifest C-terminal portions differently but have a similar first 13 amino acids N-terminal. PTHrP modifies itself into a configuration and binds to the PTH receptor. It can simultaneously bind to other PTH receptors and utilise different effects from PTH [18]. PTHrP binds to receptors of PTH in the kidney and bone. This subsequently influences the bone resorption and renal control of electrolytes, namely phosphate and calcium. PTHrP does not effect on the vitamin D3 1-alpha hydroxylase action unlike parathyroid hormone. In some animal model studies, in a squamous-cell carcinoma cell line, the amphiregulin-EGFR signalling system reconstitution caused HHM [19].

In patients with tumours originating from the lungs [8], including several other organ NETs, hypercalcaemia secondary to PTH secretion has been described [20].

Rarely, tumour cells can release interleukins that can activate the osteoclasts (like IL-1) which can be the cause of malignancy-related hypercalcaemia [21].

Clinical features and diagnosis

In lung cancer, HHM incidence is more frequent in those patients with significant disease burden (locally advanced or metastatic disease) [21]. The symptom level depends on the serum calcium concentration (calcium levels more than 14 mg/dL are considered severe), timing of the onset and the patient's precancer neurologic and renal function [22, 23].

Table 4. Humoral hypercalcaemia of malignancy causes

Parathyroid hormone-related protein
1.25 dihydroxy vitamin D
Parathyroid hormone
Granulocyte colony-stimulating factor

Moderate hypercalcaemia (serum level of 12–14 mg/dL) may result in neuromuscular symptoms (proximal myopathy, fatigue), neuropsychiatric symptoms (anxiety, confusion), circulatory disturbances (polydipsia, severe dehydration causing acute renal failure, polyuria), and gastrointestinal manifestations (abdominal pain, constipation, and rarely pancreatitis),

Hypercalcaemia, when severe, can cause cognitive impairment, confusion, and even coma. Cardiac conduction disturbance and hypotension causing death may also be seen [5, 6, 13, 22].

Many lung cancer patients can be emaciated due to cancer-related cachexia with low serum protein values. So, their serum calcium levels need correction depending on the serum albumin values. Patient's calcium and albumin values estimation should be always made simultaneously [6].

The diagnosis is confirmed by the following laboratory tests: high serum levels of ionized and total calcium, low to normal parathyroid hormone (PTH) level and high PTH-related protein (PTHrP) concentration [8].

Treatment

Treatment of the underlying malignancy as radically as possible is the most successful therapy strategy [6]. The goal of medical care should be attaining electrolyte equilibrium and restoring intravascular volume to prevent the immediate acute complications of serum hypercalcaemia. Two to three liters of intravenous normal 0.9% saline solution will achieve this. Fluid substitution decreases calcium reabsorption in the kidney by increasing the glomerular filtration rate [7, 22]. Medications causing hypercalcaemia (like calcium-containing antacids, supplements of calcium, vitamin D, diuretics like thiazides) or that aggravating mental status changes should be stopped whenever possible [9].

In refractory persistent hypercalcaemia, treatment to reduce the elevated calcium levels should be done as per latest guidelines (including bisphosphonates and possibly denosumab) [6, 23]. Serum calcium level starts to decrease within a day

and normal values of calcium can be seen within a week following administration of intravenous bisphosphonates. Bisphosphonates have an influence on serum calcium levels for up to 3 weeks [5, 24]. In treating HHM, bisphosphonates are proven to be the safest and most effective agents [24]. They also enhance the survival of patients with bone metastasis [23]. The important complications of bisphosphonates are jaw osteonecrosis due to local vessel vasoconstriction and acute renal failure. Jaw osteonecrosis can cause severe pain locally, jaw swelling, tooth loss, and, rarelysoft tissue loss exposing the underlying bone [5, 6].

In cases of PTHrP-related hypercalcaemia, cinacalcet can be helpful [25].

A low calcium diet and steroids are usually less effective.

Dialysis is generally reserved for those with severe hypercalcaemia causing life-threatening symptoms or in patients with acute renal failure.

Prognosis

In lung cancer, those with significant disease burden (locally advanced or metastatic disease) have more chances of having HHM with mean survival of only around 2 months and poor prognosis [26].

In a study of 1,149 histologically proven lung cancer patients, hypercalcaemia was seen in 6%. Among those with hypercalcaemia, squamous-cell carcinoma was found in 51%, adenocarcinoma in 22% and small-cell carcinoma in 15%. Those with hypercalcaemia mostly had advanced disease (stage 3 or more) with a median survival of only a few months [11].

Serum PTHrP levels in patients with HHM can give information regarding prognosis. In some cases, it can be used to assess tumour response posttreatment. It may also predict the reaction to bisphosphonates. Studies have shown that concentrations above 12 pmol/L are frequently associated with both a smaller reduction in hypercalcaemia and with recurrence of hypercalcaemia within fourteen days of therapy [27, 28].

Prognosis of lung cancer-associated hypercalcaemia is generally poor. Those who become normocalcaemic after bisphosphonate therapy survive better (53 days *vs* 19 days) [27].

Syndrome of inappropriate antidiuretic hormone secretion

Incidence

A syndrome of inappropriate antidiuretic hormone secretion (SIADH) causing hyponatraemia with cancer background was noticed in 1957 for the first time. In 1963, from a lung tumour sample (small-cell carcinoma), an agent with ADH-like action was isolated [29, 30]. This syndrome manifests as hyponatraemia with euvolemia clinically, low serum osmolality and unsuitably high urine osmolality. Diagnosis has a specific criterion (Table 4). The tumour cell more commonly produces antidiuretic hormone and rarely atrial natriuretic peptide leading to paraneoplastic hyponatraemia. Antidiuretic hormone increases reabsorption of free water and atrial natriuretic peptide induces natriuresis [31].

SIADH is found in 1–2% of patients with lung cancer. SIADH causing clinical symptoms has been seen to occur in around 10% of lung cancer patients, mainly with small-cell carcinoma histology. Less than one percent of paraneoplastic hyponatraemia is caused by NSCLC. In SCLC, tumour stage does not correlate with the incidence of SIADH. Overall, around 70% of paraneoplastic SIADH cases are caused by SCLC [32–34].

Biology/mechanism

ADH is a 9-amino acid peptide usually produced by neurohypophysis. The peptide binds to receptors in the kidney to reduce the excretion of free water. When plasma osmolality exceeds 280 mOsm/kg, the pituitary increases ADH release, causing the kidney to retain more free water and maintain fluid and osmolar balance.

In patients with SCLC, ectopic ADH production causes hyponatraemia by inhibiting free-water excretion in the distal tubule of the kidney. ADH mRNA is expressed in SCLC cells and the peptide is translated and secreted. Measured levels of ADH in plasma are often increased in SCLC [35]. A subgroup of hyponatraemia and concurrent SCLC cases have no detectable levels of plasma ADH. The tumours in these patients express ANP mRNA, secrete the peptide, and have high plasma ANP levels [36].

Clinical findings/diagnosis

In SIADH, the level of sodium in serum and its time of onset determines the symptoms. Initially, the patients can have only headache and fatigue as presenting complaints. However, acute onset (< 48 hours) and severe hyponatraemia (serum sodium level of less than 120 meq/L) can cause general seizures, altered mental status and rarely death due to cerebral oedema. Usually, chronic hyponatraemia patients are asymptomatic, especially when it's mild to a moderate degree.

In the background of hyponatraemia with clinical euvolemia status, > 40 mmol/L of sodium

Table 5. Syndrome of inappropriate antidiuretic hormone secretion diagnosis criteria

Serum sodium level of less than 134 mEq/L
Osmolality of plasma less than 275 mOsm/kg
Osmolality of urine more than 500 mOsm/kg
Concentration in urine more than 20 mEq/L
All should be absent: hypovolaemia, adrenal insufficiency, and hypothyroidism

in urine or > 100 mOsm/kg of osmolality in urine gives a clue to the diagnosis of SIADH [37]. The diagnosis of SIADH is confirmed by laboratory tests as given in Table 5.

Always, in patients having hyponatraemia in the setting of lung cancer, other causes of low serum sodium like inadequate sodium intake, drug-induced kidney injury or excess intravenous hypotonic solutions usage should be excluded.

Treatment

Surgical excision of the tumour is always the ideal treatment and when successful, can bring back the serum sodium level to normal in only some patients [33]. In SCLC, post-chemotherapy symptoms resolution can be seen in up to 80% of cases, but the syndrome would relapse along with the tumour (in 60–70% of patients) [33, 34]. In SCLC, rarely can chemotherapy result in tumour lysis syndrome contributing to acute SIADH [38].

As in any SIADH case, in asymptomatic milder hyponatraemia patients, reduced (1 L/day) intake of free water is the first step. In symptomatic hyponatraemia with serum sodium < 120 mEq, intravenous administration of 3% sodium chloride solution with an infusion speed of up to 1 mL/kg/hour is necessary for the first few hours at least. Correction of serum sodium level should be gradual as acute restoration can lead to irreversible demyelination.

Pharmacologic treatments can be used when conservative measures fail. Demeclocycline lowers renal response to ADH. Vasopressin receptor antagonists like conivaptan and tolvaptan enhance urine excretion of free water. These are effective in some cases.

Prognosis

SIADH *per se* carries an independent poor prognosis in malignancy [39].

In one study, SCLC patients with persistent hyponatraemia due to SIADH had worse survival. In this study, 61 patients had sodium level of less than 130 mEq/L and received at least two cycles of chemotherapy. Among these 61 subjects, compared to 46 patients whose sodium normalised (to 136mEq/L), the 15 patients in whom there was persistent hyponatraemia (< 136mEq/L) post-chemotherapy had worse survival [40].

Ectopic Cushing's syndrome (ECS)

Incidence

In 1962, for the first time, connection between CS and ectopic production of adrenocorticotrophic hormone (ACTH) was established in a patient with severe hyperadrenocorticism who was found to have SCLC [41]. In Cushing's syndrome, in up to 10% of cases, the cause can be paraneoplastic [44]. In most of these cases, malignancy involved is lung NET (small-cell carcinoma or bronchial carcinoids) [41–44]. In non-neuroendocrine tumours, rarely are ECS reported [45].

Biology and mechanism

Ectopic production of ACTH happens to be the foremost cause of this endocrine syndrome in lung cancer patients [46]. Rarely, it is caused by corticotropin-releasing hormone (CRH) secretion from tumour cells [47].

The precursor gene, proopiomelanocortin (POMC), is expressed more in the cancer cells from which a 241-amino acid prohormone is translated and then cleaved into ACTH (39 amino acids), melanocyte-stimulating hormone, and opiate-like hormones. The ACTH binds to receptors in the adrenal gland, causing them to produce excessive glucocorticoid and mineralocorticoid hormones [48]. CRH is a 41-amino acid peptide produced and released in the hypothalamus paraventricular nuclei that stimulates the release of ACTH from the pituitary.

Clinical features and diagnosis

Systemic manifestations in this syndrome are mainly due to increased serum cortisol levels. The common clinical features of ECS are skin purple striae, moon like face, acne, proximal myopathy, oedema of the periphery, systemic hypertension, primary metabolic alkalosis, and persistent serum hypokalaemia. Weight gain (due to chronic water retention or centripetal fat distribution) may be one of the rare features in those with lung cancer-related ECS unlike in those without ECS where weight loss is seen because of cachexia [7]. Most of them also have hyperglycaemia [48, 49]. In ECS, due to SCLC, classical signs of Cushing's syndrome are rare. An important reason for this finding could be the aggressive nature of SCLC causing only brief exposure to excessive ACTH [5, 50].

Those having SCLC with ECS getting chemotherapy are at increased risk for opportunistic infections [51]. ECS is also a risk factor for VTE (2%) [52]. This risk further may increase after surgery of the tumour (4%) [53].

Diagnostic laboratory findings include the following [54]:

1. Minimum of 2 increased measurements of 24-h urine free cortisol;
2. Salivary cortisol sample > 145 ng/dL between 23:00–24:00;
3. 1 mg of dexamethasone suppression test.

An ACTH level can differentiate ECS from Cushing's in cases of proven serum or urine hypercortisolism. ECS suspicion is raised when an elevated morning ACTH is seen along with the absent pituitary tumour in brain CT or MRI. High-dose dexamethasone will not suppress an ectopic source (like in lung cancer) of ACTH. Bronchial carcinoids are an exception, because in some cases with this tumour type, serum ACTH and cortisol levels have been suppressed by high-dose dexamethasone [55].

To locate the primary tumour in the lungs, whole body somatostatin receptor scintigraphy or thorax imaging like CT can be used.

Treatment

Radical excision of the tumour is the best treatment [56]. When radical therapy of the tumour is unattainable, medications directed to cease the secretion of cortisol (ketoconazole, metyrapone and other drugs) or block (octreotide may block the release of ACTH) are required [7, 57]. With careful monitoring of serum potassium, antihypertensive agents and diuretics can also be used to control symptoms.

Bilateral adrenalectomy is the option used as a last resort in case of no response to medications [5].

Prognosis

Prognosis is affected by tumour type and degree of cortisol level as both these factors influence mortality and morbidity [56].

Most of the patients with SCLC and ECS present at an advanced stage. Even with chemotherapy, their mortality is significantly high since many of these tumours are chemoresistant [5, 7, 56].

Carcinoid syndrome

Incidence

Neuroendocrine tumours of the bronchopulmonary system account for around 20% of all lung malignancies and include typical carcinoid, atypical carcinoid, large-call carcinoma and SCLC [58].

In neuroendocrine lung cancer, the release of serotonin by tumour cells might trigger the syndrome only in 1–5% of the cases [59]. A lower rate of carcinoid syndrome is seen in lung NET as they produce less serotonin than midgut NET [58]. In patients having localised disease (like in most of the cases of lung NET), carcinoid syndrome is seen most often with tumours of bigger size (> 5 cm) and those with concurrent liver metastasis [60].

Biology and mechanism

Carcinoid syndrome results from the release of vasoactive substances such as serotonin into the systemic circulation. As many as 40 substances (dopamine and many others) related to carcinoid syndrome have been identified as being the potential causes. The release of these substances can be triggered by increased adrenergic activities, such as physical exercise, or increased intake of foods rich in amines (chocolate, kiwi, avocado banana, and nuts) or alcohol.

Clinical features and diagnosis

Some may have acute symptoms like cutaneous flushing, secretory diarrhoea, and bronchospasm. The long-term results of persistently elevated hormone levels include telangiectasias of veins, valvular heart disease (right side more commonly involved), and retroperitoneum fibrosis. Flushing of the skin can be prolonged in the setting of carcinoid syndrome due to lung NET and it occurs more in the upper anterior part of the body [61, 62].

Carcinoid syndrome-associated bronchospasm is less typical and these patients usually have concurrent flushing, sneezing and dyspnoea [63]. In a retrospective study of 748 carcinoid syndrome patients, bronchospasm was seen in 15% [63].

In few cases, due to excessive production of serotonin and its release into the systemic circulation, an acute form of the syndrome can be seen. This is known as carcinoid crisis. These patients can have tachycardia, hypotension, bronchospasm, and even rarely sudden death. Carcinoid crisis is more common after stressful procedures such as anaesthesia, surgery or even radiologic interventions [64]. It can also happen spontaneously.

The evaluation of carcinoid syndrome is with a 24-hour urine collection for the most crucial metabolite of serotonin, 5-Hydroxyindoleacetic

acid (5-HIAA). This test has a specificity of approximately 90% [65].

Radiolabelled octreotide can be used to detect lung neuroendocrine tumours with ectopic hormone production as almost 80% of them demonstrate somatostatin receptors [66].

Treatment

Excision of the tumour is the best treatment [67]. Unlike other organ NETs (even with significant liver metastasis), carcinoid crisis risk with lung NETs is low, and hence prophylactic administration of octreotide before any tumour manipulation (biopsy or resection) is not recommended [62]. But still, when handling such tumours, clinicians should be aware of the possibility of the carcinoid crisis and the benefit of octreotide in such a scenario.

Rare syndromes

Hypoglycaemia

Lung cancer-associated paraneoplastic hypoglycaemia is rare. Non-isletcell tumours with insulin secretion and tumours releasing substances which can cause hypoglycaemia by non-insulin based mechanism are the main causes [68]. This condition is labelled as non-islet cell tumour hypoglycaemia (NICTH). Pulmonary tumours causing this condition are malignant mesothelioma, solitary fibrous tumour and adenocarcinomas [6].

In most of the cases, hypoglycaemia is caused by excess insulin release due to the secretion of peptides like precursors of insulin-like growth factor 2 (IGF-2), insulin-like growth factor 1, and sometimes glucagon-like peptide-1 which are capable of causing glucose utilisation by different mechanisms [68]. Rarely high tumour load having excess glucose utilisation, significant liver infiltration by tumour *per se*, or tumour metastasis to the endocrine gland (pituitary or adrenal) causing their destruction is the cause of hypoglycaemia in these patients [68].

NICTH is clinically characterised by recurrent hypoglycaemic episodes which affect elderly patients more often. In some, these hypoglycaemic episodes may direct towards the underlying undiagnosed cancer [69, 70].

Diagnosis depends on tumour type the patient is having. In NICTH acute phase, we can find decreased values of following substances in serum-insulin (normal range: 1.44–3.6 μIU/mL) and C-peptide (normal range: 0.3 ng/mL). They would also have increased levels of the following substances in serum: growth hormone, insulin-like growth factor 1, insulin-like growth factor 2 and IGF2:IGF1 ratio. In insulinoma acute phase, we can find increased value of both insulin and C-peptide levels in serum [6, 70].

Surgery of the tumour is the best management option in these patients. The essential goal in case of any hypoglycaemic emergencies is to bring back blood glucose to near expected values with 25% or 50% solution of dextrose. Oral glucose will help in few cases. In the long run, treatment of hypoglycaemia due to this syndrome may require glucagon, growth hormone and corticosteroids [6, 69–71].

Acromegaly

Only 1% of acromegaly is caused by growth hormone releasing hormone or growth hormone ectopic secretion by tumour cells. Of these, the majority are caused by carcinoid tumours of the lung and intestine [72]. In most cases, the GHRH gene is expressed by the lung cancer cells, and a 40–44 amino acid peptide is produced. Circulating GHRH peptide binds to receptors in the pituitary gland resulting in the production of excessive amounts of GH 73]. Rarely, lung carcinoid tumours express immunoreactive GHRH and result in abnormal GH secretion [74].

The earliest features of GH excess are hypertrophy of the extremities and face. The diagnosis of ectopic acromegaly is established by elevated serum levels of GHRH or GH, the absence of a pituitary tumour, complete recovery following lung tumour resection, positive GHRH immunostaining, detection of GHRH mRNA, positive bioassay (pituitary cells of rat on culture produce GH when subjected to tumour extract), or GHRH extraction from the tumour tissue [75]. However, coincidental pituitary tumours and lung solid tumours have also been described.

Management of ectopic acromegaly should be surgical resection of the tumour and is often curative in those with lung carcinoid. In those with unresectable or metastatic cancers, medical therapy with somatostatin analogues, such as octreotide and bromocriptine, have been shown to be effective [76].

Ectopic secretion of chorionic gonadotropin

This syndrome has been reported in large-cell lung carcinoma (LCLC). The syndrome causes Leydig cell hyperplasia which results in raised oestrogen levels and reduced testosterone production. This gives rise to atrophy of testicles with repression of spermatogenesis, and gynecomastia [77].

Conclusion

Despite the availability of new diagnostic techniques and biological and surgical treatment development, lung malignancy mortality is the foremost cause of cancer-related deaths since 1985.

In lung cancer, the small-cell histology type is commonly associated with endocrine paraneoplastic syndromes. These syndromes are easy to diagnose and treat because they have a clear pathogenic pathway. Endocrine paraneoplastic syndromes have both a prognostic role as well as a predictive function in tumour treatment success. The prognosis also depends on the cure of the underlying tumour. Tumour progression can present along with syndrome recurrence. The most common syndrome is humoral hypercalcaemia of malignancy seen in squamous-cell histology and measurement of PTHrP can delineate it from primary hyperthyroidism.

Conflict of interest

None declared.

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VIA MEDICA

REVIEW PAPER

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Role of ivermectin in patients hospitalized with COVID-19: a systematic review of literature

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected almost every country in the world since December 2019. Despite the efforts of the human race to combat the virus, we are still looking for an evidence-based permanent cure for the disease. Ivermectin has recently emerged as one of the therapies having a beneficial effect on COVID-19. Ivermectin, owing to its properties, continues to be a possible treatment against the COVID-19 disease. Already being a mainstream drug with minimal adverse effects, it garners valid consideration. It's use in hospitalized patients, randomized controlled trials, and observational studies has also supported its implementation. In this article, we have reviewed recent studies and explored the effectiveness of ivermectin in hospitalized COVID-19 patients.

Key words: ivermectin, COVID-19, SARS-CoV-2, virus, treatment, therapy

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Introduction

SARS-CoV-2 is a single-stranded RNA virus from the Coronaviridae family. There are 7 known species of the coronavirus that have the ability to infect humans. Its predecessor, SARS-CoV-1, can also cause severe respiratory disease. It comes as no surprise that, with little risk involved, there was not enough research done on SARS viruses which meant that we had to face a pandemic without much information about the causative agent. As of 21 February 2021, over 110 million people have been infected by the virus and about 2.4 million have lost their lives [1]. COVID-19 has also had a significant impact on the economic state of the whole world accounting for huge losses and unemployment. With so much going on, there has been immense pressure to find a suitable treatment for the disease. As such, interferon, hydroxychloroquine, chloroquine, conventional anti-virals, monoclonal antibodies, convalescent plasma therapy, and tocilizumab have been suggested as possible therapeutic options. However, studies have shown non-conclusive or insignificant evidence when it comes to patient mortality and other outcomes like disease progression, time to clinical stability, need of invasive ventilation, and duration of hospital stay [2–7]. This leaves the door open for debates on the efficacy of these drugs and whether clinicians should consider using them. So far, only corticosteroids have shown consistent encouraging signs towards a favorable prognosis of the disease [8].

Ivermectin has recently surfaced as one of the medicines showing promise in the therapy of COVID-19. They belong to the class of anti-parasitics called avermectins. First discovered in the 1970s, it has been recognized as a 'wonder drug' and its discovery earned a Nobel Prize for Physiology or Medicine in 2015 [9]. Since then, the drug has been used against a wide range of parasitic diseases like onchocerciasis, strongyloidiasis, and ascariasis both in humans and animals. It is currently FDA-approved and continues to be sold across the globe. It may be used orally or applied topically depending on the infection site.

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Mechanism of action

Ivermectin primarily amplifies the activity of GABA receptors or glutamate-gated chloride ion channels which leads to the inhibition of paralysis of somatic muscles via inhibition of myosin chain phosphorylation [10]. It is the blood-brain barrier (BBB) in vertebrates that protects them from the harmful effects of the drug in the central nervous system (CNS). Invertebrates, in contrast, are vulnerable to the actions of the drug due to a lack of the BBB. The fascinating part about ivermectin is its ability to affect a broad-range of diseases — it has shown anti-microbial, anti-cancer [11], and anti-viral properties.

The anti-viral properties of ivermectin mainly stem from its capacity to hinder the transport of viral proteins into the host nucleus via inhibition of the importin (IMP) α/β receptor. This allows it to inhibit the replication processes in various RNA and DNA viruses (e.g. Influenza, Zika Virus, Dengue Virus, Porcine circovirus, and others) [12, 13]. Indeed, in-vitro models support the theory that in SARS-CoV-2 a similar inhibitory effect leading to decreased replication will be seen [14]. Computer simulations claim that ivermectin might also bind to the S protein of the virus or to ACE-2 in humans thereby warding of host cells from COVID-19 [15]. Another possible means by which ivermectin mediates its anti-viral properties is via allosteric modulation of the P2X4 receptor, which leads to the secretion of CCL-5 using ATP [16]. Some studies suggest that ivermectin might have immunosuppressive effects [17, 18]. This is particularly important since it may point to the possibility of ivermectin playing a complementary role of mitigating the inflammatory response during severe acute respiratory syndrome. As a matter of fact, evidence supports the use of ivermectin as an anti-Inflammatory drug in the treatment of existing diseases like Rosacea [19]. The results were quite optimistic in animal models as well [20].

The aim of this review is to provide a synopsis of the literature on the interaction between COVID-19 and ivermectin while trying to gauge the potential use of the drug against this devastating disease.

Materials and methods

We searched peer-reviewed databases such as PubMed and reviewed pre-print articles. We chose randomized controlled trials (RCTs) and observational studies in the English language that evaluated the effectiveness of ivermectin on COVID-19 patients compared against standard treatment protocol, placebo, or other prospective medications in their study. Our review includes studies that were done on PCR-confirmed hospitalized COVID-19 patients. Factors such as dosage, timing, frequency, control group exposure, or publication status were not considered as appropriate filters.

The outcomes taken into consideration include:

1. Patient Mortality
2. C-Reactive Protein level
3. Time to discharge from the hospital
4. Viral load/clearance

Data extraction was carried out by the 2 reviewers independently. Articles were excluded if they were commentaries or opinion pieces. Studies examining the prophylactic effects of ivermectin in SARS-CoV-2 were also excluded. Duplicate articles, if any, were removed with the help of Mendeley software. Citations and data were included when considered appropriate. Any disagreement between the two reviewers was settled by a third, independent reviewer.

Results

After going through the databases, 14 studies were included in this review. Out of these, 8 were randomized controlled trials and 6 were observational studies. Of these 14 studies, 6 studies were peer-reviewed while 8 were preprints. A cumulative total of 7,744 laboratory-confirmed COVID-19 patients were involved — with 1,330 patients being a part of the ivermectin exposed group.

Patient mortality was reported in 8 studies which included a total of 6,770 patients. Out of these 6,770 patients, mortality was seen in 17.57% (n = 1,190) of patients, of which 82 were from the ivermectin exposed group. Only two studies did not classify patients according to severity of disease (Babalola *et al.* [21] and Soto-Beccerra *et al.* [22]). The rest of the trials included mild, moderate, and severely ill COVID-19 patients. A majority of subjects had comorbidities such as diabetes mellitus, hypertension, or various pulmonary diseases.

Table 1 describes the details of these studies in terms of design, dosage, size, and outcomes.

Administration and dosage of ivermectin varied across all studies. The lowest dosage used was 0.2 mg/kg, whereas the highest was 0.4 mg/kg. The most frequent dosage used, however, was 0.2 mg/kg. In some cases, absolute values such as

Table 1. Sample size and characteristics of the selected studies

Study name and design	Size	Intervention (dosage, frequency, duration)	Control	All-cause mortality	Other notable outcomes
Elgazzar *et al*. [23] RCT	n = 400	0.4 mg/kg IVM + ST Once daily for 4 days	HCQ + ST	1% *vs* 12%	Reduced CRP levels (90% and 56% *vs* 84% and 14%) and hospital stay
Niaee *et al*. [24] RCT	n = 180	0.2–0.4 mg/kg IVM Once daily or on 3 interval days	ST or ST + Placebo	3.3% *vs* 18.3%	Reduced CRP levels, DLO, hospital stay
Hashim *et al*. [25] RCT	n = 140 (44 HP)	0.2 mg/kg IVM (once for 2–3 days) + DC (twice for 5 days) + ST	ST	0% *vs* 27.3% in severely ill HP	Lower rate of progression (9% *vs* 31.8%) and hospital stay (avg. 7 days)
Kirti *et al*. [26] RCT	n=112	12 mg IVM (days 1 and 2) + ST (days 3–6)	ST + Placebo 6 days	0% *vs* 7%	Did not seem to affect negative RT-PCR (23.6% *vs* 31.6%)
*Spoorthi *et al*. [31] RCT	n=100	0.2 mg/kg IVM once and/or DC	Placebo	N/A	Shorter clinical recovery (3.7 *vs* 4.7 days) and stay (6.7 *vs* 7.9 days)
Ahmed *et al*. [30] RCT	n = 72	12 mg IVM + ST Once daily for 1 or 5 day(s)	Placebo + ST	N/A	Faster viral clearance (11.5 and 9.7 *vs* 12.7 days)
Babalola *et al*. [21] RCT	n = 62	6 mg or 12mg IVM + ST Twice a week for 2 weeks	LPV/r + ST Daily	N/A	Faster viral clearance (4.7 and 6 *vs* 9 days)
Chachar *et al*. [32] RCT	n = 50	12 mg IVM + ST 3 doses in 2 days	ST	N/A	Difference in clinical recovery did not reach statistically significant levels
Soto-Beccerra *et al*. [22] OBS	n = 5683	IVM Within 48 hours	ST	51.4% *vs* 42.6%	—
*Rahman *et al*. [34] OBS	n = 400	18 mg IVM once + 100 mg DC twice daily for 5 days	HCQ + AZIT	N/A	Faster and better viral clearance (16.5% on day 6 *vs* 18.5% on day 12)
*Rajter *et al*. [27] OBS	n = 280	0.2 mg/kg IVM + ST Mostly once	ST with or without HCQ/AZIT	15.0% *vs* 25.2% (38.8% *vs* 80.7% in severe patients)	No difference found in the length of stay
*Khan *et al*. [28] OBS	n = 248	12 mg IVM Once	ST	0.9% *vs* 6.8%	Shorter recovery time (9 days *vs* 15 days) and faster viral clearance (4 days *vs* 15 days)
*Gorial *et al.* [29] OBS	n = 87	0.2 mg/kg IVM + HCQ + AZIT Once	HCQ + AZT	0% *vs* 2.8%	Shorter duration of stay (7.6 days *vs* 13.2 days) and faster viral clearance (7 days *vs* 12 days)
*Camprubi *et al*. [33] OBS	n = 26	0.2 mg/kg IVM + IS Once on the onset of symptoms	IS	N/A	Small differences in discharges, need for ICU

*Studies with one dose of ivermectin.

AZIT — azithromycin; CRP — C-reactive protein; DC — doxycycline; DLO — duration of low O_2 saturation; HCQ — hydroxychloroquine; HP — hospitalized patients; IS —immunosuppressants; IVM — ivermectin; LPV/r — lopinavir/ritonavir; N/A — not applicable; OBS — observational study; RCT — randomized controlled trial; ST — standard therapy

6, 12, or 18 mg were used. Differences in one day and multi-day dosing were also noted. Just over a third of trials exposed the clinical group to another medicine (excluding those in the standard of care) such as doxycycline, azithromycin, etc. Mortality was noted across eight different reports, and in almost all of them death occurrence was more prominent in the control arm [23–29]. The

only study whose findings were not consistent with the rest is the retrospective cohort in Peru by Soto-Beccerra *et al*. [22] which found a higher mortality rate in the interventional group.

Viral clearance was recorded in 5 studies, all of which were able to show a reduced time required for viral clearance or viral load after a set time in the study group [21, 23, 28–30]. Perhaps the most pronounced effects were seen in the retrospective study by Khan *et al*. [28] in which the median time required for viral clearance decreased from 15 days to just 4 days.

Eight trials measured the duration of hospitalization as an outcome. Six out of these revealed a shorter stay in hospitals in the ivermectin arm compared to the control group [23–25, 28, 29, 31]. In two studies, the results did not reach statistical significance [27, 32].

Out of the studies, two did not show a significant decrease in C-reactive protein (CRP) levels compared to the control group [29, 33], whereas three highlighted a statistically significant decrease [23, 24, 30]. In a randomized multi-center trial conducted by Niaee *et al*. [24] with $p < 0.001$, reduced CRP levels across all arms were observed. Out of all 5, Elgazzar *et al*. [23] and Niaee *et al*. [24] were the only two studies with sample sizes of over 100 subjects.

Patterns may also suggest the existence of a dose-response relationship. In one of two studies, Ahmed et al. illustrated that taking ivermectin for 5 days instead of 1 day increased the rate of viral clearance [30]. Similarly, Babalola *et al*. [21] found that the arm exposed to 12mg ivermectin instead of 6mg had faster clearance by over 1 day. However, in one case, where 3 dosing strategies were utilized against a control of standard therapy and placebo, there were similar mortality rates and length of hospital stays across interventional arms [24].

We also noticed one-dose trials, such as the one conducted by Gorial *et al*. [29], which saw all members of the study group cured against 97.2% of the control group. Meanwhile, the dichotomy in mortality rates was more pronounced in most multi-day dose trials (as summarized in Table 1), supporting the above hypothesis. Statistically, insignificant findings in length of hospitalization [27, 33] were also noted in one-time exposure studies, whereas only one multi-exposure study yielded similarly insignificant results [32]. However, a few one-dose studies did show major changes in at least one of the 3 categories [27–29, 31, 34], thereby resisting the theory of there being a relationship.

Discussion

This review suggests that ivermectin reduces mortality, CRP levels, and lengths of stay in the hospital while enhancing viral clearance in SARS-CoV-2 hospitalized patients in different populations around the world.

In the study carried out by Soto-Beccerra *et al*. [22], which pushes back against the notion of ivermectin decreasing mortality in COVID-19 patients, it should be noted that the same report found increased weighted hazard or unweighted hazard ratios in all interventional groups (others included azithromycin, hydroxychloroquine, or a combination of all 3 drugs). The data could be limited since zero deaths occurred on the second day in the control arm whereas mortality was reported in all other groups (deaths in the first 24 hours were not included for both arms).

These findings are also supported by a systematic review and meta-analysis performed by Padhy *et al*. [35]. However, it should be noticed that they used ivermectin as an adjuvant, only chose 4 observational studies, and included outpatient data. The studies chosen in this review are specific to hospitalized COVID-19 patients, larger in quantity, and include RCTs. A recent case series also supported these findings as 34 subjects treated with ivermectin all survived and mortality was observed in the other group(s) [36].

This is particularly encouraging keeping in view the current situation of the pandemic. The anti-viral activity of ivermectin seems to make it a very viable option for the treatment of such patients. The safety of this drug is well documented [37, 38]. A study that compiled data from other studies, including 50,000 subjects in Cameroon, found that only 20 faced serious complications [39]. Being relatively affordable and readily available makes it economically/logistically feasible.

However, we urge medical professionals to exercise prudence until more high-quality evidence is available. The COVID-19 Treatment Guidelines Panel at NIH stated on January 14, 2021 that there is 'insufficient data to recommend either for or against the use of ivermectin for the treatment of COVID-19' due to the fact that 'significant methodological limitations and incomplete information' were visible across trials [40]. In addition, Merck & Co. (known as MSD outside USA and Canada), which originally marketed the drug, identified a lack of evidence on February 4, 2021 with regards to the use of ivermectin in COVID-19 [41]. In a recent randomized clinical trial in patients with mild COVID-19 treated

with ivermectin, Lopez-Medina *et al*. [42] found no significant difference in time to resolution of symptoms in comparison with placebo. We too believe it is early to include ivermectin in existing protocols for similar reasons. There are other issues that may make ivermectin unsuitable. It is poor as far as water solubility is concerned, which is why it is not absorbed well via the oral route [43]. Nevertheless, it should be mentioned that liposomal and inhalational therapy might help tackle this issue. Although ivermectin is not able to cross the BBB, in patients with hyper-inflammation, a docile BBB may permit passage into the CNS. As a matter of fact, we ask that quantitative analyses and better designed high-powered RCTs be run on the efficacy of the drug.

In the case of the dose-response relationship mentioned above, there is insufficient data to conclude anything. The only thing we consistently saw across studies was a decrease in viral load at higher doses when directly compared to lower doses [21, 23]. Although some one-dose studies did show smaller changes when compared to their multi-dosage counterparts, others found significant results in a few outcomes. It should be noted that exclusive administration of ivermectin across most of the aforementioned studies was scarce. It is possible that the significant reduction in viral clearance by the drug does not necessarily translate into early discharge from the hospital or a decrease in all-cause mortality. However, we cannot merely surmise that. We encourage other researchers to investigate this question while exploring the drug and its potential as a therapeutic option for said population.

Limitations

Due to the current COVID-19 pandemic, the reviewers agreed to include pre-print data in the article, which is not peer-reviewed. Standard treatment regimens, dosage, duration, inclusion/exclusion criteria, and definition of severity of disease differed across all studies. Furthermore, in some trials, ivermectin was used in conjunction with either standard treatment or other drugs (azithromycin, doxycycline, etc.). Publication bias might exist in some studies.

Conclusion

Ivermectin, owing to its properties, continues to be a potential therapeutic option against the COVID-19 disease that we are facing. Already being a mainstream drug with minimal adverse effects, it garners valid consideration and attention in these times. In hospitalized patients, RCTs and observational studies have supported its use. Still, there needs to be more high-quality proof and quantitative analysis in order to legitimize its use as part of general protocol. As for now, we shall have to wait for the final verdict on the capabilities of ivermectin.

Conflict of interest

None declared.

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Proteomic biomarkers of non-small cell lung cancer patients

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Abstract

Lung cancer is a disease with a very low 5-year survival rate (6–13%) worldwide. The most frequently diagnosed histological type of this cancer is non-small cell lung cancer (NSCLC). Poor prognosis for lung cancer — including NSCLC — is mainly related to the fact that patients are diagnosed in the advanced stages of the disease. The aim of this study is to summarize data that concerns new directions of research regarding diagnostic biomarkers that could be used to support the routine diagnosis of this cancer. In recent years, proteomic analysis has become an important tool for cancer biology research, complementing genetic analysis. Among the numerous methods of proteomic analysis, mass spectrometry techniques enable the extremely accurate qualitative and quantitative identification of hundreds of proteins in small volumes of various biological samples. Such analyses may soon become the basis of improvement in lung cancer diagnostic procedures. This study presents the latest reports in proteomic research concerning the diagnosis of NSCLC. New potential proteomic biomarkers, whose presence indicates the development of a neoplastic process at an early stage, are presented. We describe biomarkers whose altered expression levels correlate with different stages of cancer. We also present protein biomarkers that help differentiate NSCLC subtypes. In the clinical workup of NSCLC patients, it is important not only to make an early diagnosis, but also to monitor the development of the neoplastic disease. Considering this fact, we also present examples of biomarkers whose abnormal expression may indicate a high risk of metastasis to the lymph nodes. This paper also emphasizes the need to conduct further research that would confirm the usefulness of the described biomarkers in clinical practice.

Key words: non–small cell lung cancer, proteomic biomarker, mass spectrometry

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Introduction

Lung cancer is one of the most commonly diagnosed cancers and is also the leading cause of cancer-related mortality. In 2018, 2,093,876 new cases of lung cancer were diagnosed, which accounts for 11.6% of total cases. 1.8 million people died (18.4% of total cancer-related deaths) [1] with a focus on geographic variability across 20 world regions. There will be an estimated 18.1 million new cancer cases (17.0 million excluding nonmelanoma skin cancer. This high mortality is mainly caused by a late diagnosis in patients with advanced-stage cancer. The early stage of the disease is characterized by a poor clinical manifestation or occurrence of unspecific symptoms, which makes the diagnostic procedure difficult. Therefore, there is an urgent need to discover a highly sensitive and specific biomarker in order to diagnose non-small cell lung cancer (NSCLC) patients at an early stage of the disease process.

Proteomic-based lung cancer biomarker search

Proteomic analysis is a powerful tool in the global assessment of protein expression and has been extensively applied to biomarker discovery in clinical diseases [2]. The rapid development of mass spectrometry techniques allows to efficiently identify hundreds of differentially expressed proteins in small quantities of various biological samples [3]. Mass spectrometry identifies unknown biomolecules based on their accurate mass and fragmentation pattern. However, for proteomic studies, this is possible only if a sample is a simple mixture or has been previously divided into simpler parts by high resolution separation methods such as two-dimensional electrophoresis, protein microarrays, and liquid chromatography [4]. Quantitative proteomics provides information about relative and absolute protein expressions within a sample [5].

The most common histological type of lung cancer is NSCLC, which accounts for 85% of all

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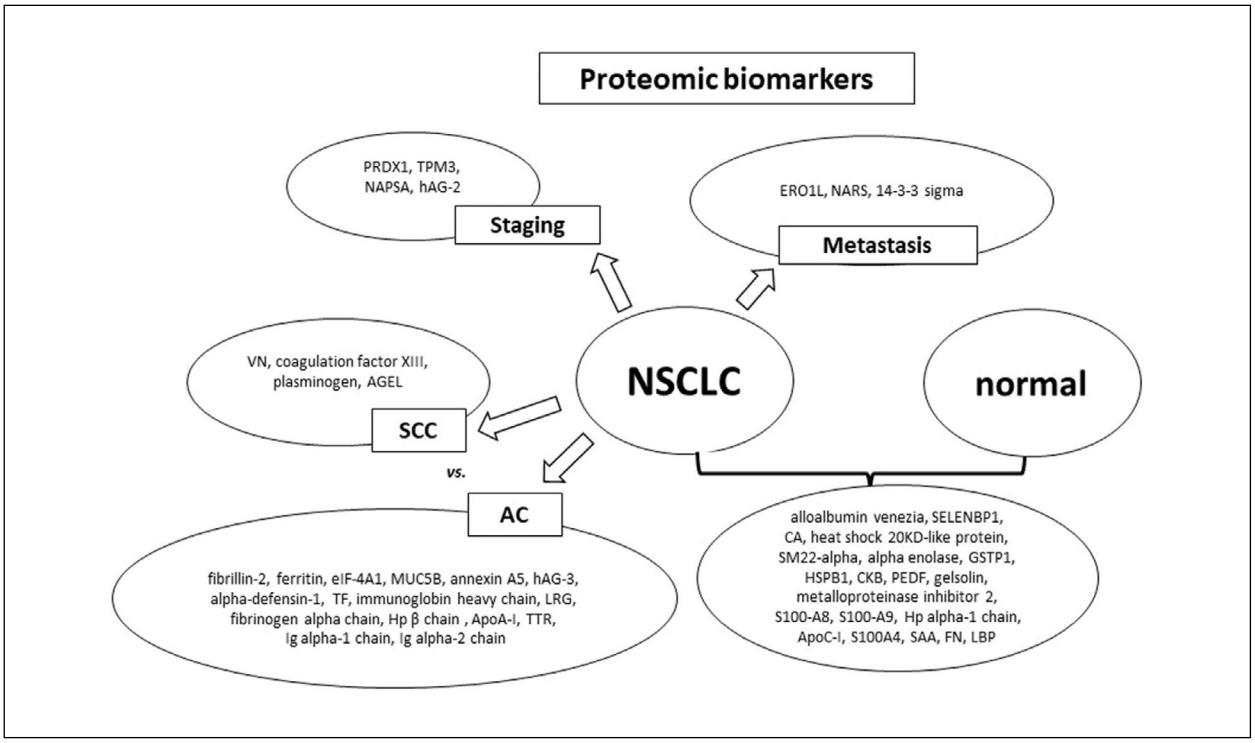

Figure 1. Proteomic biomarkers of NSCLC patients

AC — adenocarcinoma; AGEL — gelsolin; CA — carbonic anhydrase; CKB — creatine kinase brain-type; eIF-4A1 — eukaryotic translation initiation factor 4A1; ERO1L — ERO1-like protein alpha; FN — fibronectin; GSTP1 — glutathione S-transferase P1; hAG-2 — anterior gradient protein 2 homolog; hAG-3 — anterior gradient protein 3; Hp — haptoglobin; HSPB1 — heat shock protein beta-1; LBP — lipopolysaccharide binding protein; LRG — leucine-rich alpha-2-glycoprotein; MUC5B — mucin-5B; NAPSA — napsin-A; NARS — asparagine-tRNA ligase; NSCLC — non-small cell lung cancer; PEDF — pigment epithelium-derived factor; PRDX1 — peroxiredoxin 1; SAA — serum amyloid A; SCC — squamous cell carcinoma; SELENBP1 — selenium-binding protein 1; TF — transferrin; TPM3 — tropomyosin alpha-3 chain; TTR — transthyretin; VN — vitronectin

cases [6]. Histological subtypes of NSCLC include adenocarcinoma (AC) (40%), squamous cell carcinoma (SCC) (25%), and many other subtypes which occur at a very low frequency [7]. The heterogeneity of NSCLC causes difficulty in appropriately diagnosing a patient and subsequently selecting an adequate treatment option, which differs significantly between subtypes [8]. Due to the high prevalence of NSCLC and its diversity, there is a need to identify specific biomarkers that could be used to support routine diagnosis of this cancer. This study presents the latest reports in proteomic research concerning new biomarkers which may be used in the diagnosis of NSCLC (Figure 1).

Protein diagnostic biomarkers detected in NSCLC patients

Proteomic studies allowed to identify a great number of proteins whose expression was diversified between tumor tissue and adjacent macroscopically-unchanged tissue, which constituted the control group in this study. As proteomic biomarkers of NSCLC, Li et al. [9] listed selenium-binding protein 1 (SELENBP1), carbonic anhydrase (CA), heat shock 20KD-like protein, transgelin (SM22-alpha), alloalbumin venezia (whose expression levels were down-regulated in lung cancer tissue), and alpha enolase (which was overexpressed). SELENBP1, a member of the selenoprotein family, mediates the intracellular transport of selenium [10], whose dietary deficiency is associated with an increased incidence of epithelial cancers, including lung cancer [11]. A progressively decreased expression level of SELENBP1 was observed by Zeng et al. [12] in the human bronchial epithelial carcinogenic process, which indicates its major role in the regulation of cancer development and progression. On the basis of the receiver operating characteristic (ROC) curve analysis, the authors revealed the ability of the SELENBP1 expression level to distinguish the normal bronchial epithelium from preneoplastic lesions with a sensitivity and specificity of 80% and 79%, respectively. Carbonic anhydrases (CAs) are enzymes involved in several fundamental biological processes including respiration, transport of CO2, pH regulation, and ion transport [13]. It has been shown that CAs are important mediators

of tumor cell pH by modulating the bicarbonate and proton concentrations for cell survival and proliferation. A proteomic study by Nigro et al. [14] concentrated on two CA isoforms, CAI and CAII, revealed a significantly downregulated expression level in the tumor tissue compared to control tissues, which indicates that these proteins could be candidates for use as diagnostic biomarkers in NSCLC patients. SM22-alpha is an actin cross-linking protein that is involved in calcium interactions and regulates contractile properties [15]. It has been found that it may play a role in cell differentiation, cell migration, cell invasion, and matrix remodeling by stabilizing the cytoskeleton through actin binding. However, the data on the abnormal expression level of SM22-alpha in lung cancer is controversial. Contrary to the results of Lie et al. [9], another proteomic study by Rho et al. [16] revealed an upregulated expression level of SM22-alpha in lung AC tissues compared to the control tissue.

Searching for biomarkers to diagnose squamous cell lung carcinoma, Zeng et al. [17] assessed the expression level of proteins in different stages of disease. The combination of three proteins, glutathione S-transferase P1 (GSTP1), heat shock protein beta-1 (HSPB1), and creatine kinase brain-type (CKB) was found to discriminate an invasive stage of cancer from the normal bronchial epithelium with a sensitivity of 92% and a specificity of 91%. Furthermore, they revealed that changes in expression levels of those proteins may be used to diagnose a patient with preneoplastic lesions with a sensitivity of 96% and a specificity of 92%. HSPB1 is a type of small Heat Shock Protein (sHSP) which is produced in cells by stressors such as hypoxia, UV light exposure, and viral agents. There is evidence that HSPB1 plays an essential role in cancer as it protects from programmed cell death (PCD) through interactions with several key regulatory proteins. GSTP1 is an enzyme catalyzing the detoxification of a variety of electrophilic compounds including oxidized lipid, DNA, and catechol products, thereby protecting cells from bioactive xenobiotics and reactive oxidative substances. The down-regulation of GSTP1 enhances the level of harmful substances and the frequency of gene mutations increasing the risk of bronchial epithelial carcinogenesis [17]. The study on human bronchial epithelial line cells revealed that GSTP1 knockdown increased the susceptibility of cell transformation induced by benzo(a)pyrene, the main lung carcinogen within tobacco smoke. CKB is one of two isoenzymes of creatine kinase which are involved in energy transduction pathways. It is predominantly expressed in the brain as well as in the lung, clearly in airway epithelial cells. The study by Hara et al. [18] demonstrated that CKB expression levels decreased in bronchial epithelial cells in the setting of cigarette smoke exposure, which is the main cause of SCC. According to the results of Zeng et al., upregulation of GSTP1 and CKB expression, and downregulation of HSPB1 expression may indicate the development of squamous cell lung carcinoma.

Pleural effusion, which is produced continuously at the parietal pleural level and reabsorbed through the lymphatic system, is a significant source of NSCLC biomarkers. In a number of disorders, including cancer, it accumulates because the rate of fluid formation exceeds the rate of its removal. It is rich in proteins, either secreted from tumor cells, derived from the circulation, or locally released by inflammation. These can potentially be used as biomarkers.

The study by Rodríguez-Piñeiro et al. [19] compared the proteome of pleural effusion samples as well as serum from NSCLC patients to those from patients with benign lung diseases such as pneumonia or tuberculosis. Their biomarker candidates comprise proteins with an increased expression in malignant pleural effusions such as pigment epithelium-derived factor (PEDF), gelsolin, and metalloproteinase inhibitor 2. Others studied included S100-A8 and S100-A9, although they had a lower expression. PEDF was the only protein found with significantly different levels both in the pleural effusion and the serum from NSCLC patients when compared with benign lung diseases. Recent studies on cell lines showed that PEDF affects migration, invasion, and motility of NSCLC cells by the regulation of thrombospondin 1 expression [20]. Previous investigations revealed that increased expression levels of PEDF were related to a counteracting activity to compensate for increased vascular endothelial growth factor (VEGF) levels [21]. VEGF is a strong angiogenic factor that is overexpressed during tumorigenesis. Therefore, an increase in PEDF would be expected to fight the spread of cancer cells.

Blood proteomic analysis may have a great advantage over the proteomics performed in lung cancer tissue due to the greater availability of blood samples. The proteomic study by Yang et al. [22] identified three serum candidate protein biomarkers for NSCLC: apolipoprotein C-I (ApoC-I), haptoglobin alpha-1 chain, and

S100A4, which can diagnose NSCLC in patients with a sensitivity and specificity of 96.56% and 94.79%, respectively. ApoC-I is a lipid carrier protein and, although previous studies mainly focused on lipoprotein metabolism, it has been reported that the ApoC-I also regulates many cellular functions such as the promotion of growth factor-mediated cell survival and apoptosis [23]. It has been demonstrated that ApoC-I has a certain anticancer effect on tumor cells, as well as the ability to decrease the expression of PCNA, Ki-67 and Bcl-2 proteins, enhance Bax protein expression, and inhibit cell proliferation [24]. S100A4, a member of the S100 family of calcium binding proteins, influences many biological processes including angiogenesis, stimulation of cell motility, upregulation of matrix metalloproteinases (MMPs), and modulation of tumor-related transcription factors [25, 26]. In addition, the tumor suppressor protein p53 has also been identified as a target for the S100A4 protein promoting its degradation and may be central for the stimulation of tumor development [27]. As a serum potential biomarker for NSCLC patients, Sung et al. indicated serum amyloid A (SAA), whose expression level was upregulated when compared to a control group [28]. Considering that SAA is a positive acute phase protein involved in the inflammatory response and that lung cancer is a chronic inflammatory disease, the elevated concentration of this protein is not surprising. However, a further study revealed that serum SAA expression levels in lung AC were significantly higher compared to other diseases of the respiratory system such as idiopathic pulmonary fibrosis and bronchial asthma, as well as in other cancers like stomach and breast cancer. The results of studies conducted by Urieli-Shoval et al. [29] revealed that the production of SAA by the alveolar lining epithelium of the lung occurred without provoking a continuous systemic acute-phase response during carcinogenesis.

In recent years, exosomes have garnered considerable attention from researchers due to their potential utility as circulating biomarkers for cancer. They are small (50–150 nm in diameter), membrane-enclosed particles containing nucleic acid and protein cargo, which have been implicated in a diverse range of physiological functions as well as pathological ones due to their capacity to convey molecules from a donor cell to a recipient cell. Tumor-derived exosomes have been demonstrated to carry the disease-associated molecular cargo and modulate the behavior of recipient cells towards a pro-oncogenic phenotype [30]. The proteomic studies assessing the profile of proteins in exosomes derived from the serum of NSCLC patients demonstrated a significantly higher expression level of fibronectin [31] and lipopolysaccharide binding protein [32]. It was shown that these proteins may be good biomarkers of NSCLC on the basis of the ROC curve (AUC was 0.833 and 0.713, respectively) and could be applied to diagnose NSCLC patients.

Subtype-specific tumor markers of blood in NSCLC patients

Adenocarcinoma and squamous cell carcinoma are the two most common NSCLC subtypes and have been shown to differ significantly both in terms of their clinical behavior and molecular signatures [33–36].

To investigate the expression of tumor-associated proteins in AC, Li et al. [36] used quantitative proteomic analyses which revealed significant differences in the expression levels of fibrillin-2, ferritin, eukaryotic translation initiation factor 4A1, annexin A5, mucin-5B (MUC5B), alpha-defensin 1, and anterior gradient protein 3, compared to the control lung tissue. Among these proteins, they found that MUC5B may be used as a good candidate biomarker in the detection of AC. MUC5B is a member of a family of high molecular-weight heavily-glycosylated proteins which are involved in the processes of epithelial differentiation, growth regulation, modulation of cell adhesion, cell signaling, and protection of the airway against environmental toxins [37, 38]. However, there is evidence that mucins also play important roles in tumor cell growth, invasion, and metastasis in cancer cells. The study by Nagashio et al. [39] confirmed that the expression level of MUC5B is higher in AC compared to SCC, as well as in patients with an advanced stage of cancer. Moreover, they noticed a correlation between the MUC5B expression level and tumor size, nodal status, and pleural invasion. These results suggest that MUC5B may not only be a useful differential diagnostic marker of AC from other histological types of lung cancer (especially from SCC), but may also serve to be a useful marker for more aggressive AC.

Another proteomic study revealed that the plasma of AC patients was characterized by a higher abundance of transferrin, immunoglobulin heavy chain, and leucine-rich alpha-2-glycoprotein [40]. A recent study by Li et al. [41] reported that the up-regulated leucine-rich alpha-2-glycoprotein expression induced the en-

hancement of cell proliferation, migration, and invasion, and mediated a proangiogenic effect via the activation of the transforming growth factor β pathway. Chang et al. [42] identified eight differentially expressed proteins between lung AC and the control group and these included: fibrinogen beta chain, fibrinogen alpha chain, haptoglobin (Hp), apolipoprotein A-I, transthyretin, serotransferrin, Ig alpha-1 chain, and Ig alpha-2 chain. Of these, haptoglobin had the highest peak ratio. Furthermore, ROC curve analysis revealed that Hp may be used as a good biomarker of AC, especially for males (AUC was 0.929). The main function of Hp is to bind free plasma hemoglobin thus preventing iron loss. However, it was also reported that body iron could accumulate in cancer cells and promote neoplastic cell growth [43]. It has also been shown that Hp has angiogenic [44] and antioxidant properties [45] and plays an important role in cell migration, contributing to cancer progression. Furthermore, Hp acts as a potent immunoreactive modulator protecting tumors against the host's immunity, which may contribute to the immune escape of the tumor [46]. The study by Abdullah et al. [47] revealed extrahepatic expression and synthesis of haptoglobin in lung tumors, especially in ACs, when compared to healthy lung tissues. The proteomic analysis by Kang et al. [49] indicated that a change in the HP molecular structure may be related to tumorigenesis. Hp is a tetrameric protein composed of $\alpha 1$, $\alpha 2$, and β chain polypeptides in differing combinations, which are connected by disulfide bridges. It has been reported that various forms of Hp have different abilities to bind hemoglobin and different properties regarding inflammatory and angiogenic functions [48]. Among the three different chains of Hp, $\alpha 2$ and β chains presented differences in the expression in AC compared to healthy controls. However, only the Hp β chain showed a significant difference between lung AC and other tumors, such as breast cancer and hepatocellular cancers, as well as other respiratory diseases (tuberculosis, idiopathic pulmonary fibrosis, and bronchial asthma) [49]. These studies demonstrated the significant role of Hp in the development and progression of AC and indicated that the Hp β chain could be a potential serum biomarker for AC patients.

The comparative analysis of serum proteome profiles conducted by Ciereszko et al. [39] revealed a higher abundance of vitronectin (VN), coagulation factor XIII, plasminogen, and gelsolin in SCC patients compared to AC patients. Recently, VN has been reported to be a potent migration-enhancing factor and plays an important role in the movement of cancer cells to lymphatics and body cavities via the interaction with the uPAR receptor [50]. It may suggest that VN plays a role in spreading cancer cells in SCC patients. Gelsolin acts as a protective protein against apoptosis in NSCLC cells, which is mediated through the inactivation of PI3K/Akt signaling [51], which may contribute to tumor progression.

Stage-specific tumor biomarkers

Based on the Tumor-Node-Metastasis (TNM) system, NSCLC patients are classified into different stages of disease (stages IA1, IA2, IA3, IB, IIA, IIB, IIIA, IIIB, IIIC, IVA, and IVB) through the assessment of primary tumors (T descriptor), regional lymph node (LN) involvement (N descriptor), and occurrence of distant metastasis (M descriptor) [52]. Depending on the stage of NSCLC disease, the 60-month overall survival rate significantly decreases from 92–68% in patients with stage I disease, to less than 10% in patients with stage IV disease [53]. Therefore, it is extremely important to discover biomarkers in the early-stage of NSCLC development.

In an attempt to find new stage-specific tumor markers, Deng et al. [54] assessed differential protein expression patterns of lung squamous carcinoma tissue collected from patients at different pathological stages. Among all identified proteins, tropomyosin alpha-3 chain (TPM3) demonstrated a decrease in the expression level with malignant progression from stage I to stage IV, while the expression level of peroxiredoxin 1 (PRDX1) was significantly increased from stage I to III and had a slight decrease at stage IV. To the best of our knowledge, this is the only study demonstrating the role of TPM3 in NSCLC progression and requires further investigation. PRDX1 is a member of the redox-regulating protein family of peroxiredoxins and has antioxidant activity protecting against Reactive Oxygen Species damage, which is attributed to its cell survival enhancing function. The study by Chen et al. [55] on cell lines confirmed that PRDX1 I influences cell proliferation by regulating the cell cycle and enhances their metastatic properties by increasing the expression of bcl-2 and VEGF proteins, contributing to tumor progression.

In order to characterize protein expression reflecting clinical stages of individual patients with AC, Kawamura et al. [56] performed proteomic analysis and identified 81 proteins with significantly different expression levels in patients

with stage IA of disease compared to patients with stage IIIA of disease. Further, proteomic analyses by Nishimura et al. [57] demonstrated that napsin-A (NAPSA) expression was significantly reduced in patients with an advanced stage of disease. Furthermore, they found a negative correlation between the expression level of NAPSA and survival time after surgery. The study by Nishimura et al. [57] also revealed that the anterior gradient protein 2 homolog (hAG-2) was highly expressed in patients with stage IIIA in comparison to those with stage IA. The higher expression level of hAG-2 was also related to the development of regional lymph node metastasis. This data suggested that the assessment of the expression level of these proteins can be used to distinguish between early and advanced stages of AC.

Metastasis-specific tumor markers

The presence of metastasis in patients with NSCLC is the major factor which influences lower survival rates. Unfortunately, it is estimated that 30–50% of NSCLC patients present with metastatic disease at the time of diagnosis [58, 59] as part of the Monitoring of Cancer Incidence in Japan (MCIJ. A better understanding of the molecular mechanism that regulates the development of metastasis is needed; such research will also reveal biomarkers predicting the progress of NSCLC.

In terms of determining metastasis-specific tumor markers, Hsu et al. [60] performed a study in lung tissue from AC patients with different extents of lymph node involvement. Their study indicated that the ERO1-like protein alpha (ERO1L) and asparagine-tRNA ligase may have a significant impact on the development of metastasis in this type of cancer [60]. They also suggested that ERO1L overexpression in primary sites of early-stage tumor tissue indicated a high risk for cancer micrometastasis. Previous studies reported that the induction of ERO1L was the key adaptive response in the HIF-1-mediated pathway under hypoxia that operates to improve VEGF secretion, facilitating local tumor progression and the formation of distant metastases [61].

The proteomic study by Li et al. performed on lung squamous carcinoma tissue indicated that 14-3-3 sigma may be a potential lymph node metastasis-related biomarker in SSC patients. The expression of this protein was significantly down-regulated in lymph node metastatic tumors compared to primary SSC. This protein is involved in the negative regulation of cell cycle progression and modulation of cell growth, differentiation, and apoptosis [63]. It has been shown that reducing the 14-3-3 sigma expression by siRNA silencing increased the in-vitro invasive ability of HTB-182 and A549 cells, while the enforced expression of ectopic 14-3-3 sigma decreased these abilities [62]. This data suggests that the downregulation of 14-3-3 sigma expression in tumor tissues may indicate an increased risk of developing lymph node metastases in SCC patients.

The proteomic approach has allowed for large-scale studies of protein expression in different tissues and body fluids which have been applied to discovering cancer biomarkers. This report reviews the major proteomic biomarkers which may be used to diagnose the development and progression of NSCLC.

Conflict of interest

None declared.

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Impact of inhalers used in the treatment of respiratory diseases on global warming

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Abstract

The term "carbon footprint" describes the emission of greenhouse gases into the environment as a result of human activities. The healthcare sector is responsible for 5–8% of the value of global greenhouse gas emissions, of which medical aerosols account for only 0.03% of the total emissions. The reduction of greenhouse gases, including those used for the production and use of medicinal products and medical devices, is part of the responsibilities that Poland and the respective countries should undertake in order to implement the assumptions of international law. At the level of medical law, this obligation correlates with the need to exercise due diligence in the process of providing health services, including the selection of low-emission medical products and devices (inhalers) and providing patients with information on how to handle used products and devices, with particular emphasis on those that imply greenhouse gas emissions. Pressurized metered dose inhalers (pMDI) containing the hydrofluoroalkane 134a demonstrate the largest carbon footprint, followed by a metered dose liquid inhaler and dry powder inhalers (DPI). The carbon footprint of DPI with a given drug is 13–32 times lower than it is in the case of the corresponding pMDI. Replacement of pMDI by DPI is one of the effective methods to reduce the carbon footprint of inhalers, and the replacement should be based on current medical knowledge. A recycling system for all types of inhalers must be urgently implemented.

Key words: carbon footprint, global warming potential, pressurized metered dose inhaler, hydrofluoroalkane, dry powder inhaler, inhalation therapy

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Introduction

The aim of the publication was to present the influence of inhalers used in the treatment of respiratory diseases on global warming. For this purpose, the literature available in the PubMed database was reviewed. Data provided by inhaler manufacturers were also used. The following parts of the article present the definitions and indicators of the carbon footprint, European and Polish legal regulations on the reduction of greenhouse gases, a short review of inhalers and inhalation drugs based on the example of the Polish market. Further section presents the results of studies on the carbon footprint of selected inhalers and methods of reducing the negative impact of inhalers on the environment, including the problem of replacing pressurized metered dose inhaler (pMDI) with dry powder inhaler (DPI).

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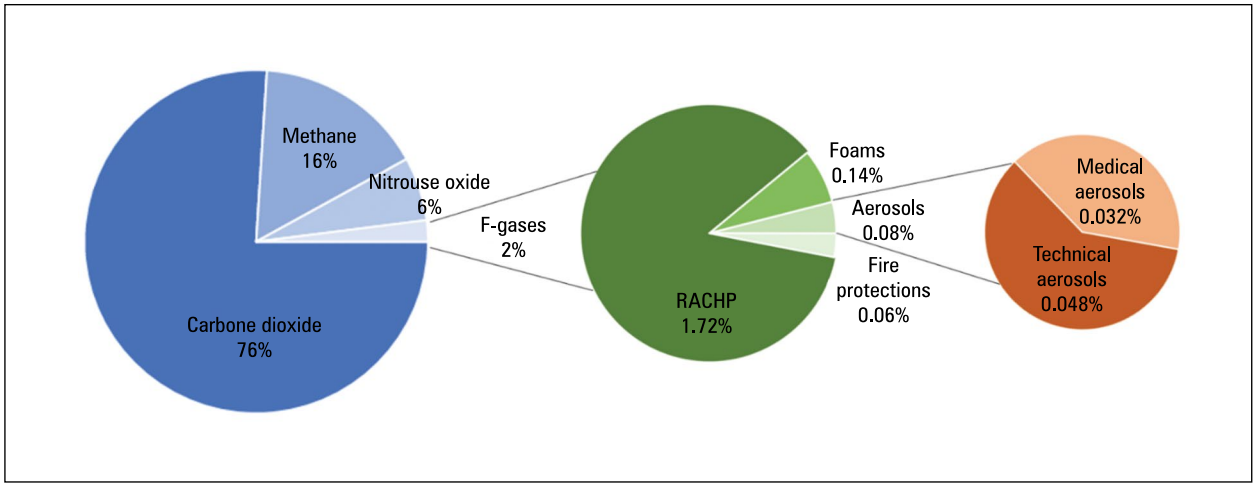

Figure 1. The share of medical aerosols in the total GHG pool in the world in 2016 [7]. RACHP — Refrigeration, Air-Conditioning, Heat Pomp

Basic definitions and indicators of the carbon footprint

Term carbon footprint describes the emission of greenhouse gases (GHG), that are generated to the environment due to human activity [1]. Carbon footprint is quantified by the global warming potential (GWP), expressed in tones, kilograms or grams of the equivalent emitted carbon dioxide (CO_2): t CO_2e, kg CO_2e or g CO_2e. GWP shows how many times the impact of a single t/kg/g of a given gas emitted to the atmosphere is higher that the greenhouse effect caused by a single t/kg/g of CO_2. For instance, GWP values for methane and hydrofluoroalkane (HFA) 134a are 23 and 1300 t CO_2e, respectively [2]. Accordingly, one tone of emitted methane causes the same effect as 23 tones of emitted CO_2, while one tone of HFA 134a — as 1300 tones of emitted CO_2. Another important indicator of the impact of GHG on the natural environment is their atmospheric persistence (stability). Methane is stable in the atmosphere for 12–15 years, whereas various HFAs — above 250 years. The stability of sulfur hexafluoride (SF6) in the atmosphere is up to 3200 years [3]. Among GHGs that are defined as natural or anthropogenic components of the atmosphere that absorb and reemit infrared radiation, we can find CO_2, CH_4, N_2O and many gases that contain fluoride (the F-gases), including: SF6, perfluorocarbons (PFCs), chloroflourocarbons (CFCs, including freons) and hydrofluorocarbons (HFCs, including HFAs). HFCs that are used in pressurized metered dose inhalers (pMDIs) are mainly HFA 134a and HFA P277 [4].

Power industry and transportation are the predominant sources of GHGs on the global scale, where CO_2 forms ¾ of the total emission. According to the available data in 2016, the global emission of CO_2 was above 34 bln tones. Different regions and countries have various input to this emission. China, USA, India and Russia are dominating being responsible for 55% of global CO_2 emission. Accoring to the same data, the input of Poland is only 0.83% of global emission of CO_2 [5]. It is also known that F-gases compose 2% of global GHG emission and they are primarily used in cooling and refrigeration, AC systems and fire fighting [6]. Only 0.03% of total GHG emission is related to medical aerosols [7] (Figure 1).

The broadly understood healthcare sector is responsible for 5–8% of the global GHG emission value [8]. In Germany, 7% of the country's carbon footprint is produced in the health sector [9]. It is not known how big this share is in Poland. Among the many elements that make up this value, inhalers, especially pMDI, occupy a certain proportion. Over 800 million HFA-based pMDIs are sold annually worldwide (> 11,500 tones/year), resulting in an estimated CO_2e of > 13 million tonnes [10, 11]. In light of the above, global and regional non-governmental organizations and governments of several countries have started implementing projects aimed at reducing GHG emissions from the healthcare sector [12]. A policy of pro-ecological public procurement is proposed and the inclusion of these considerations in the decision-making process on purchasing and financing medical technologies. Reducing CO_2 production has become the goal of the sustainable development of pharmaceutical companies. In a more patient-centered healthcare ecosystem, patients are increasingly acting as consumers and

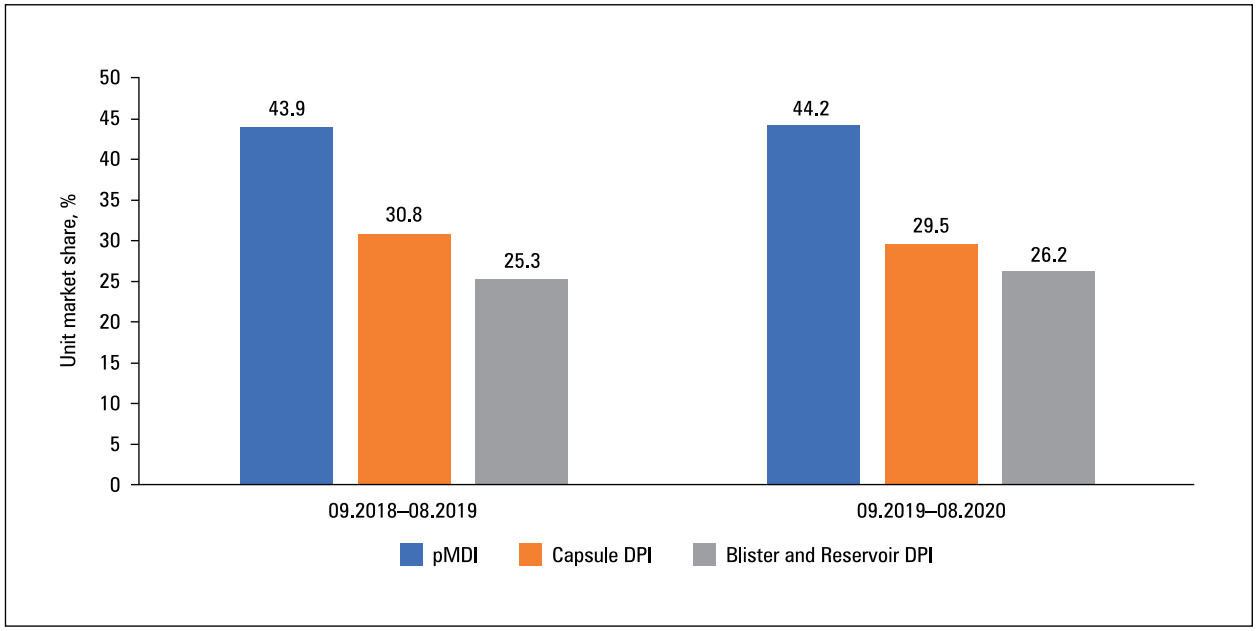

Figure 2. Sale of pMDI and DPI inhalers to pharmacies in 12-month periods from 09.2018 to 08.2019 and from 09.2019 to 08.2020 (data from the Pharmaceutical Database, IQVIA 08/2020 sell in.). DPI — dry powder inhaler; pMDI — pressurized metered dose inhaler

Table 1. Availability of inhaled drugs in Poland in the respective types of inhalers (as of 01/01/2021)

Inhaler/Drug	ICS	LABA	ICS + LABA	SABA	SAMA	SABA + SAMA	LAMA	LABA + LAMA	ICS + LABA + LAMA
pMDI	+	+	+	+	+	+	–	–	–
pMDI-BA	+	–	–	–	–	–	–	–	–
DPIs	+	+	+	+	–	–	+	+	+
Nebulizer	+	+	–	+	+	+	–	–	–

DPIs — dry powder inhalers; ICS — inhaled corticosteroid; LABA — long acting beta-2 agonist; LAMA — long acting anti-muscarinic agent; pMDI — pressurized metered dose inhaler; pMDI-BA — pressurized metered dose inhaler-breath actuated; SABA — short acting beta-2 agonist; SAMA — short acting anti-muscarinic agent

may prefer environmentally friendly products (including inhalers) [13].

Review of inhalers and inhalation drugs on the example of the Polish market

The inhalation route is the most important method to administer majority of drugs used in asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis and other acute or chronic respiratory diseases [14–16]. Aerosol therapy can be carried out with several groups of inhalation devices (inhalers), such as:

— pressurized metered dose inhaler (pMDI) with its variant — a pressurized metered dose inhaler — breath actuated — pMDI-BA;
— dry powder inhaler (DPI) — a lot of different types (generations) of inhalers;
— metered dose liquid inhaler (MDLI) — one inhaler on the market in the country;
— nebulizers (pneumatic and ultrasonic, including mesh nebulizers) — a lot of devices that are technically very diverse.

There is huge variation between the respective countries in the share of inhalers being used. For example, in 2011 in Sweden, about 90% of inhaled corticosteroids (ICS) were inhaled using DPI, while in the UK about 80% were inhaled using pMDI [17]. The share of individual types of inhalers in the Polish market is shown in Figure 2.

The data contained in the BAZYL Pharmaceutical Database, which are partially presented in Figure 2, show that a little over 13 million pMDI and DPI packages are sold in Poland per year. Of these, pMDI accounts for approx. 44%, single-dose capsule DPIs for approx. 30%, and multi-dose blister and reservoir DPIs for approx. 26% of this market. The list does not include MDLI (Respimat) and nebuliser devices.

Table 2. Medicines registered in Poland in the respective types of DPI (as of 01/10/2020)

Inhaler's trade name	DPI type	Medicines available
Aerolizer®	Capsule	Budesonide, formoterol
CNG Fantasmino®	Capsule	Budesonide, fluticasone propionate, formoterol, salmeterol
CNG Breezhaler®*	Capsule	Budesonide, mometazon/indacaterol, indacaterol/glycopyrronium, mometazon/indacaterol/glycopyrronium
Diskus®*	Blister	Fluticasone propionate, salbutamol, salmeterol, fluticasone propionate/salmeterol
Generic Diskus (Aerostar®, G7)	Blister	Fluticasone propionate/salmeterol
Easyhaler®	Reservoir	Budezonid, salbutamol, formoterol, budezonid/formoterol
Ellipta®*	Blister	Umeclidinium, fluticasone furoate/vilanterol, umeclidinium/vilanterol, Umeclidinium/fluticasone furoate/vilanterol
Forspiro®	Blister	Fluticasone propionate/salmeterol, budezonid/formoterol
Genuair®	Reservoir	Umeclidinium, Umeclidinium/formoterol
Handihaler®	Capsule	Tiotropium
Nexthaler®	Reservoir	Beklometazone/formoterol
Novolizer®	Reservoir	Budesonide, salbutamol, formoterol
Podhaler®	Capsule	Tobramycin
Spiromax®*	Reservoir**	Budesonide/formoterol
Turbuhaler®*	Reservoir	Budesonide, formoterol, Budesonide/formoterol
Twisthaler®	Reservoir	Mometasone
Zonda®	Capsule	Tiotropium

*Inhalers also available in an electronic version (sensor recording the use of an inhaler and/or measuring the inspiratory flow), but currently not available in Poland.
**Spiromax® — multidose, reservoir, III generation.
DPI — dry powder inhaler

Table 1 presents various inhaled medications used in the treatment of asthma or COPD available on the Polish market in each type of inhaler, and Table 2 presents the drugs registered in Poland in each DPI.

The wide variety of DPIs is, on the one hand, a good solution for patients and doctors, as it allows individual selection of the appropriate inhaler. However, on the other hand, it causes difficulties in choosing DPI and the need to educate medical personnel and patients. Choosing the right inhaler for a given patient depends on many elements and it is subject to established rules depending on the type of the disease (asthma *vs* COPD *vs* cystic fibrosis), age of patients (children *vs* adults), and other variables, not only clinical [14–19].

European and Polish legislation on greenhouse gas reduction

The issues of reducing GHG are regulated both under European and Polish law. The preamble to the United Nations Framework Convention already States that climate change and its negative effects are one of the key problems facing humanity [20]. It was noted that the highly developed countries have the largest share of global GHG emissions. The purpose of this Convention is, in accordance with the wording of Article 2 thereof, to "achieve (…) stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system (…)" [20]. Another important legal step was the Kyoto Protocol of 11.12.1997, which required 38 developed countries to reduce GHG emissions [21, 22].

On 12 December 2015 in Paris, at the Conference of the Parties to the United Nations Framework Convention on Climate change, 195 States adopted the text of the new climate agreement, the Paris Agreement signed in New York on 22 April 2016, which became applicable at the beginning of 2020, Replacing the Kyoto Protocol [23]. The agreement imposes an obligation on individual States to take two types of action: To reduce CO_2 emissions and to extend their absorption, inter alia, by increasing forestation. In accordance with Article 4 of that Agreement, the reduction of CO_2 emissions is to be achieved as soon as possi-

ble. Individual States are required to identify their contributions (Intended Nationally Determined Contributions) to the fight against climate change and to gradually increase it.

Further legislation relevant to this issue concerns the protection of the ozone layer of the atmosphere. These include Regulations (EC) No 1005/2009 of the European Parliament and of the Council [24] and No 517/2014 [25]. In view of the direct effectiveness of EU regulations, national legislation on this issue is complementary and implementing to EU law, and is intended primarily to enable the latter to be properly applied.

National legislation on the protection of the ozone layer is in force under the Act of 15 may 2015 on substances that deplete the ozone layer and on certain fluorinated greenhouse gases (F-gases) [26]. The provisions of Regulation (EU) NO 517/2014 of the European Parliament and of the Council on fluorinated greenhouse gases regulate environmental issues by reducing emissions of such gases [27].

The first important international document which directly referred to ozone-depleting substances was the Montreal Protocol of 16 September 1987 [26]. The Protocol was amended by an amendment from Kigali of 15 October 2016 [28], which was ratified by Poland on the basis of Article 89(1) of the RP Constitution [29]. On 18 December 2018, the Law of 9 November 2018 on the ratification of the amendments to the Montreal Protocol [30] entered into force. The first result of the adoption of the Kigali amendment is:

1. Extension of the list of controlled substances to 19 HFC substances commonly used as substitutes for ozone-depleting substances but to be GHG with very high GWP values;
2. Introducing a timetable for reducing HFCs, which is separate for developed and developing countries;
3. Extension of the obligation to submit annual reports on HFC production, import and export [31];
4. Extension of the obligation to license imports and exports to HFC;
5. Extending the withdrawal of HFCs in developing countries to the multilateral Fund Protocol funding scheme.

According to Article 4 of the Act on the professions of doctor and dental practitioner, 'a doctor is required to practice the profession, as indicated by current medical knowledge, by the methods and means available to him to prevent, recognize and treat diseases, in accordance with the principles of professional ethics and due diligence' [32]. Due diligence in the treatment process should be understood, inter alia, to eliminate activities which involve the risk of adverse effects for the person being treated or for the general public (even after many years). The use of ecological inhalers prevents distant effects in the area of climate change, which has a direct impact on improving quality of life and health protection. An example of a lack of due diligence can be the choice and use of medicinal products with negative environmental consequences. It should be noted that where the patient declares that he is only in agreement with the handling of non-organic products that are still in circulation, the doctor cannot implement a treatment contrary to the patient's will, even if it considers it to be the optimal way of medical treatment.

In analysing the context of due diligence in the area of GHG reduction, attention should be paid, inter alia, to the British Thoracic Society guidelines, which stress the importance of selecting DPI as an alternative to pMDI, and to informing patients about the possibility of low-carbon inhalation therapy [33]. At the same time, the above-mentioned guidelines emphasize the need to inform patients that optimizing the use of medicinal products involves the use of existing products and also the proper segregation of used packaging of medicinal products.

The last of the topics discussed is important under Polish law. According to it, the packaging of used, expired or damaged medicinal products or medical devices (including inhalers) should be placed in labelled containers, which may be placed, inter alia, in publicly available pharmacies [34]. In the context of the due diligence to which the doctor is responsible, it should be noted that it will inevitably be an element of informing the patient about the handling of used packaging of medicinal products and medical devices (including inhalers), which should be properly disposed of due to the loss of therapeutic value.

In conclusion, the reduction of greenhouse gases, including those used in the manufacture and use of medicinal products and medical devices, falls within the scope of the obligations which Poland and the individual countries should undertake to implement the principles of international law. At the level of medical law, this obligation implies due diligence in the process of providing health services, including the selection of low-carbon products and products (inhalers), and information to patients on how to deal with used products and products, with particular

attention to those which imply greenhouse gas emissions.

Carbon footprint of selected inhalers

In the analysis of the carbon footprint of a given product, including an inhaler, its full "life" cycle should be considered — from its production, through its use, to the disposal of its waste [35]. Comprehensive analysis is possible with the use of a special LCA methodology — life cycle analysis [36, 37]. In order to perform this analysis in relation to medical inhalers, complete information is required about each stage of the process:

1. Manufacturing of the inhaler;
2. Manufacturing of the drug contained therein (that usually includes a proprietary know-how);
3. Distribution and sales channels as well as warehousing of the inhaler;
4. Use of the drug;
5. Maintaining hygiene of the inhaler;
6. Managing (partial or complete) waste of the inhaler and the drug.

For each of these "life" stages of the inhaler, the carbon footprint would have to be determined separately in terms of GWP values (e.g., per delivered dose of the drug or per 100 doses) and then summed up. Accurate data on this subject is not available for many inhalers, making it difficult to reliably quantify and compare inhalation products in terms of their carbon footprint.

The study by Goulet *et al.* [36] is an example of an analysis of the carbon footprint for various inhalers. The authors attempted to compare the carbon footprint of two types of inhalers: pMDI HFA 134a with albuterol 200 μg/dose (Proventil, Merk & Co., Inc., Kenilworth, NJ, USA) and the DeVillbis Pulmo-Aide continuous pneumatic nebulizer (DeVilbiss, Port Washington, NY, USA) using the standard dose of 3 mg of albuterol. In the case of pMDI, the authors analyzed not only the HFA 134a carrier released into the atmosphere during drug administration, but also other components of the inhaler, including an aluminum drug container (canister), dosing valve or polypropylene inhaler housing, carrying a specific carbon footprint. The pneumatic nebulizer, although it does not emit greenhouse gases directly when inhaling the drug, is electrically powered, and consists of many metal and plastic elements that relate to carbon footprint. Even the washing method (by hand or in the dishwasher) and the possible sterilization of the nebulization chamber also contribute to the carbon footprint. The authors cited above showed that the carbon footprint of pMDI HFA 134a is two to three times higher than the carbon footprint of the nebulizer (per dose), the difference is mainly caused by the emission of HFA, a gas with a high GWP value. In the case of a nebulizer, its carbon footprint is significantly influenced by the method of washing the nebulization chamber and mouthpiece — the GWP significantly increases in the case of manual washing. The contribution of other factors, due to the long time of using the device (compressor, nebulization chamber, connecting tubes), remains at a very low level. The authors omitted the issue of the carbon footprint resulting from the management of the used pMDI inhaler and the complete nebulizer, and did not consider the inhalation filter in the nebulizer. Similarly, they considered the contribution of the transport of both inhalers to the carbon footprint to be insignificant. There are no data available on the carbon footprint of mesh nebulizers, although theoretical considerations may indicate lower GWP values vs pneumatic nebulizers (in-house data, unpublished). Another study showed that a GWP of Atrovent pMDI HFA 134a is approx. 14.6 kg CO_2e, and a GWP of Berodual™ pMDI HFA 134a is approx. 16.5 kg CO_2e and these values are approx. 20 times higher than those obtained for drugs administered with MDLI such as Spiriva Respimat® or Berodual Respimat® preparations – both approx. 0.78 kg of CO_2e [38]. In the case of MDLI of the Respimat type, depending on the number of uses (refillable cartridge), the inhaler "produces" between 0.77 and 1.03 kg of CO_2 [39]. In pMDI, more than 95% of GWP comes from the HFA carrier, and the additional effect comes from the inhaler itself (approx. 1%), drug formulation, and other components (approx. 0.8%), as well as from manufacturing and distribution processes ($< 0.5\%$) [38]. This study was methodologically correct, as it covered all stages of the "life" of the inhalers tested (acquisition and initial processing of materials, production, distribution, use and disposal of the inhaler — LCA methodology). Similar data apply to other drugs with pMDI HFA 134a [40]. It is worth recalling that the HFA 227a propellant contained, for example, in the GKSw/LABA Flutiform™ 120 doses, demonstrates even higher GWP value — 295 g CO_2e per dose [41] *vs* Ventolin™ pMDI 134a 200 doses — approx. 120 g CO_2e per dose [42]. Also, the HFA 227a contained in pMDI Symbicort™ shows a very high GWP value [43]. Both, Flutiform™ pMDI and Symbicort™ pMDI, are not available on the Polish market.

The comparisons of pMDI HFA 134a with DPI for inhalers available on the Polish market are interesting. In a recently published study, Janson *et al.* [44] assessed the total annual carbon footprint of pMDI and DPI of Accuhaler (Diskus)™ and Ellipta™ types (Table 3).

The presented data show that the combination of fluticasone propionate with salmeterol in DPI results in a carbon footprint 32 times lower than in pMDI HFA 134a. On the other hand, preparations containing two or three medicinal substances in one inhaler resulted in a lower carbon footprint than using them in separate inhalers: by 12.6% for pMDI and by 23.2% for DPI [44]. Similar results are found in the publication by Wilkinson *et al.* [40].

Slightly different results were presented by Panigone et al., who were analyzing some inhalation drugs by Chiesi Farmaceutici S.p.A., also with the new HFA-152a propellant (drugs with this propellant are under study) (Table 4) [45]. It presents data on inhalers containing 120 doses and providing 200 μg beclometasone dipropionate/6 μg formoterol/nominal dose (Foster®) or 100 μg beclometasone/6 μg formoterol/12.5 μg glycopyrronium bromide per metered dose (Trimbow®).

The carbon footprint of the NEXThaler type DPI is approx. 15 times lower than the corresponding combination in case of pMDI HFA 134a.

Table 3. Comparison of GWP for frequently used pMDI and DPI (own modification according to [44])

Inhalers/drugs	GWP kg CO_2e/year
pMDI — Ventolin Evohaler®	205
pMDI — Seretide Evohaler®	234
Total	439
DPI — Seretide Accuhaler (Diskus)®	7.3
DPI — Relvar Ellipta®	9.5
Total	16.8

DPI — dry powder inhaler; GWP — global warming potential; pMDI — pressurized metered dose inhaler

On the other hand, the use of the new HFA 152a carrier in pMDI reduces the carbon footprint of the assessed preparations by approx. 8 times and it is only approx. 2 times higher than it is in case of NEXThaler. Recently, data on the Breezhaler® capsule type DPI was provided by Novartis AG for its combination drugs: indacaterol (IND)/mometasone furoate (MF) and IND/MF/glycopyrronium (IND/MF/GLY) [46]. These tests were performed in accordance with the recommended standards (GHG Protocol). The evaluation covered the entire life cycle of the product, including the device, active pharmaceutical ingredient (drugs) and optional sensor. The carbon footprint comparison was carried out for these products in 4 countries: France, Germany, the UK and Japan. Data from France are shown in Figure 3. For the first time, the authors reported the carbon footprint of the sensor, an optional electronic device (Propeller Health Sensor) facilitating the control of patient adherence to prescribed inhalation treatment, and registered by the European Medical Agency in 2020 for use with DPI Breezhaler [47].

They show that a Breezhaler containing IND/MF or ING/MF/GLY without a sensor "produces" approx. 0.4 kg CO_2e and approx. 0.38 kg CO_2e, respectively, per month of use. However, an inhaler with ING/MF/GLY with a sensor produces as much as about 0.5 kg of CO_2e per month.

Recently, Orion Pharma reported the carbon footprint of the Easyhaler® DPI, and the study was performed according to the LCA methodology (Table 5) [48 ,49].

Manufacturing process of the device, drug substance, lactose carrier, packaging and package leaflet for the patient turned out to be the most important source of emissions constituting approx. 60% of the total carbon footprint (CO_2e) of the product. By comparison, the distribution of the inhaler constitutes less than 2% of the total carbon footprint. Salbutamol Easyhaler® shows the highest carbon footprint associated with its production, as it requires more lactose than other medicines manufactured by this company.

Table 4. Carbon footprint of selected inhaled drugs by Chiesi Farmaceutici S.p.A. [45]

Inhalers/drugs	GWP g CO_2e/dose	GWP kg CO_2e/inhaler
pMDI — Foster® HFA 134a*	118.56	14.23
pMDI — Foster® HFA 152a*	14.50	1.74
pMDI — Trimbow® HFA 134a	118.99	14.28
pMDI — Trimbow® HFA 152a	14.34	1.61
DPI — Foster NEXThaler®	7.64	0,92

DPI — dry powder inhaler; GWP — global warming potential; pMDI — pressurized metered dose inhaler

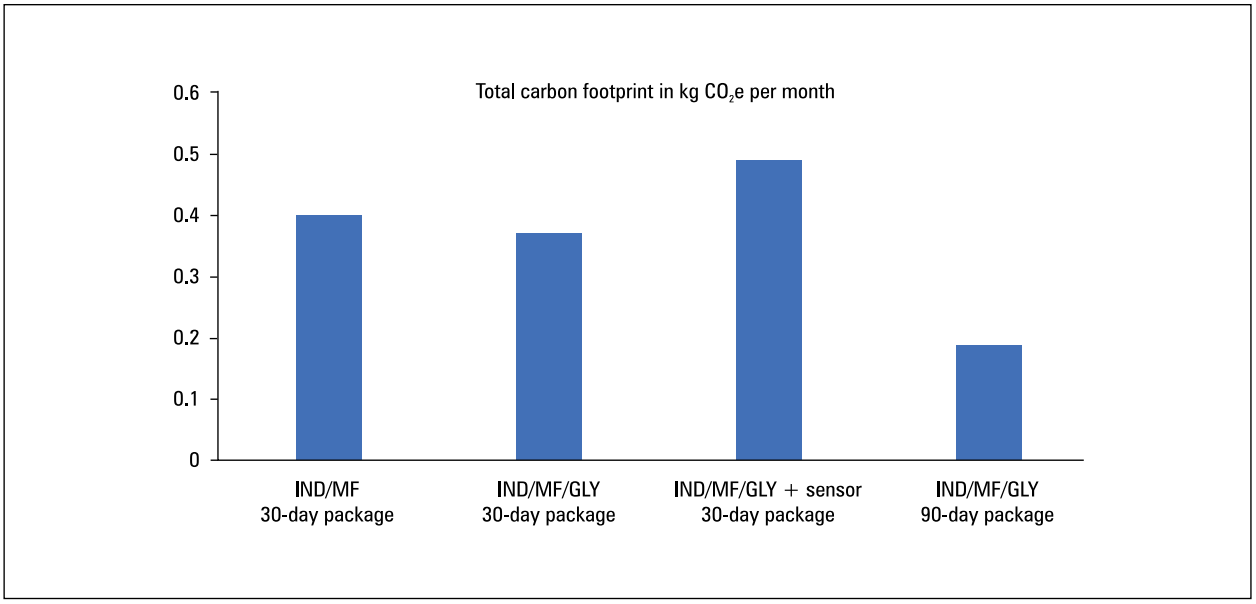

Figure 3. Carbon footprint in kg of CO_2e per month of medication use with DPI Breezhaler [46].
IND — indacaterol maleate; MF — mometasone furoate; GLY — glycopyrronium bromide

Not all pharmaceutical companies have disclosed the carbon footprint of their inhalers. For example, there are no data or only estimates for such popular DPIs as Turbuhaler® (AstraZeneca), Forspiro® (Sandoz AG) or Spiromax® (Teva Pharmaceuticals Industries Ltd.). There are also no data on inhalation chambers necessary for the use of pMDI in children and in some groups of adults.

Methods of reducing the negative environmental impact of inhalation drugs — a responsible view of inhalers in the context of the carbon footprint

Offered inhalers and the method of their use (until their disposal) will undoubtedly move towards reducing their carbon footprint, as this will be enforced by signed obligations and created law. Reducing the negative impact of inhalers on the environment can be achieved through a number of activities that involve inhaler and drug manufacturers, the payer, medical staff, and the patients themselves. Detailed actions should include the following [45, 50, 51]:

1. Implementation and strict adherence to an effective individual inhalation treatment plan (physician, patient);
2. Education and continuous verification of the correctness of the inhalation technique (health educator, physician, nurse, patient);
3. Reducing the use of SABA "on demand" in all types of inhalers by improving asthma and COPD control (physician, patient);
4. Optimal use of the inhalation chamber, usually associated with the improvement of the clinical efficacy of pMDI drugs (physician, patient);
5. Using inhalers for the last dose and not wasting doses by releasing the drug into the atmosphere (patient);
6. Introduction of pMDI with new propellants with lower GWP values, for example: HFA 152a (manufacturer, payer, physician);
7. Rational replacement of pMDI by DPI or MDLI (doctor);
8. Reducing the number of inhalers in a given patient through the wider use of drugs combined in one inhaler and the introduction of new two- or three-component formulations (manufacturer, physician, payer);
9. Creating DPI and MDLI inhalers with replaceable cartridges extending the time of using the inhaler (manufacturer);
10. Using DPI capsule for a larger number of doses, which requires actions that improve the inhalers (manufacturer);
11. Promoting the recycling of all inhalers (manufacturer, pharmacy, patient).

Replacing pMDI with DPI

Replacing pMDI with DPI is one of the ways to reduce the carbon footprint of inhalers, which was suggested a few years ago [52]. For example, it has been shown that reducing the number of pMDIs in favor of DPI in the UK from 70% to 13%

Table 5. Components of the carbon footprint level for various drugs in Easyhaler® DPI [49]

	Carbon footprint (g CO_2e) per inhaler		
Components influencing the carbon footprint	**Salbutamol**	**Fluticasone/Salmeterol**	**Budosonide/Formoterol**
Dose size in μg Number of doses	100 200	250/50 60	160/4.5 120
Raw materials for the production of inhaler components, packaging and patient information leaflets	142.3	142.3	142.3
Raw materials needed for drug and carrier production	0.74	1.9	0,50
Transportation of raw materials	11.5	11.50	11.40
Drug and carrier production	314.1	250.4	164.7
Assembling of the finished product	76.4	76.4	76.4
Product distribution	8.5	8.4	8.3
Utilization	72.4	72.4	72.4
Total	664.1	601.8	514.5

Table 6. Percent change in costs resulting from the replacement of various drug classes from pMDI to DPI — Poland compared to other European countries, data from European markets with the highest value (65 in-house modification).

	Poland [%]	Germany [%]	United Kingdom [%]	France [%]	Italy [%]	Spain [%]
SABA	290	147	290	171	277	304
SABA/SAMA	—*	205	—*	—*	—*	—*
ICS	80	81	121	101	107	99
LABA	90	92	92	100	99	107
ICS/LABA	93	91	95	97	100	92
ICS/LABA/LAMA	—*	155	148	142	161	183
Sum	96	102	107	107	106	104
Market value in mln $	271	1394	1293	894	685	751

*No equivalent in DPI.
DPI — dry powder inhaler; ICS — inhaled corticosteroid; LABA — long acting beta-2 agonist; LAMA — long acting anti-muscarinic agent; pMDI — pressurized metered dose inhaler; SABA — short acting beta-2 agonist; SAMA — short acting anti-muscarinic agent

will reduce CO_2 emissions by over 550 kt/year [53]. However, inhalers are not easily interchangeable and the selection of the correct device depends on many factors [54]. The best inhaler for a given patient should be chosen, following the principle of "the right inhaler for a given patient" and not "the same inhaler for all patients" [18, 51, 55, 56]. Each type of inhaler requires specific instructions for use and a new inhaler can be a problem for the patient, even if it would be better for some reason in the opinion of the doctor. Changing the inhaler may lead to a deterioration of the treatment effect [57, 58]. However, switching (both to a generic inhaler and to another one) in clinically justified cases in patients with asthma or COPD may reduce exacerbations and improve adherence as well as it can be a cheaper treatment [59]. It seems to be influenced by various local factors, therefore, data from one country (market) and a given type of inhaler cannot be uncritically transferred to other countries (markets) and inhalers [60]. As a general rule, if an obstructive bronchial disease is well controlled, the inhaler should not be changed without good reason. The change of each inhalation device should be agreed with the patient, who should be trained in the use of the new inhaler, and the use of the inhaler and inhalation technique should be controlled [54, 61, 62]. The limitation of the necessary inhaled drugs (regular and emergency) to one type of device (pMDI or DPI or MDLI or nebulizer), and in the case of DPI — to inhalers of the same generation is a significant facilitation for the patient [63, 64]. Switching drugs administered from pMDI to DPI may be associated with an increase in direct costs for most large EU countries, but not for Poland (Table 6) [65].

The highest cost of replacing pMDI with DPI will relate to SABA, also in Poland (growth by 290%). In the case of other drug classes in the country, lower costs of DPI vs. pMDI can be expected. There are substitutions in DPI for majority of pMDI drugs. Exception in the country includes ciclesonide, fenoterol, ipratropium bromide, fenoterol/ipratropium bromide and beclomethasone/formoterol/glycopyronium occurring only in pMDI.

Summary and conclusions

Precise determination of the carbon footprint for a given inhaler is not easy, so comparing different inhalers in this respect is a major challenge. In articular, the variety of DPI and nebulizer designs makes it difficult to perform simple comparisons of the carbon footprint between different device classes. There is not enough data on the carbon footprint of nebulizers to form a reliable opinion. So far, the assessment of the carbon footprint of pMDI inhalation chambers, nebulizer exhaled aerosol filters and many electronic devices (sensors) attached to or incorporated into the pMDI or DPI has been omitted. There are also no generally applicable uniform methods for assessing the carbon footprint of inhalers. The reduction of GHG related to the production and use of inhalers, despite a relatively low share of inhalation products in the total GHG emission, is part of the obligations that individual countries should undertake in order to implement the principles of international law. At the level of medical law, this obligation correlates with the need to exercise due diligence in the process of providing health services, including the selection of low-emission inhalers and providing patients with information on how to deal with inhalers.

Conclusions

1. The vast majority of inhalation drugs used in the treatment of asthma or COPD available in Poland are available in pMDI and DPI.
2. pMDI HFA 134a shows the highest carbon footprint, followed by MDLI and DPI. There is insufficient data on nebulizers to assess this group of inhalation devices.
3. The carbon footprint of DPI with a given drug is 13–32 times lower than it is in the corresponding pMDI.
4. It is necessary to disseminate new pMDI propellants with low greenhouse potential.
5. All types of inhalers should be available, as there are numerous groups of patients who cannot use DPI (children under 4–6 years of age, elderly COPD patients, severe forms of COPD/asthma with inspiratory flow < 30 L/min, in case of the drugs available only in pMDI).
6. We recommend caution and the use of current medical knowledge when replacing pMDI inhalers with DPI in patients with asthma or COPD. Changing the inhaler type solely on the basis of the dose equivalence is not appropriate.
7. There is an urgent need to implement a recycling system for all types of inhalers.

Conflict of interest

None declared.

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The effect of buprenorphine *vs* methadone on sleep breathing disorders

Abstract

Opioids are used widely as analgesics and can play an important role in agonist maintenance therapy for opium dependence. Despite their benefits, the negative effects on the respiratory system remain an important side effect to be considered. Ataxic breathing, obstructive sleep apnea, and most of all central sleep apnea are among these concerns. Obstructive sleep apnea leads to various metabolic, cardiovascular, cognitive, and mental side effects and may result in abrupt mortality. Buprenorphine is a semisynthetic opioid, a partial mu-opioid agonist with limited respiratory toxicity preferably used by these patients, as it is accompanied by significantly lower risk factors in the development of obstructive and central sleep apnea. In this manuscript, the case of a patient is reported who underwent methadone maintenance therapy which was shifted to buprenorphine in order to observe possible changes in sleep-related breathing disorders. The results of this study indicate a reduction in these problems through the desaturation and apnea hypopnea index of methadone substituted by buprenorphine while no change in sleepiness was observed.

Key words: opioid, methadone, buprenorphine, sleep breathing disorders

Adv Respir Med. 2020; 88: 439–443

Introduction

Opioids are compounds that affect opioid by G-protein receptors. Three main subgroups of these G protein receptors are mu, kappa, and delta. Although these receptors are present in the respiratory system, the important point to consider about their respiratory effect is the influence of the respiratory center located on the brain stem, precisely in pre-Botzinger complex, which seemingly is the area with respiratory rhythm-generating neurons [1]. These receptors are mainly of the mu-type and their stimulation by drugs such as chronic use of methadone is at risk because of breathing disorders of complicated and potentially lethal nature, including central and obstructive apneas, hypopnea, ataxic breathing and nonapnoeic hypoxemia during sleep [2]. Opioids were used in order to relieve pain and also, to act as a main part of opioid (e.g. methadone or buprenorphine) agonist maintenance therapy [3]. Buprenorphine is a semisynthetic opioid, i.e., a partial mu-opioid agonist with limited respiratory toxicity. But it has an antagonistic effect on kappa and delta receptors and through the effect of the opioid receptor-like (ORL-1). Meanwhile, it is an agonist that induces the analgesic effects [4]. Nowadays, ORL1 is the same as Nociceptine/orphanin FQ receptors which are widely used because of the contributing result of their function, the treatment of opioid dependency and chronic nonmalignant pain. Although buprenorphine maintains an analgesic dose response across all levels, it appears to have flat or inverted U-shaped biological response on respiratory suppression via mu receptors.

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The more the dosage, the lower the incidents of breathing disorders, that is, higher doses do not lead to higher effect in both animal and human studies, for example, the ventilatory response to hypercapnia does not continually decrease with progressively greater doses, while the analgesic effect is maintained. In analgesic effect, it does not follow a U-shaped curve in dose-response pattern. The incidence of tolerance to the analgesic effects of buprenorphine is relatively low in high doses [5].

On the other hand, the estimated yearly death rate (1994–1998) for methadone was at least threefold greater than the death rate related to buprenorphine. There are two well-known treatments to harm reduction in opioid dependent patients, maintenance therapy with methadone (MMT) and buprenorphine (BMT) [3]. However, their risk of sleep apnea with these alternative therapies still exists. Research suggests increased central sleep apnea due to chronic opioid use, although few studies have reported obstructive sleep apnea [5, 6]. In another study by Grote *et al.* which investigated the results of 14 clinical trials in remifentanil, benzodiazepine, only mild deteriorations in overnight oxygenation and apneic events have been shown; no systematic increases in the apnea hypopnea index (AHI) have been observed [6]. It should be emphasized that evidence based on facts and meta-analyses does not always explain us everything. It is noteworthy that in 3,325 case reports of sleep apnea syndrome (SAS) as adverse drug reaction (ADR), the polysomnography record couldn't have been verified. This is necessary for true diagnosis of central sleep apnea (CSA) and obstructive sleep apnea (OSA). The data wasn't systematically recorded in VigiBase [7].

Concerning the harm reduction approach with opioid agonist in opioid users, it is necessary to select a drug with a minimum risk of developing respiratory depression. In this paper, the case of a patient is discussed with more prominently marked decrease in central than obstructive sleep apnea when changing the therapeutic plan from MMT to BMT.

Case report

A 36-year-old man with symptoms of insomnia and daily hypersomnolence (ESS = 16) visited the Sleep Clinic of Masih Daneshvari Hospital in Tehran. Given his long history of opium use, he consumed 25 mg of methadone equivalent to 75 mg oral morphine per day. The patient had a history of mood disorder and consumption of citalopram 40 mg, 400 mg sodium valproate, and clonazepam 1 mg on a daily basis. Neck circumference and body mass index (BMI) were 42 cm, and 31.1 kg/m^2, respectively, while the PCO_2 was 37 mm Hg. After one night of adaptation, polysomnography was performed, by G3 Phillips Respironic software. The analysis was carried out considering the AASM 2017 manual of sleep scoring (Table 1). Concerning the continuation of events during titration, BiPAP-S/T (S/T bilevel positive airway pressure — spontaneous/timed) was ultimately applied, set on 24/20/2/12. Compliance of the patient was acceptable. During one year the man used the device 4 hours and 45 min, and AHI is equal to 4/h.

One year after the last visit, the patient was advised by his psychiatrist to add 100 mg of quetiapine and 50 mg of lamotrigine to his previous medications and also to replace methadone with sublingual buprenorphine 4 mg (equivalent dose of 160–320 mg of oral morphine) over the last three months. The patient's weight rose by 9 kg and his BMI to 34.3 kg/m^2, he could not tolerate the BIPAP st device. Considering the fact that his drugs and dosage were changed, polysomnography was repeated. The comparative polysomnography results related to methadone and buprenorphine are presented in Table 1 and Figures 1, 2.

Discussion

The major finding of this report was the complete elimination of obstructive and central sleep apnea and partial decrease of hypopnea by replacing methadone with buprenorphine.

Despite the fact that a certain dosage of buprenorphine had manifold effect compared with methadone, it is a partial agonist of the mu-receptor. Although the mortality risk related to buprenorphine overdose is lower than that of methadone, the rate of sleep breathing disorders, especially central apnea, have been reported to be higher in patients who underwent methadone and buprenorphine maintenance therapy rather than the control group [8]. However, the occurrence of sleep apnea was not affected by such factors as buprenorphine dosage, benzodiazepine and quetiapine use, or other apnea risk factors [9, 10]. Nociceptine/orphanin FQ receptors (NOP) receptor activation has a clear modulatory role on mu opioid receptor-mediated actions and thereby affects opioid analgesia positively, while leading to the tolerance of respiratory suppression. Buprenorphine can act through this receptor [8, 10].

Table 1. Polysomnographic findings on methadone and buprenorphine maintenance therapy

Polysomnography items	Methadone	Buprenorphine
Recording duration [min]	528.0	375.5
Total sleep time [min]	450.0	325.0
Sleep onset latency [min]	5.0	18.2
Sleep efficiency%	95.9	88.3
Wake after sleep onset	14	25.0
Sleep stages		
N1 [%]	20.8	6.6
N2 [%]	58.3	72.6
N3 [%]	6.4	2.0
REM [%]	15.4	18.8
REM sleep latency [min]	86.0	90.5
Number of REM	5	8
Respiratory events		
Number of events/hour		
Central sleep apneas	69 (9.2)	0 (0)
Obstructive sleep apneas	4 (0.5)	0 (0)
Mixed sleep apnea	7 (0.9)	0 (0)
Hypopnea	183 (24.4)	94 (17.4)
RERA	1 (0.1)	12 (2.2)
Apnea/hypopnea index	**263** (35.1)	94 **(17.4)**
Respiratory disturbance index	264 **(35.2)**	106 **(19.6)**
Oximetry		
Average $SatO_2$ [%]	95	92
Lowest Detected $SatO_2$ [%]	85	83
$SatO_2 < 90\%$ (duration %TIB)	5.7	
$SatO_2 < 88\%$ (duration %TIB)	0.1	
Desaturation index	290 (37.6)	102 (18.0)
Arousals		
Number index	29 (4.0)	30 (5.7)
Arousals associated with leg movement	0	0
Arousals with respiratory events and desaturation	17	15
Periodic leg movement	0 (0.0)	0 (0.0)
PCO_2 mmHg	37	39
HCO_3	20.7	23

REM — rapid eye movement sleep; RERA — respiratory effort–related arousal; SatO2 — oxygen saturation

This is a stunning result that can be helpful in prescribing less harmful opioid medication during maintenance therapy and other treatment processes. Given the high number of patients treated with these morphine agonists, it is important to know which of the two drugs is associated with a lower risk of developing sleep breathing disorders. In the only available case report so far, it was suggested that significant reduction in CSA together with improved hypoxia and normalized awake ventilatory control following a change from methadone to buprenorphine-naloxone therapy occurred [11].

Nociceptin opioid peptide receptors include MOP (μ), KOP (κ), and DOP (δ) discovered so far. They are found in many parts of the body, especially in breathing control centers. These areas include pre-Bötzinger complex, retro-trapezoid, and para-facial respiratory group (RTN/pFRG) located in PONS which contribute to the con-

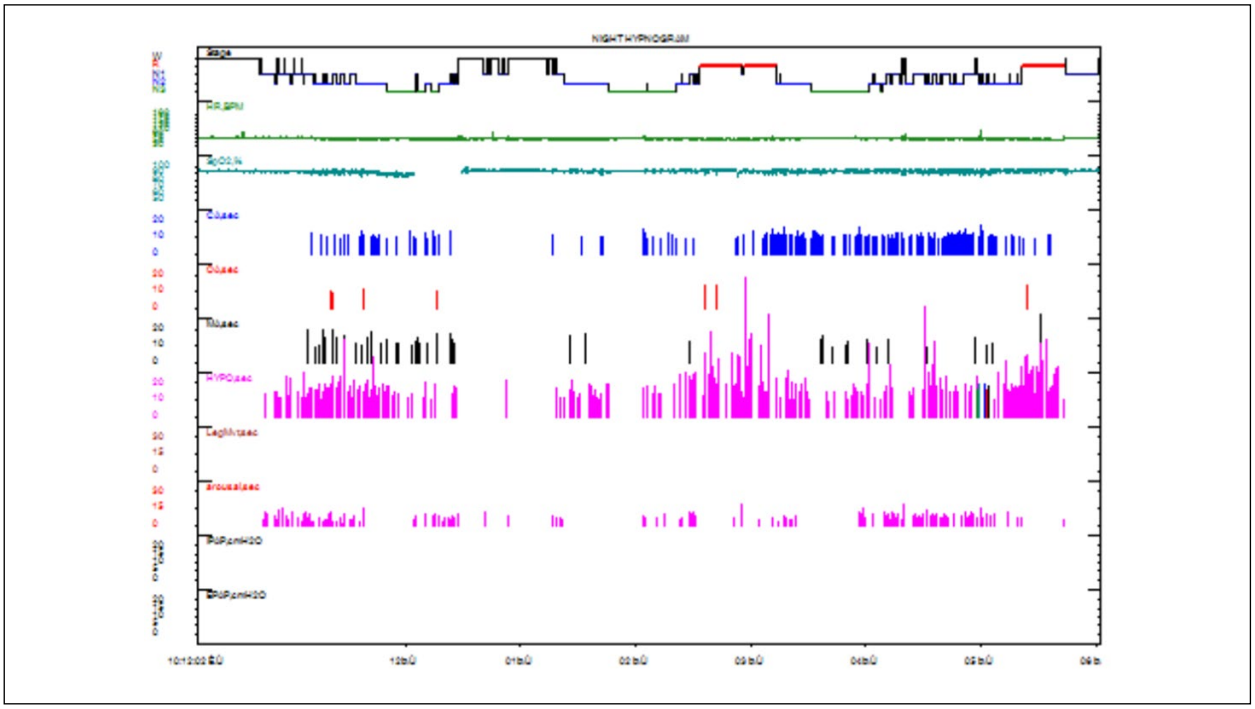

Figure 1. Patient on methadone

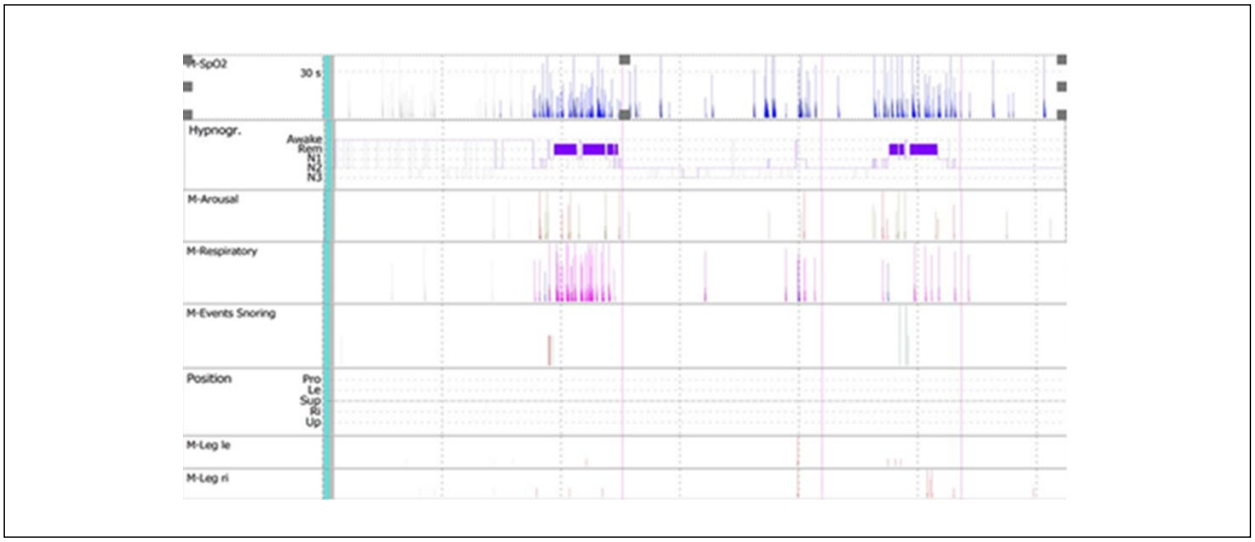

Figure 2. Patient on buprenorphine

trol of the breathing rhythm and are affected by wakeful stimulants and central and peripheral chemoreceptors [12].

While NOP receptors activation tends to synergize with mu-receptor-mediated actions, it sometimes tends to oppose them. Accordingly, gaining an insight into NOP receptors pharmacology in the context of these interactions with the opioid receptors shall significantly contribute to the development of novel and innovative therapeutic methods that engage the NOP receptors. Buprenorphine, despite methadone, is an agonist of this receptor.

The affinity of these receptors to bind with the opioid agonists (buprenorphine) is extremely low; therefore, a high concentration of opioid drugs can stimulate them while inhibiting them can be achieved by a high concentration of naloxone. These receptors have a regulatory effect on morphine receptors; their activity is so that they have minimum effect on the breathing system [12].

That is why a high dosage of buprenorphine in this patient led to a reduction in terms of the effect of the mu-receptors and also to a decrease in ventilation suppression, CSA, and OSA. This is a case in point when it comes to reverse pharmacology effect.

Despite the weight gain of 9 kg, the CSA index dropped to zero, and a good response to titration was observed over methadone consumption. Noticeably, the equivalent morphine dose of buprenorphine was higher than that of methadone.

Moreover, the patient's drowsiness was still present; the intensity of sleepiness related to methadone and buprenorphine was 16 and 18, respectively.

Sleepiness continued although taking modafinil with buprenorphine could be attributed to the concomitant use of quetiapine. Despite the increase in taking sedative medications, he still complained of early insomnia, however, sleep latency and sleep efficiency were within the normal range. Slow wave sleep was reduced when the patient was taking buprenorphine and methadone, but it could also be attributed to the concurrent use of benzodiazepine. The relative reduction of REM in addition could be explained by taking citalopram. Lamotrigine, quetiapine and benzodiazepine can increase obstructive apnea. In the Mason's study, it was noted that the pharmacological effects of these drugs haven't had deleterious effect on severity of AHI and ODI, but in the case of remifentanil, benzodiazepine in the OSA subgroup, minimum oxygen saturation was reduced [13], but in this case, diminishing of both type of apnea was noted.

Conclusions

In this case, a marked decrease in CSA and OSA was observed after switching from methadone to buprenorphine.

Considering the importance of sleep apnea and the widespread use of methadone and buprenorphine, controlled clinical trials are required to assess sleep-related breathing disorders in buprenorphine MT. Safe and beneficial prescription of MMT depends greatly on a careful patient selection and treatment follow-up.

Conflict of interest

None declared.

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Diagnosis and management of combined post- and precapillary pulmonary hypertension in a patient with multiple comorbidities

Abstract

Diagnosis of pulmonary hypertension requires a laborious investigation that must be performed in accordance with international guidelines. Right-heart catheterization is the gold standard examination to assess the degree of hemodynamic impairment of post- or precapillary origin, guiding management. The presence of comorbidities is becoming rather frequent in real-life pulmonary hypertension cases, thus creating diagnostic and therapeutic complexity. We present a case of combined post- and precapillary pulmonary hypertension in a patient with ischemic heart disease and combined pulmonary fibrosis and emphysema, in order to describe the diagnostic algorithm for pulmonary hypertension and elucidate the problematic aspects of managing this debilitating disease in a patient with several comorbidities. Current guidelines do not support the use of specific vasodilator treatment in group II -due to heart disease and group III-due to lung disease pulmonary hypertension, unless the patient presents with severe pulmonary hypertension (mean pulmonary artery pressure > 35 mm Hg or cardiac index < 2.0 L/min) with right ventricular dysfunction and is treated in an expert center and preferably in the context of a randomized control trial. In the case presented, therapeutic management focused, firstly, on treatment of the underlying heart and lung disease and, subsequently, on specific vasoactive therapy, due to severe hemodynamic deterioration.

Key words: diagnostic algorithm, combined post- and precapillary pulmonary hypertension, combined pulmonary fibrosis and emphysema

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Introduction

Pulmonary hypertension (PH) arises as a complication of chronic lung or heart disease, increasing morbidity and mortality of the primary disease. Diagnosis requires early clinical suspicion and a laborious investigation. Currently, there is no approved treatment for PH associated with chronic lung disease and heart failure. The objective of this presentation is to describe the diagnostic algorithm for pulmonary hypertension and elucidate the problematic aspects of managing this debilitating disease in a patient with several comorbidities.

Material and methods

A 70-year-old white male (BMI 30.6 kg/m^2) presented with worsening dyspnea and fatigue during the last 6 months. He also reported retrosternal chest pain during activity lasting less than 10 minutes. He suffered from emphysema and bronchiectasis and had a history of pneumothorax that was surgically managed by lung decortication 15 years ago. He was a former smoker with a smoking history of 45 pack/years. His medical history also included atrial fibrillation, coronary artery disease that led to coronary artery bypass grafting 10 years ago, goitre and gastroesophageal reflux.

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On clinical examination, he presented normal blood pressure (125/72 mm Hg), tachycardia (95 beats/min), elevated respiratory rate (19 breaths/min) and low oxygen saturation (SaO_2% 88%) on room air. On chest auscultation, lung sounds were diminished uniformly, and bibasilar crackles were found. He presented jugular venous enlargement and mild leg edemas. Cardiac pulse was irregular and loud P2 sound was present. Examination of the abdomen was normal.

Blood cell count and the basic biochemical panel were normal, along with thyroid function. The BNP value was 307 ng/L. Blood gas analysis revealed mild hypercapnia and hypoxemia on room air. Diffusion capacity (D_{LCO}: 25%) was severely diminished, and lung volumes were mildly abnormal (FEV_1: 80%, FVC: 84%, FEV_1%: 72, TLC: 62%, RV: 38%), implying coexistence of restrictive and vascular pathophysiology. 6-minute walking test (6MWT) was 328 m with desaturation from 92% to 84% while receiving oxygen at a flow of 4 lt/min.

Chest X-ray revealed a reticular pattern in the lower lung area bilaterally, and high-resolution computed tomography (HRCT) confirmed new moderate fibrosis of usual interstitial pneumonia (UIP) pattern in the lower lobes (Figure 1). The upper lobes presented emphysema, comprising the diagnosis of combined pulmonary fibrosis and emphysema (CPFE).

Heart ultrasonography revealed an ejection fraction of 50% and diastolic dysfunction of the left ventricle, severe dilatation of the right ventricle with impaired systolic function, moderate insufficiency of the tricuspid valve and an estimated systolic pressure of the right ventricle of 70 mm Hg. Ventilation-perfusion scintigraphy was negative for pulmonary embolism.

Cardiopulmonary exercise testing (CPET) followed in order to discriminate between cardiac and respiratory cause of dyspnea, assess functional capacity, the degree of desaturation and the need for oxygen therapy. The patient had reduced peak oxygen consumption (65% pred), decreased anaerobic threshold (54% pred), elevated ratio of minute ventilation to CO_2 production (46), reduced ventilatory reserve (10lt), and a nadir saturation of 82%. These values were compatible with lowered functional capacity due to respiratory limitation and increased pulmonary vascular resistance.

The patient underwent right-heart catheterization (RHC) revealing combined pre-and post-capillary PH (CpcPH). He had a mean pulmonary pressure (mPAP) of 32 mm Hg, pulmonary wedge pressure (PAWP) of 16 mm Hg, pulmonary vascular resistance (PVR) of 3.2 WI, cardiac index (CI) of 3.5 L/min/m^2 and a diastolic pulmonary gradient (DPG) of 9 mmHg. According to hemodynamic values, the man was classified as group II-due to heart disease and group III-due to lung disease PH. Current guidelines do not support the use of the approved therapies for pulmonary arterial hypertension (PAH) in these two groups, unless the patient presents with severe pulmonary hypertension (mPAP > 35 mm Hg or CI < 2.0 L/min) with right ventricular dysfunction and is treated in an expert center and preferably in the context of a randomized control trial. Furosemide and continuous oxygen therapy were initiated. The patient was already receiving perindopril, bisoprolol, rivaroxaban, simvastatin, and omeprazole. Low-salt and low-fat intake and mild exercise, as tolerated, were recommended. Moreover, the man received nintedanib, an anti-fibrotic agent as treatment for his lung disease. However, he presented severe gastroesophageal reflux disease that led to immediate discontinuation of treatment. Pirfenidone could not be prescribed as alternative anti-fibrotic treatment due to insurance coverage reasons.

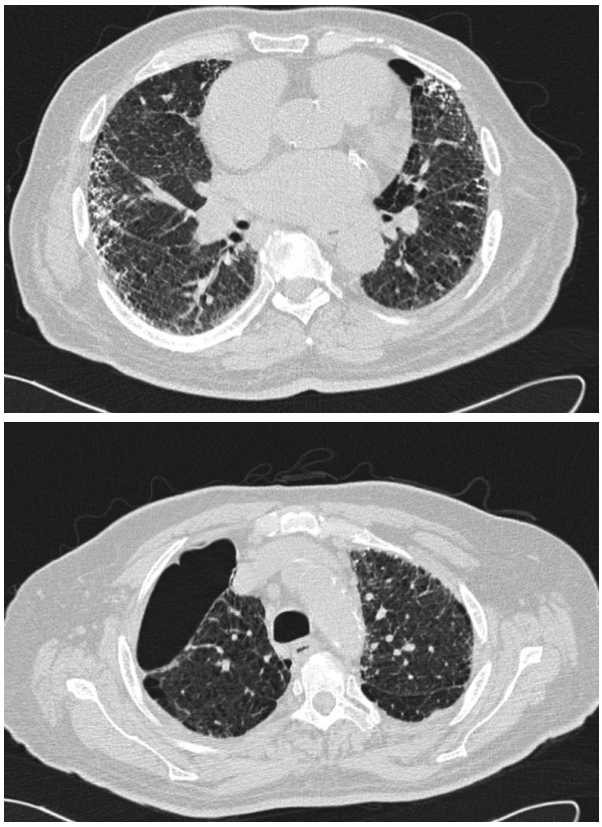

Figure 1. Chest computed tomography

The patient did not present for follow-up and returned deteriorated one year after. His dyspnea occurred in simple tasks, with immediate severe desaturation. We repeated a CT angiography that was negative for pulmonary embolism. Heart ultrasonography presented deterioration with severe enlargement of the right ventricle with flattening of intraventricular septum and a D-shaped left ventricle. The BNP value increased to 897 ng/L. A second RHC revealed an elevated mPAP of 49 mm Hg, PVR of 4.3 WI and normal wedge pressure under diuretic treatment. We decided to initiate sildenafil, a PDE-5 inhibitor, in combination with inhaled iloprost, a prostanoid analogue, due to the severe impairment of hemodynamics. The patient improved transiently but disease progression led to the unfortunate event of death four months later.

Discussion

The case presented is characterized by challenging complexity. The first RHC revealed CpcPH, as PAWP was > 15 mm Hg and PVR was > 3 WI [1]. Ischemic heart disease explained the post-capillary element of the hemodynamic derangement. The precapillary element was attributed to chronic lung disease, based on the extensive findings on HRCT. The discrimination between group 1 and 3 PH often requires a comprehensive investigation of several criteria, including spirometry, CPET, hemodynamic profile, radiologic findings and PAH risk factors [2]. In our case, the spirometry was not representative of the extent of lung disease and could mistakenly favor the diagnosis of group 1 PAH, as in CPFE, lung volumes appear normal or mildly abnormal due to the opposite effects of expiratory flow and lung volumes [3].

Moreover, at the time of the first RHC, PH was classified as non-severe, and treatment was focused on the underlying lung and heart disease [4]. Currently, the well-established PAH therapies are not approved in groups 2 and 3 PH, as the results from existing studies have been unfavorable [4–7].

Specifically, no multicenter trial exists to support a benefit of vasoactive drugs in group 2 PH [1, 4]. On the contrary, disappointing results from trials testing the efficacy of sildenafil, macitentan, riociguat, epoprostenol and others have accumulated [1]. However, several trials are ongoing to examine the use of these drugs in patients with heart failure with preserved ejection fraction (HFpEF) and CpcPH [1, 8]. A promising signal for this selected subgroup of patients arise from a few studies on sildenafil and riociguat, encouraging further research [9, 10].

Similarly, the use of vasodilator agents in group 3 PH is generally contraindicated. Endothelin receptor antagonists and riociguat have been deemed harmful, while sildenafil has occasionally been used in group 3 PH with conflicting results [3, 6, 7, 11]. Positive effects have also been reported regarding inhaled iloprost in COPD patients, inhaled and intravenous treprostinil use in patients with severe group 3 PH, with severe right ventricular dysfunction [12, 13].

Existing literature involving the aforementioned group provides heterogenous results. Brewis *et al* reported an absence of change in 6MWT and FC, and an improvement of BNP levels ($p = 0.015$) after specific PH therapy in 118 patients with severe group 3 PH [5]. Furthermore, the treatment effect was found dependent on lung disease phenotype, with CPFE presenting the worst outcome [5]. However, the study was limited by the absence of a control group that did not receive treatment [5].

Moreover, results from a subgroup of 151 patients of the COMPERA registry with chronic fibrosing interstitial pneumonias and severe PH indicated that PDE5-i long-term use increased 6MWT and functional class in the short term, with unknown effects on survival [14].

Another promising single arm prospective study included 14 patients with severe PH associated with lung disease, 6 of which were diagnosed with CPFE. Repeated cardiac magnetic resonance imaging revealed improvement of right ventricle dilatation and dysfunction after 3 months of treatment with sildenafil, implying potential benefit in this group of patients [15].

Additional trials are currently ongoing to further evaluate long-term efficacy of PAH therapies in severe group 3 PH [7]. Possibly, selected subgroups could be identified to benefit from these drugs, altering therapeutic strategies in the future [7]. To date, recent guidelines and recommendations underline the significance of referral to an expert center for the individualized care of a patient presenting severe hemodynamic derangement with coexisting lung disease [2, 4]. Cautious use of PH specific therapy could be considered, preferably in the context of a randomized control trial, with frequent follow-up assessing treatment effect [4, 7].

Conflict of interest

None declared.

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Acute hypoxemia due to lung collapse in COVID-19: the role of therapeutic bronchoscopy

Abstract

Bronchoscopy is an aerosol-generating procedure and involves a high risk of transmission of SARS-CoV-2 to health care workers. There are very few indications for performing bronchoscopy in a patient with confirmed COVID-19. These include atelectasis, foreign body aspiration, and suspected superinfection in immunocompromised patients. Proper use of standard personal protective equipment is mandatory to reduce the risk of transmission to health care workers. In this article, we describe a case of acute lung collapse in a 16-year-old boy with cerebral palsy who was infected with COVID-19. This patient responded to therapeutic bronchoscopy and had complete resolution of lung collapse within 24 hours of the procedure.

Key words: bronchoscopy, SARS-CoV-2, COVID-19, atelectasis

Adv Respir Med. 2021; 89: 448–450

Introduction

Therapeutic bronchoscopy is frequently used in the intensive care unit (ICU) due to lobar atelectasis that is unresponsive to chest physiotherapy [1]. It is an aerosol-generating procedure that has a high risk of transmission of coronavirus disease 2019 (COVID-19) to health care workers (HCWs). Specific guidelines have been introduced for performing bronchoscopy during the time of the COVID-19 pandemic, and these guidelines include instructions on the use of adequate personal protective equipment (PPE) [2–5]. Bronchoscopy in confirmed COVID-19 patients is indicated in conditions such as lung collapse due to mucus plugging or foreign body aspiration, suspected superinfections in immunocompromised patients, management of massive hemoptysis (in conjunction with other measures), central airway obstruction, bronchoscopic intubation, and percutaneous tracheostomy [3]. However, the role of bronchoscopy is limited in the diagnosis of COVID-19 due to the substantial risk of transmission of the disease to health care workers, and because of the availability of low-risk and non-invasive upper respiratory samples obtained from oropharyngeal and nasopharyngeal swabs. Here, we present a case of a young male who presented with acute worsening hypoxemia and left lung collapse with a history of contact with a confirmed COVID-19 patient who was managed with therapeutic bronchoscopy.

Case presentation

A 16-year-old boy presented to the emergency department with a history of progressive shortness of breath for three days. There was a doubtful history of aspiration of a piece of a toy (in the form of a wool ball) three days ago during an episode of a seizure. There was no history of upper respiratory symptoms or fever. The patient was previously diagnosed with cerebral palsy with spastic diplegia, and also had a seizure disorder. He had been bedridden for three years and was dependant for all activities of daily living. His mother was the primary caretaker and was detected to be COVID-19 positive about two weeks back, but was asymptomatic. On examination, he had tachypnea with a respiratory rate of

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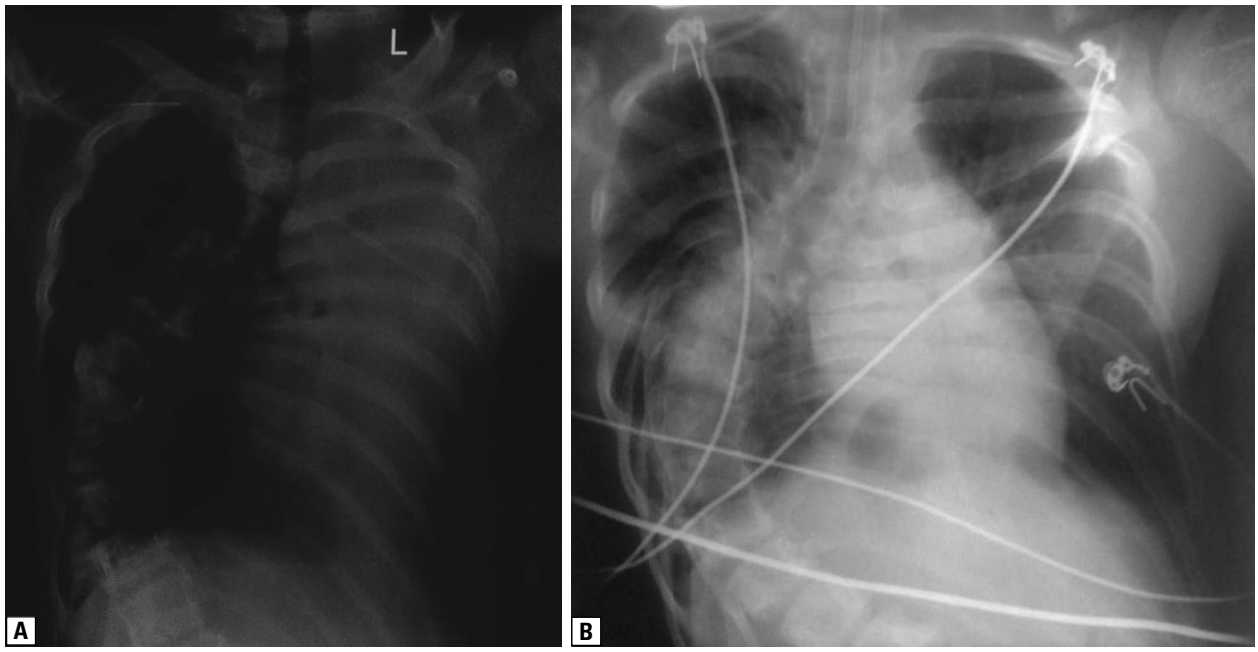

Figure 1. A. The chest radiograph demonstrating scoliosis with complete opacification of the left hemithorax with a mediastinal shift to the same side suggesting left lung collapse; **B.** The chest radiograph following bronchoscopy demonstrating complete clearance of the left lung with aeration

30 per minute, and a saturation of 95% on oxygen inhalation at 8 L/min with a facemask. He was hemodynamically stable. His nasopharyngeal and oropharyngeal swabs were sent for COVID-19 RT-PCR and came back positive. Chest X-ray demonstrated left lung collapse with scoliosis (Figure 1A). A contrast-enhanced computed tomography was ordered because of the suspicion for foreign body aspiration. It revealed an oval, smooth lesion in the distal left main bronchus which was suspicious for a foreign body or mucous plugging. There was also evidence of a collapsed left upper and lower lobe. There was a gross scoliotic deformity with convexity towards the right and a resultant deviation of the mediastinum and vascular structures to the left hemithorax. He was transferred to the ICU as a result of an increased need for oxygen. Due to worsening hypoxemia, a decision to begin therapeutic bronchoscopy was undertaken. For the procedure, all the necessary equipment and materials were prepared outside the COVID-19 ICU. This included saline, syringes, and the bronchoscopy system (therapeutic bronchoscope with a monitor). A negative pressure room was not available for the procedure. As per recommendations, all PPE were used. These included an N95 mask, goggles, two sets of gloves, and a plastic protective gown which included a head and neck cover. Flexible bronchoscopy was performed via the oral route under sedation. The sedative agents used included propofol and fentanyl. Topical anesthesia via oropharyngeal spray and the 'bronchoscopic spray as you go' method were provided using 2% lidocaine. The left main bronchus showed a large thick mucus plug which was removed with thorough suctioning following saline instillation. No foreign body could be identified. Following the procedure, the patient required intubation due to worsening hypoxia. A chest X-ray performed 24-hours following the procedure showed complete expansion of the left lung (Figure 1B). He was further managed using standard COVID-19 care protocols which included administration of steroids and hydroxychloroquine. He was subsequently extubated on day three of admission and was discharged home on day 12 with a room air oxygen saturation of 97%.

Discussion

This case describes the therapeutic role of bronchoscopy in a patient with confirmed COVID-19. The use of therapeutic bronchoscopy in this patient facilitated his recovery from acute hypoxemic respiratory failure.

Across numerous studies, flexible bronchoscopy has been shown to be effective in removing secretions and improving atelectasis with success rates between 79–89% [6]. Lobar atelectasis responds better than subsegmental atelectasis. This is likely due to the larger size of mucus plugs which are amenable for bronchoscopic removal.

Other modalities for the treatment of atelectasis (i.e. chest physiotherapy) are also useful in the majority of individuals. A randomized controlled trial studying the use of an aggressive chest physiotherapy regimen versus the use of bronchoalveolar lavage for the treatment of acute atelectasis showed the same extent of improvement at 24 and 48 hours in terms of the radiological resolution of the atelectasis [6]. Patients with restrictive lung diseases, such as neuromuscular diseases, are more prone to develop atelectasis due to mucous plugging. If these patients develop pneumonia, lobar or complete collapse of the lung can occur. In such conditions, bronchoscopy remains the procedure of choice for its diagnostic and therapeutic value. This was affirmatively the case in our patient as there was suspicion of an aspirated foreign body and an inability to cooperate with aggressive chest physiotherapy due to his severe hypoxia and his comorbid cerebral palsy. As a result, we decided to treat the patient with therapeutic bronchoscopy. Bronchoscopy, in critically ill patients, is not without risk. The overall incidence of complications and mortality during fiberoptic bronchoscopy based on a large prospective study covering 19 Italian centres encompassing 20,986 bronchoscopies was approximately between 0.02% and 1.1% [7]. The most common complications of bronchoscopy are bronchospasm, hypoxemia, cardiac arrhythmias, hypotension, bleeding and hemoptysis, pneumothorax, myocardial infarction/pulmonary edema, and death [8]. Our patient also needed intubation in the post-procedural period due to hypoxia. His oxygen requirement prior to the procedure was high. We initially planned to electively intubate him before bronchoscopy. However, keeping in mind the poor prognosis of COVID-19 patients on invasive mechanical ventilation, and after discussion with family members, a decision for proceeding with bronchoscopy was made.

A previous study described 101 bronchoscopies in 93 patients with COVID-19 on invasive mechanical ventilation with the major indication being superinfection (63/101), followed by airway secretion management with or without atelectasis (38/101) [9]. Ninety-five per cent of the patients showed thick white gelatinous secretions that were difficult to suction. This could be the result of either direct viral effect or because of heat moisture exchanger use among ventilated patients. One bronchoscopist out of the three performing the procedure was infected, but it is unclear whether the infection was procedure-related. In our patient, none of the healthcare workers involved in bronchoscopy were diagnosed with COVID-19 during the four week period following bronchoscopy.

Conclusion

In cases with definitive indications, therapeutic bronchoscopy should not be delayed in COVID-19 confirmed cases as the procedure may be lifesaving. Proper use of PPE helps in reducing the risk of transmission of the disease to health care workers.

Conflict of interest

None declared.

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Vanishing lung syndrome with community-acquired pneumonia and infection of bullae

Abstract

We present a case of a 36-year old male who was a long-term smoker and was found to have giant bullous emphysema on chest imaging as an accidental finding. At the time, when his first chest CT was obtained, he was asymptomatic and was recommended to consult a pulmonologist but was lost to follow-up for a year until he presented to the emergency department with fever, dyspnea, and chest pain. He was admitted to a pulmonology department. Chest CT was performed and it revealed infected bullae containing air-fluid levels as a complication of community-acquired pneumonia. After successful antibacterial treatment, the patient was discharged and recommended to consult with a thoracic surgeon. A few months later, he had video-assisted thoracoscopic surgery and left upper lobectomy as part of definitive treatment.

Key words: giant bullous emphysema, vanishing lung syndrome, infected bullae, pneumonia

Adv Respir Med. 2021; 89: 451–455

Introduction

Giant bullous emphysema (GBE), also known as vanishing lung disease or type I bullous disease, is defined by giant bullae in one or both upper lobes occupying at least one-third of the hemithorax and compressing the normal surrounding parenchyma [1]. It is a progressive disease in which the affected regions of the lung do not participate in gas exchange often leading to dyspnea, hypoxia, chest tightness, and spontaneous pneumothorax. Vanishing lung disease can often be asymptomatic, especially in younger patients, and manifests only when complications occur – most commonly infection of bullae or pneumothorax. The development of GBE is associated with smoking, inhaled drug abuse, alpha-1 antitrypsin deficiency, and connective tissue disorders.

Material and methods

A 36 year-old patient was admitted to the emergency department complaining of left-sided chest pain worsened by coughing and deep inspiration, cough with yellow-greenish discharge, non-foul smelling sputum, and dyspnea of a few days duration. Two days before hospitalization, he developed chills and fever up to 39°C. Upon admission, he had fever of 38.5°C, respiratory rate of 24×/min, and SpO_2 at 93% on room air. Respiratory examination revealed crackles and wheezing on the left side. The examination of other systems was unremarkable.

Laboratory examination revealed neutrophilic leukocytosis (leukocytes 18.8 × 10^9/L) and increased CRP (211.3 mg/L). Other results were within reference ranges. Chest X-ray was performed and revealed consolidations on the left side with air-fluid levels and blunting of the left costophrenic angle (Figure 1). There was suspicion of possible empyema with free air in the pleural space.

For further investigations and treatment, the patient was admitted to the pulmonology department. Before initiating therapy with amoxicillin/clavulanic acid, sputum and blood cultures were drawn. A chest CT scan was performed and revealed centrilobular and paraseptal emphysema predominantly in apical parts of the lungs and

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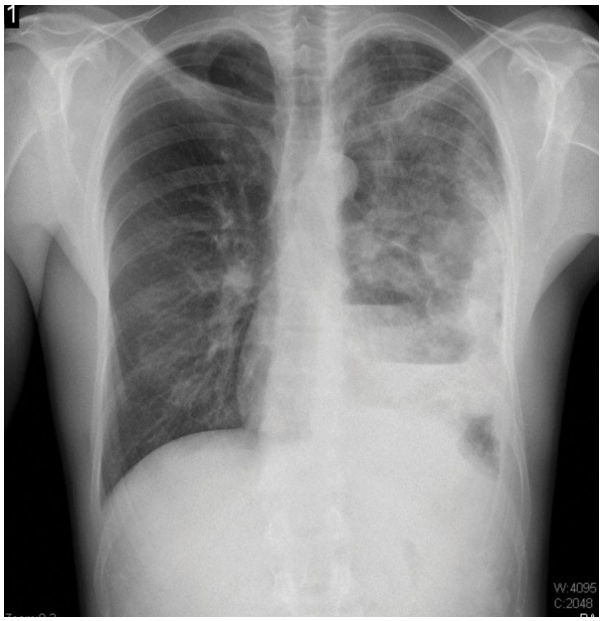

Figure 1. Chest X-ray PA revealing consolidations of the left lung with air-fluid levels and blunting of left costophrenic angle

a bulla in the left apex (~16 × 8.6 × 7.3 cm) with air-fluid levels. Another perihilar cavity with air-fluid levels was seen between the 6th and 8th segment together with smaller bullae in the 2nd and 9th segment. Free fluid in the left pleural space was noted up to 3.6 cm (Figure 2).

Even though the patient had purulent sputum, the sputum culture came back unspecific revealing only normal upper respiratory flora. Blood cultures were negative. Treatment with amoxicillin/clavulanic acid was ineffective and patient still had a fever up to 39°C. Inflammatory markers were increasing which prompted a change in antibacterial treatment to piperacillin/tazobactam. On this regiment, the patient's overall condition rapidly improved and he became afebrile in 4 days. One week later, his inflammatory markers markedly decreased (leukocytes — 15.29 × 10^9/L, CRP — 17.4 mg/L). A follow-up CT scan 2 weeks after initiation of piperacillin/tazobactam revealed improvement in pneumatization of the

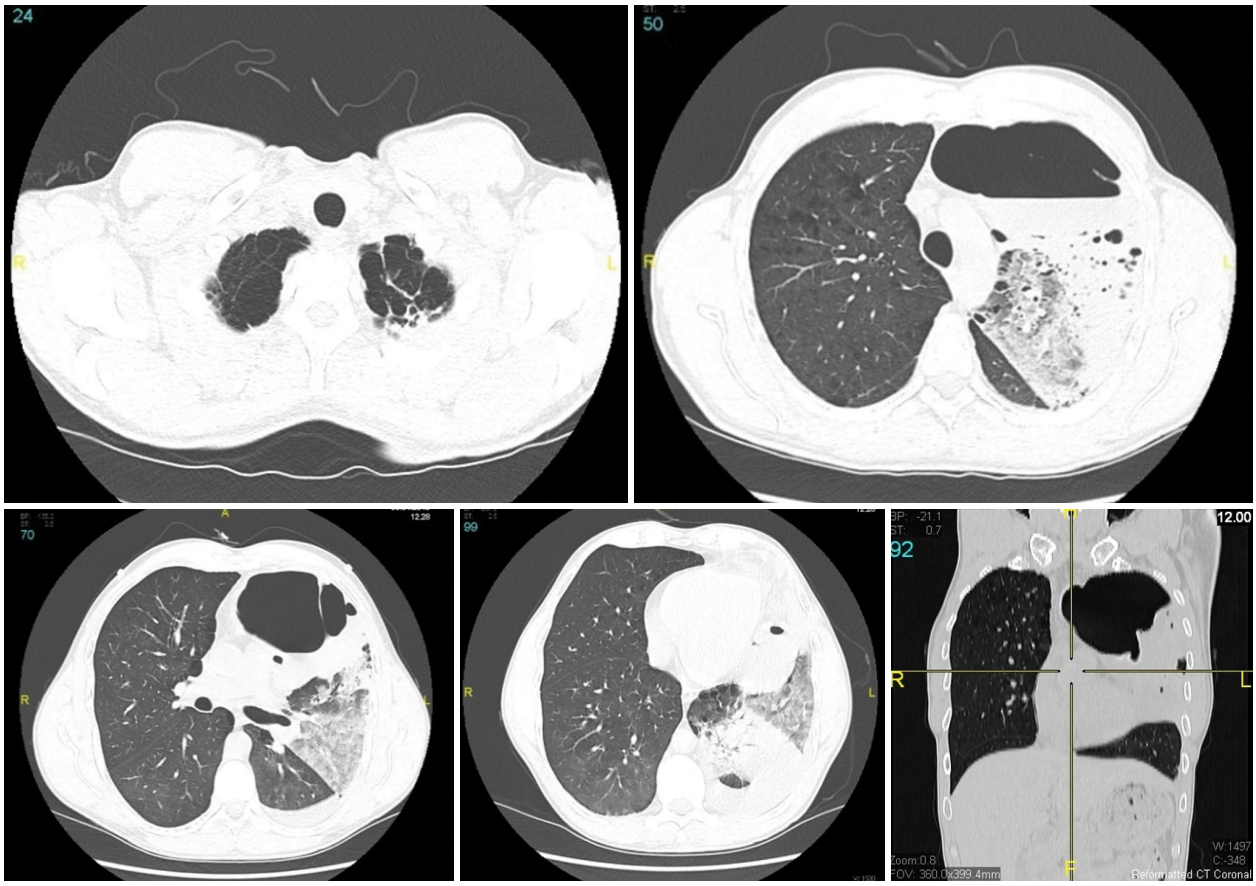

Figure 2. Chest computed tomography in coronal and axillar plane revealing bulla on the left side with air fluid levels, adjacent consolidation of the lung, and pleural effusion

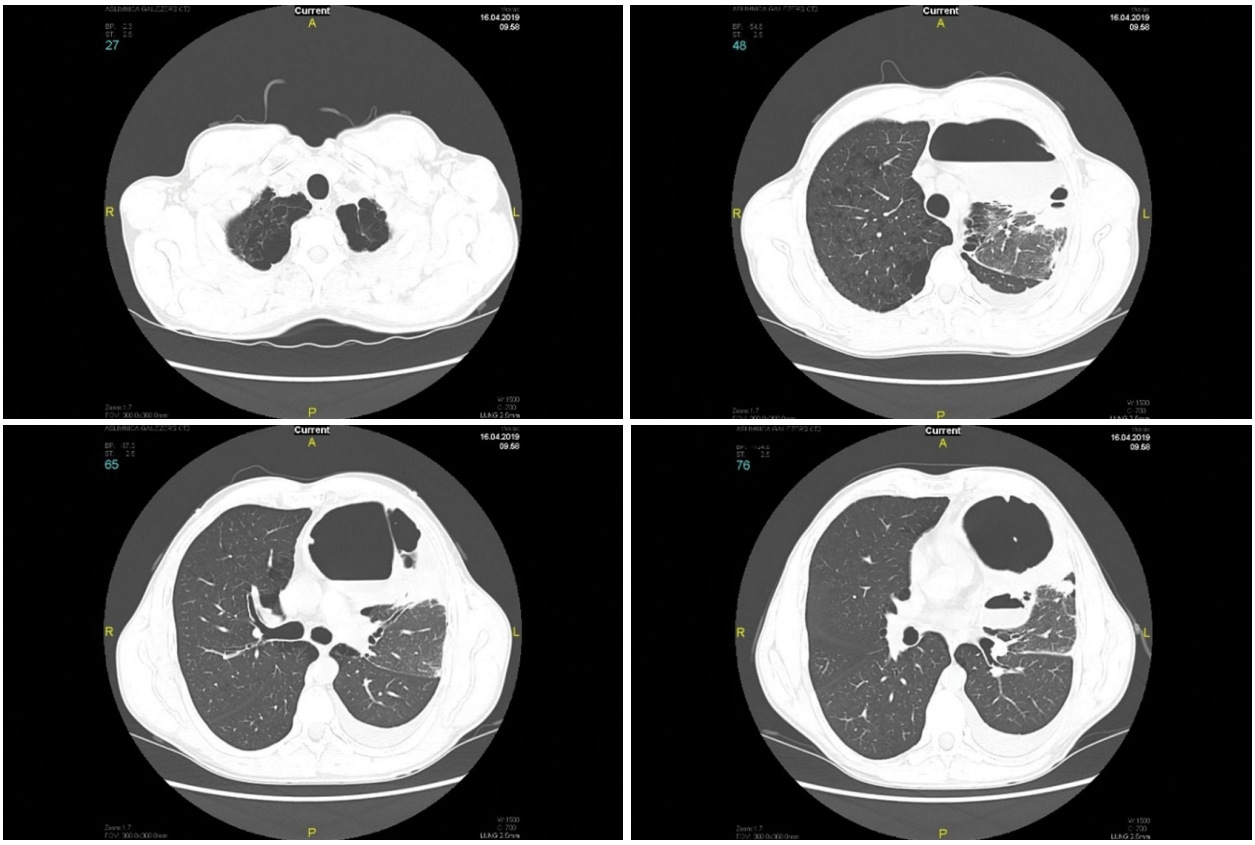

Figure 3. Chest computed tomography 2 weeks after initiation of antibacterial treatment with piperacillin/tazobactam. Air-fluid level in the bullae still present, but marked improvement in pneumatization of the lung is seen

left lung, but the bullae were still filled with fluid (Figure 3). The patient was referred to a thoracic surgeon and uniportal video-assisted thoracoscopy (VATS) with left upper lobectomy was indicated. The patient didn't agree to any further treatment in the hospital and, against medical advice, was discharged with recommendations to consult a thoracic surgeon. Two months later, the patient was seen by a surgeon and was admitted to the thoracic surgery department for VATS. After a successful postsurgical period, the patient was lost for further follow-up as was expected considering his low adherence to treatment and recommendations.

During the time of hospitalization and after additional questioning of the patient, it was determined that he was a long-term smoker who had been smoking approximately 30 cigarettes a day since the age of 6 (45 pack years). As well, he unwillingly revealed to have often smoked marijuana as well. At the time of hospitalization, he had switched to electronic cigarettes, but was not able to quit smoking completely. He admitted that a year prior to his present hospitalization episode, he was hospitalized at another hospital with acute pancreatitis. There, a CT was performed that accidentally revealed giant bullous emphysema. The bullae had a "mass effect", protruding and restricting left lung upper segments and moving the heart shadow to the right (Figure 4).

After marked improvement of the patient's overall condition, full pulmonary function tests were performed which revealed air trapping (defined as an increase in RV and FRC) and decreased diffusing capacity — FEV1/FVC 80.30%, FEV1 82.5%, TLC 102.7%, RV 164%, FRC 175%, DLCO 65.7%. α1-antitrypsin plasma levels were within the normal range (1.8 g/L; reference values 0.92–2.0 g/L).

Discussion

Giant bullous emphysema (GBE), also referred to as vanishing lung syndrome, is a rare condition that typically presents in young male smokers or α_1-antitrypsin deficient (AATD) patients. Bullae are formed by the destruction of interalveolar walls due to chronic or acute stretch injury with increased intraalveolar pressure [2]. On chest radiograph, a bulla has to take no less

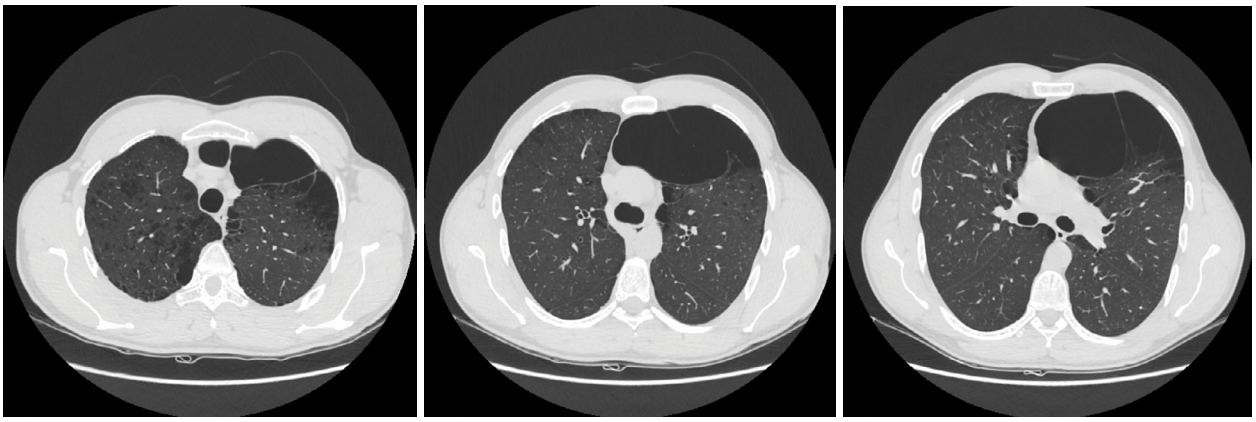

Figure 4. Chest CT performed one year prior, revealing giant bullous emphysema

than 30% of the hemithorax to be considered a giant bulla. Commonly, such patients present with progressive dyspnea due to the bulla compressing surrounding lung tissue and disturbing optimal gas exchange, but sometimes these patients can be asymptomatic and be unaware of the existing pathology [3].

The exact prevalence of vanishing lung syndrome remains unknown as only a small number of case reports and series have been reported. The specific cause of the pathology is obscure, but male predisposition and smoking (both cigarette and marijuana abuse) have been observed to be risk factors [4]. Similar to how only about 30% of smokers develop emphysema (mostly panacinar), it is reasonable to assume that yet to be identified triggers and predisposing factors (acquired or inherited) lead to the development of GBE in the same population. On the other hand, the pathogenesis of bulla formation is similar and is due to an imbalance of proteases/antiproteases and oxidants/antioxidants in the lung. This disequilibrium causes a chain reaction at a cellular level leading to the eventual destruction of alveolar walls resulting in permanent and abnormal enlargement of distal airspaces [5]. Bullae developing in emphysema are air-filled, thin-walled cavities larger than 1cm in diameter leading to poor gas exchange due to reduced total alveolar surface area and fibrosis of the alveolar membrane [6].

In patients with established GBE, pulmonary function tests and α_1-antitrypsin serum level measurements must be performed. In the reported patient, AATD was initially highly suspected. However, as plasma levels were normal, no further genetic studies were performed because it is not reimbursed by the government in our country. There is no precise data of α_1-antitrypsin deficiency prevalence in Latvia. The European data is scarce — it varies from 1 in 1,368 in Denmark to 1 in 58,319 in Poland [7].

The natural history of vanishing lung syndrome is reported to be a progressive one, but the rate of it is highly variable [8]. Bullae can enlarge, compressing healthy lung parenchyma and interfering with normal respiratory mechanics and gas exchange (increase in dead space fraction). Lung compliance decreases and work of breathing increases [5]. Usually, in patients with emphysema, we would expect to have a typical obstructive pattern in spirometry. However, as demonstrated by this patient, it is not always the case in GBE. This is also why the use of spirometry as a follow-up tool is questionable. Regarding follow-up, the most important issue is to monitor the patient closely for worsening of symptoms and for the development of possible complications.

In patients with GBE, important steps in treatment include smoking cessation and adherence to vaccination schedules. It is assumed that in asymptomatic patients with GBE, a reasonable first-line approach is the use of bronchodilators. However, as patients become more symptomatic or develop complications, especially pneumothorax, surgical intervention is required.

The treatment of choice in GBE is bullectomy. Available data suggests that elective bullectomy has good results. In a 5-year follow-up study of 41 patients with giant bullous emphysema who had undergone elective bullectomy, improvements in symptoms and lung function (significant improvements in lung volumes, FEV1, and the FEV1/ FVC ratio over baseline) were noted. As well, bullae did not reappear and residual bullae did not become enlarged at the site of the bullectomy. During follow-up, the dyspnea score was reduced significantly soon after bullectomy

and remained so even up to the fourth year of follow-up. The bullectomy approach is associated with a decreased risk of infection and pneumothorax [9]. As of now, there is no consensus available on the ideal timing for surgery or even follow-up of GBE patients.

The reported patient was asymptomatic and was unaware of the existing pathology until hospitalization due to acute pancreatitis and accidental CT findings of bullous emphysema. At that moment, his pulmonary function tests were better than expected – the deviations seen in this patient's case were increased RV and FRC (above 120%) with normal TLC levels, as well as decrease in DLCO due to bullae leading to a decrease in surface area where gas exchange takes place. As a result, the consulting pulmonologist prescribed treatment with bronchodilators. However, the patient was not adherent to the treatment protocol as the bullous changes were not causing any symptoms and did not interfere with his daily activities. One year later, he presented with acute worsening of the symptoms and community-acquired pneumonia with infection of the bullae which prompted a lengthy stay in the hospital and surgical intervention. This case shows that even though the disease is asymptomatic, follow-up is important as pathology can lead to serious complications such as infection or rupture of bullae, total pneumothorax, or infection-linked life-threatening conditions.

Conflict of interest

None declared.

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Probe-based confocal laser endomicroscopy in COVID-19

Abstract

Probe-based confocal laser endomicroscopy (pCLE) is a method that produces microscopic imaging of a lung tissue during bronchoscopy. We report a case ot a patient with negative nasopharyngeal swabs and suspected lung cancer who underwent pCLE. The diagnosis of COVID-19 was confirmed by PCR analyses of lavage fluid and transbrohial biopsy. The pCLE image shows density of alveolar thickened fibres, disorganization of elastin network, and multiple large drops of intraalveolar secretions. As far as we know, this is the first pCLE image discribed in patient with COVID-19 at that moment.

Key words: confocal laser endomicroscopy, optical biopsy, COVID-19, SARS-CoV-2

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Introduction

Coronavirus disease 2019 (COVID-19) is a type of lower respiratory tract infection with the potential to cause severe and possibly fatal atypical pneumonia in humans caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), initially named novel coronavirus or 2019-nCoV [1]. The first cases of COVID-19 were reported in Wuhan, China in early December 2019 [2]. Since then, COVID-19 has become a global pandemic due to its transmissibility resulting in it spreading across continents with the number of cases and deaths rising daily [3].

Probe-based confocal laser endomicroscopy (pCLE) is a technique that produces microscopic imaging of living tissue through a fiberoptic miniprobe that can be introduced into the working channel of the bronchoscope [4]. This method could be used in the diagnosis of lung cancer and interstitial lung diseases [5, 6]. We did not find any scientific articles about pCLE images of COVID-19. We introduce a case where pCLE was used in a patient with suspected lung cancer who had subsequently confirmed COVID-19.

Case Report

An 87-year-old male who was a non-smoker was admitted to our clinic with complaints of weakness, shortness of breath on exertion, and cough. From May 12, 2020, the patient developed cough and fever up to 38.0°C after experiencing hypothermia. Nasopharyngeal swabs for RT-PCR SARS-CoV-2 were negative. CT scans revealed obstruction of the segmental bronchi of the upper lobe of the left lung with development of obstructive pneumonitis and pleurisy (Figure 1). The patient underwent a pleural puncture and 160 mL of cloudy, bright yellow liquid with reddish loose sediment was evacuated.

The effusion had no signs of malignancy and contained blood elements, lymphocytes, histiocytes, and mesothelial cells.

The patient was hospitalized in the clinic in order to diagnose his pneumonia as being post-ob-

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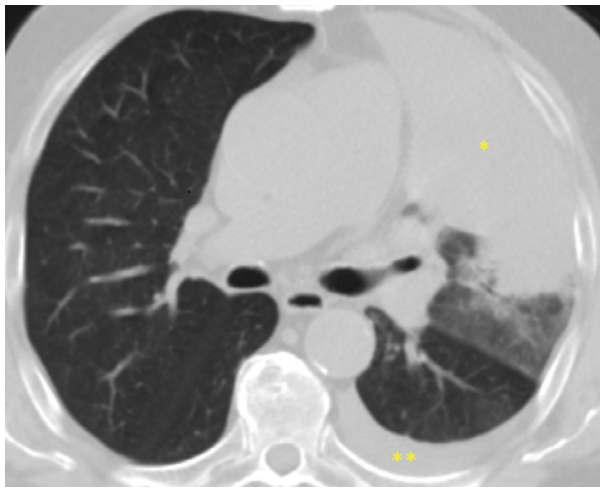

Figure 1. CT-scans showed a large area of consolidation in the left upper lobe (*) and moderate pleural effusion (**)

structive or bacterial/viral in origin. Lung cancer was also suspected.

His comorbidities include coronary artery disease, aortic valve replacement for severe aortic stenosis (2013), superficial thrombophlebitis of the saphenous veins of both legs, type 2 diabetes mellitus with diabetic polyneuropathy and diabetic nephropathy, autoimmune thyroiditis, cholecystectomy (1986), and chronic prostatitis.

After the first day of hospitalization, the patient experienced a sharp deterioration in breathing and a decrease in blood oxygen saturation (89%) which required artificial ventilation.

Because of the negative smears for RT-PCR SARS-CoV-2 and suspected lung cancer, it was decided to perform bronchoscopy with transbronchial biopsy and confocal laser endomicroscopy.

Bronchoscopy was performed in the intensive care unit by a limited number of staff who donned personal protective equipment (PPE). During bronchoscopy, a moderate amount of liquid-mucous hemorrhagic secretion was noted on both sides, and the bronchus of left segment 3 was obturated with a blood clot. The lumens of the other bronchi were ordinary. pCLE was performed using the Cellvizio system and 1.4-mm probe Alveoflex (Mauna Kea Technologies, Paris, France). After removal of the clot, no endobronchial lesions were found. The miniprobe was introduced into all subsegments of the left upper lobe. The pCLE image showed changes in the alveolar frame with increased density up to the point where alveolar structures were absent, thickening of elastic fibers, and a large amount of intra-alveolar secretions (Figure 2). Transbronchial lung biopsy (TBB) was performed from the zones of moderate and severe disorganization of lung tissue by pCLE image data. Also, bronchoalveolar lavage from left upper segmental bronchi for RT-PCR SARS-CoV-2 was performed.

Histological examination revealed signs of diffuse alveolar damage with fibrin, pneumocytes, and macrophages present without any atypical cells (Figure 3). PCR analysis of bronchoalveolar lavage fluid yielded a positive result for SARS-CoV-2. Appropriate treatment was started. Unfortunately, the pneumonia evolved and became bilateral. It eventually progressed to respiratory failure and subsequently caused the death of the patient. Postmortem examination did not reveal any signs of malignancy, and the pneumonia was confirmed.

Discussion

The diagnosis of COVID-19 is based on specific symptoms, history of contact with an infected person, and bilateral lung involvement on CT-scans. The diagnosis is confirmed by a positive nucleic acid test for SARS-CoV-2 commonly from oropharyngeal and nasopharyngeal swabs or bronchoalveolar lavage fluid (BALF) [7]. A nasopharyngeal or oropharyngeal swab for the SARS-CoV-2 RT-PCR test is the primary and preferred method for diagnosis of COVID-19, but its sensitivity varies from 53.6% to 73.3% and from 11.1% to 61.3%, respectively. Their accuracy also depends on how many days have passed since illness onset [8]. Positive rates from BALF in infected patients have been noted in up to 100% of patients, but all guidelines suggest that bronchoscopy is relatively contraindicated in COVID-19 and/or should play a limited role in diagnosis and management because of the high risk of spreading the infection to the staff involved in the procedure [9]. However, the use of bronchoscopy may be justified if an alternative diagnosis which would change management is suspected [7, 9].

In our case, the diagnosis of COVID-19 was not clear because of the initially unilateral lung infiltrate and pleurisy on CT-scans, and negative nasopharyngeal swab tests. The patient was hospitalized with suspected lung cancer. Probe-based confocal laser endomicroscopy showed density of alveolar thickened fibres, thickening of elastin fibres, and multiple large drops of intraalveolar secretions. In pCLE images, no solid pattern consistent with a malignant disease was noted which allowed us to exclude lung cancer in the differential diagnosis [5]. Histological examina-

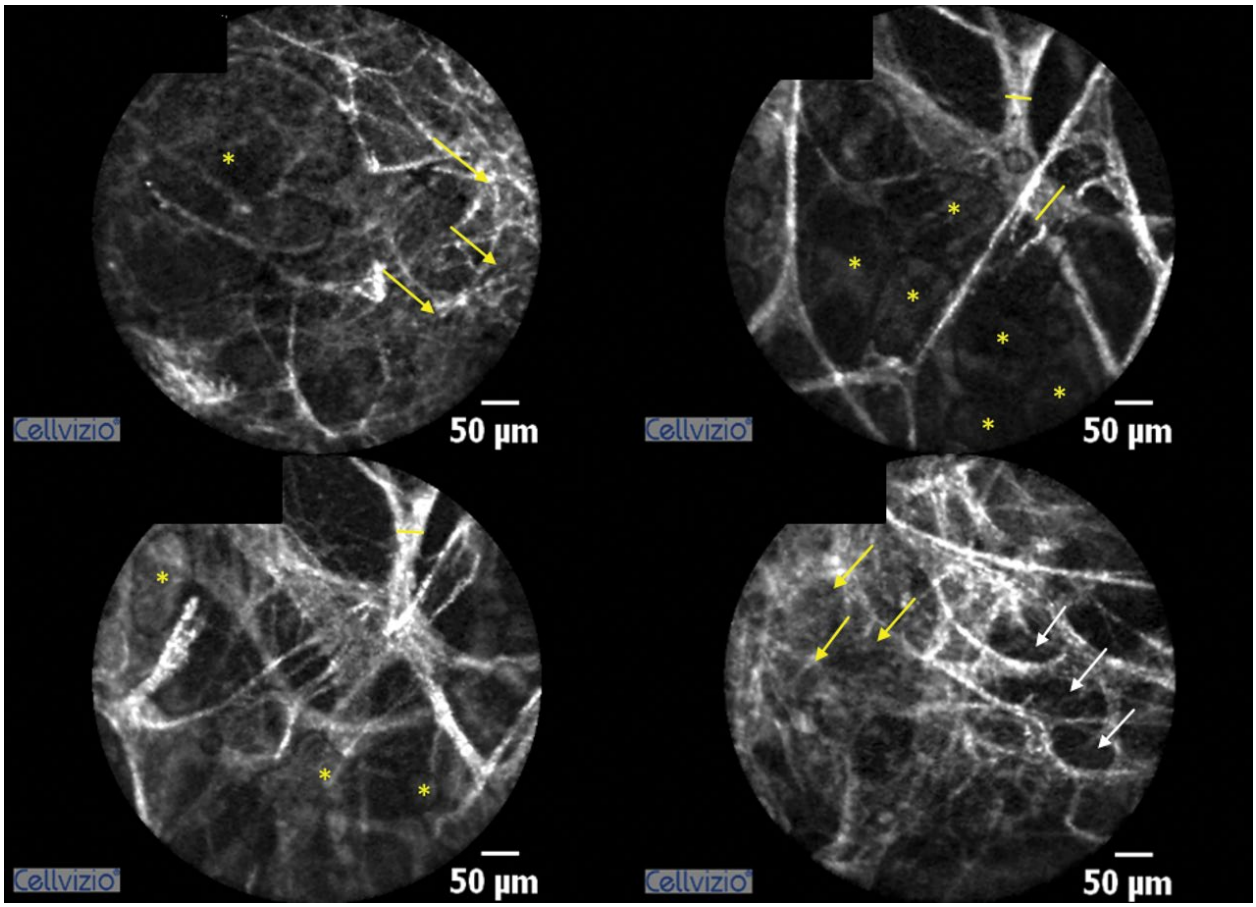

Figure 2. pCLE images of lung tissue of the left upper lobe. Intraalveolar fiber thickness (yellow line), increased density of elastic structures (white arrow) up to disappearance of alveolar structures (yellow arrow), and large drops of intraalveolar secretions (*)

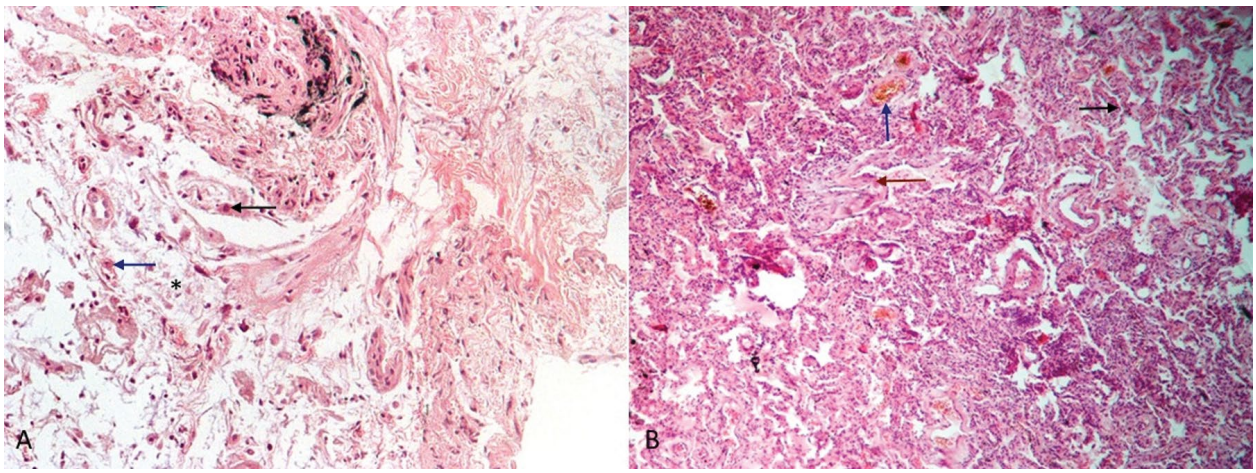

Figure 3. Pathological examination of TBB samples. **A.** Diffuse alveolar damage, proliferative phase: there is a small amount of pneumocytes in the alveoli (black arrow), macrophages (blue arrow), and fibrin (*); × 100, stained with hematoxylin and eosin. **B.** Diffuse alveolar damage, proliferative phase: accumulation of fibrin in the lumen of the alveoli with polypoid growths of connective tissue (brown arrow), interstitial inflammation with thickening and edema of interalveolar septa (black arrow), and microangiopathy and thrombosis of the vascular bed (blue arrow), stained with hematoxylin and eosin, × 40

tion of TBB specimens revealed signs of diffuse alveolar damage described in COVID-19 [10]. To the best of our knowledge, this is the first pCLE image of a patient with COVID-19. This image was not significantly different from other images seen in interstitial lung diseases [6].

Conclusion

pCLE can be a useful method for suspecting or excluding a diagnosis of COVID-19 in real time if nasopharyngeal swab tests are negative and bronchoscopy is required to establish the diagnosis.

Conflict of interest

None declared.

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Unusual presentation of tuberculosis in pregnancy: a diagnostic difficulty

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In India, pulmonary tuberculosis (TB) is highly prevalent but has rarely been reported as an underlying cause of Acute Respiratory Distress Syndrome (ARDS). Nonspecific symptoms and progressive respiratory failure in immunocompetent individuals affected by disseminated TB, especially during the perinatal period, can complicate the diagnosis. We report a case of a 24-year-old second gravida ante-partum woman of 38-week gestation presenting with high-grade fever and 3-day cough. There were no known systemic or gestational comorbidities. BP — 110/70 mm Hg, PR — 102/min, respiratory rate-28 cycles/min, SPO_2 — 89% room air at the time of presentation. She underwent normal vaginal delivery on the same day. Chorioamniomitis was suspected in view of a foul-smelling discharge, and antibiotics were started. She was intubated for respiratory distress one day after delivery. Arterial blood gas analysis showed reduction in arterial partial pressure of oxygen (PaO_2) and fraction of inspired oxygen (FiO_2) ratio of 190, suggestive of moderate-ARDS fulfilling the Berlins-criteria [1].

Her blood, urine and endocervical swab cultures were normal. WBC count was 9700Cells/mm^3 (normal: 4,000–10,000 cells/mm^3) and C-reactive protein was 4.2 mg/L (positive > 5 mg/L). Pro-calcitonin levels were 0.46 ng/mL (normal < 0.25 ng/mL) — suggestive of possible infection. 2-dimensional echocardiography was normal. Chest computed tomography showed diffuse nodular opacities and consolidation changes with minimal pleural effusion on right side (Figure 1). Repeated tracheal aspirate bacterial cultures and CBNAAT were

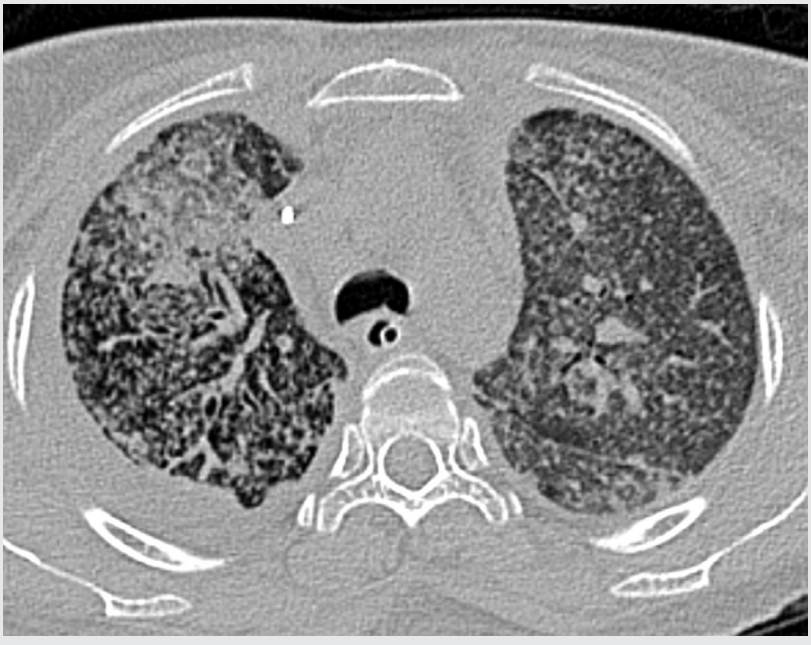

Figure 1. Computerized tomography scan of thorax

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normal. Diagnostic pleural fluid aspiration was suggestive of exudative effusion with adenosine deaminase of 52 IU/L. Pleural fluid CBNAAT detected *Mycobacterium tuberculosis* with no rifampicin resistance. Patient's clinical condition was improved after 8 days of antituberculosis therapy and was weaned off from ventilator in due course.

Diagnosing tuberculosis in pregnancy is difficult because of the nonspecific presentation and reluctance to order radiological investigations [2]. Tuberculosis presenting as ARDS is rare and will occur because of physiological immunosuppression and inflammation due to massive release of tubercular antigens, such as lipoarabinomannan, tumor necrosis factor-alpha [TNF-a] and interleukin-1b [IL-1b]. During pregnancy, the mother's immune system acquires a physiologically immunosuppressive state via suppression of Th1 cytokines, such as IL-12 and interferon-gamma (IFNγ), which are detrimental to the fetus, and by causing an elevation in pregnancy-supportive of Th2 (IL-10) cytokines [3]. Reversal of Th2 to Th1 in the immediate post-pregnancy period may be associated with a heightened inflammatory response indicating underlying latent infectious diseases, such as TB [4]. One should always suspect TB as an etiological agent in young immunocompetent peripartum individuals presenting with ARDS, especially in countries where TB burden is very high.

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An uncommon cause of a benign posterior mediastinal mass in an adult

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A 63 year old homemaker with a history of diagnosed type II diabetes mellitus treated with oral hypoglycemics presented with complaints of breathlessness (mMRC grade II) that had been present for 3 months. Chest radiography showed a well-defined mass in the left upper and mid-zone with no mediastinal shift. Contrast-enhanced chest tomography (CECT) of the thorax showed a large well-defined mass with soft tissue density in the upper left hemithorax extending to the posterior mediastinum. It measured 11.3 mm × 9.1 mm × 12.7 mm and extended from the level of the thoracic inlet to the level of D7 with no evidence of chest wall invasion (Figure 1A). A CT-guided core needle biopsy of the mass was done and showed sheets of haphazardly arranged benign spindle cells showing positivity to beta catenin. EMA, TTF, SMA, and CD-34 were all negative. The differential diagnosis included chest wall fibromatosis and solitary fibrous tumors of the pleura.

She underwent left posterolateral thoracotomy for resection of the mass. Intraoperatively, neural elements were attached to the mass but no chest wall invasion was noted. The resected mass (13 × 12 cm) contained tumor cells which were spindle-shaped with wavy nuclei and scanty eosinophilic cytoplasm with abundant myxoid stroma, a few tiny nests, and singly scattered ganglion cells (Figure 1B). They stained positive for synaptophysin. The histopathology confirmed the findings to be that of a ganglioneuroma. After surgical intervention, the left lung expanded.

Ganglioneuromas are a distinct type of well-differentiated benign tumors comprised mainly of mature ganglion cells and schwann cells without the presence of a true capsule forming a mature spectrum of sympathetic neuroectodermal tumors [1]. The most commonly involved locations that ganglioneuromas affect include the adrenal medulla (35%), the extra-adrenal retroperitoneum (30–35%), and the posterior mediastinum (20%) [2]. Under macroscopic examination, an excised mass with an attached nerve trunk may facilitate the

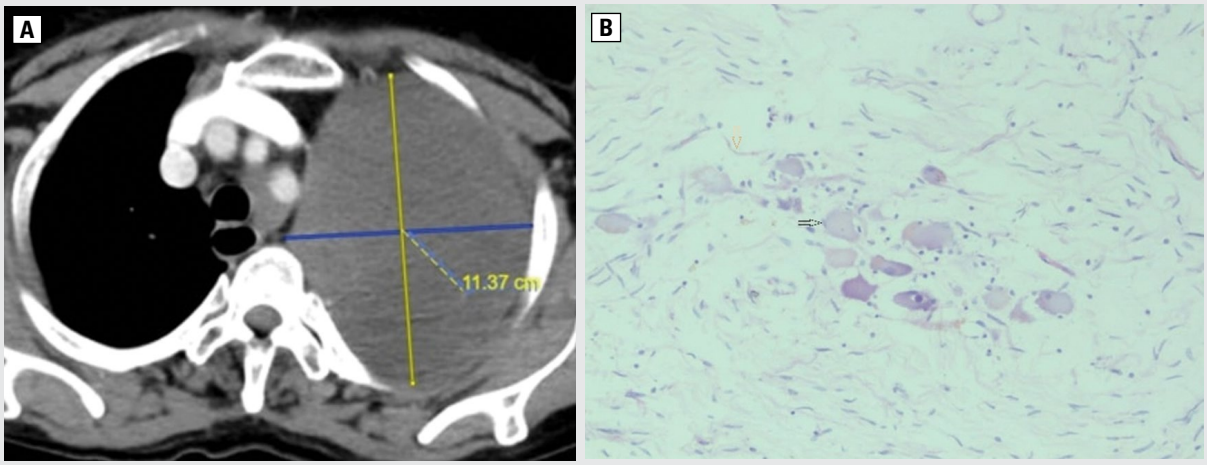

Figure 1. A. CECT of the thorax in axial view showing a large well-defined mass in the upper left hemithorax extending to the posterior mediastinum; **B.** Microscopic H&E image of the specimen under 200× power showing a ganglion cell (black arrow) with Schwann cells (orange arrow) in the background of the myxoid stroma.

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diagnosis of a ganglioneuroma**.** Histologically, they are believed to originate from ganglion cells where mature ganglion cells are seen against sheets of schwann cells [3]. Total surgical resection or subtotal resection (< 2 cms) has been observed to offer a good prognosis.

In reporting this uncommon presentation, we advise fellow clinicians to consider ganglioneuromas in the differential diagnosis in adults with a posterior mediastinal mass because it is an uncommon tumor with uncommon locations in adults.

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Acute pulmonary histoplasmosis masquerading as miliary tuberculosis in a non-endemic region

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A 28-year-old female who worked as a house maid presented with fever, troublesome cough, progressive breathlessness, and anorexia which had been present for 25 days. She had tachypnea, tachycardia, and a falling peripheral oxygen saturation. Bilateral inspiratory crepitations were heard on chest auscultation. An arterial blood gas measurement showed hypoxemic respiratory failure. She was in close contact with an active case of pulmonary tuberculosis. A sputum test for acid fast bacilli (AFB) and GeneXpert were negative for tuberculosis. Chest radiograph revealed bilateral nodular opacities. High-resolution computerized tomography (HRCT) of the thorax disclosed bilateral pulmonary nodules in a random distribution (miliary pattern) as well as mediastinal lymphadenopathy (Figure 1A). She was diagnosed clinically with pulmonary tuberculosis and initiated on anti-tubercular treatment (ATT). However, her symptoms worsened and ATT was discontinued. There was a palpable right supraclavicular lymph node and an excision biopsy revealed granulomatous inflammation (Figure 1B). Ziehl-Neelsen stain stain for AFB and GeneXpert were negative. Fungal staining revealed Histoplasma capsulatum (Figure 1C). She was diagnosed with acute pulmonary histoplasmosis and initiated on Amphotericin-B liposomal injection 4 mg/kg/day intravenously for 14 days followed by oral Itraconazole 200 mg twice daily. There was a significant clinico-radiological response to the antifungal therapy.

Histoplasma capsulatum is a dimorphic fungus which grows as a mould in soil (infective form) and as yeast in the host's tissue (pathogenic form) [1]. Two varieties of histoplasma are known to infect humans — *H. capsulatum var. capsulatum* and *H. capsulatum var. duboisii*. It is endemic in Central and North America but sporadic cases have been reported in eastern India along the Gangetic plains. It can involve almost any organ system but pulmonary and mediastinal involvement are most common [2]. Acute pulmonary histoplasmosis may vary in its clinical presentation. It may present as an asymptomatic illness or as a severe disease progressing to acute respiratory failure. Clinical features include fever, malaise, breathlessness, dry cough,

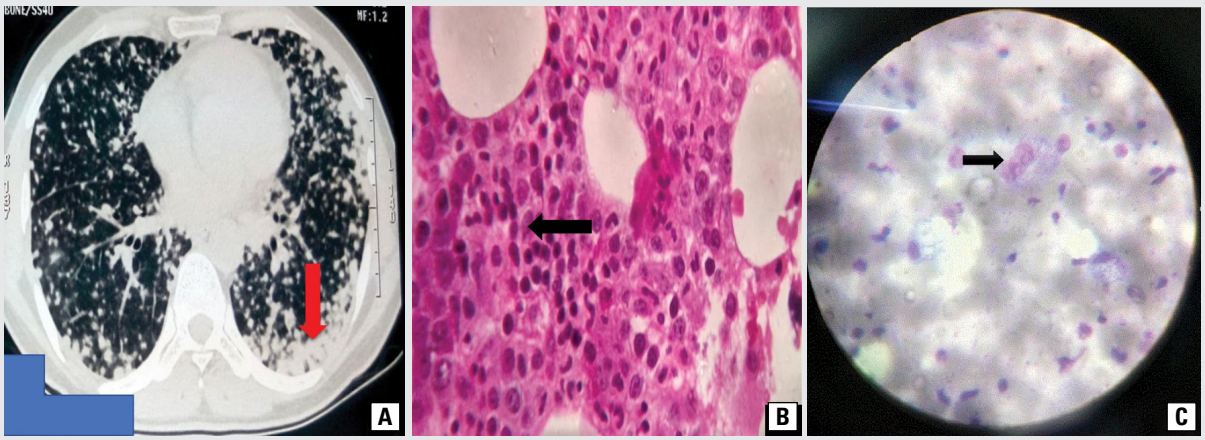

Figure 1. A. HRCT thorax axial reformatted images at the level of inferior pulmonary vein showing bilateral nodules in random distribution with conglomeration of nodules seen at lung bases (red arrow); **B.** Lymph node biopsy sections H&E stain 400× magnification showing multiple epithelioid granuloma (black arrow); **C.** Lymph node biopsy section Giemsa stain 1000x magnification showing capsulated yeast form of histoplasma (black arrow)

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and chest pain. Chest imaging usually reveals nodular opacities mimicking miliary shadows and mediastinal adenopathy. Although miliary opacities on chest imaging have a long list of differentials, patients are frequently diagnosed as having either tuberculosis (in the developing world) or sarcoidosis (in the developed world) and are initiated on empirical therapy [3]. Since, the symptomatic and severe form of acute pulmonary histoplasmosis can be fatal, early and definitive diagnosis is imperative. The best method is via direct detection of the organism by histopathology or cytology. Other diagnostic options include antigen detection, culture, serology, skin prick tests, and molecular tests [4]. Treatment is not required in asymptomatic or mild cases. Severe cases are treated with liposomal Amphotericin B (3-5mg/kg/day) or deoxycholate Amp B (0.7–1 mg/kg/day) intravenously for 7–14 days followed by oral Itraconazole (200 mg thrice a day for 3 days followed by twice daily) for a total of 6 weeks [5].

Histoplasmosis is not uncommon in non-endemic regions but is an under-recognized entity because the clinico-radiological and pathological profile mimics tuberculosis, the most common granulomatous disease in this part of the world. Besides, the environmental cause of fungal infections in non-endemic regions is yet to be explored leading to most cases being misdiagnosed. Hence, a high clinical suspicion and awareness is required for its diagnosis.

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An unusual origin of Ewing sarcoma

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An 18-year-old boy presented with right-sided chest tightness for 2 months. There was no fever, cough or wheeze. There was loss of weight and appetite for 1 month. There was no relevant medical or surgical history in the past. On examination, there were decreased movement, dull percussion note and decreased breath sounds in mammary, infraaxillary and infrascapular areas of the right side.

The chest roentgenogram showed a homogeneous opacity in right mid and lower zones silhouetting the right heart border, cardiophrenic angle and diaphragm. A bedside ultrasonography revealed a complex lesion with thick septations and few solid areas. The differentials included sarcomas of the chest wall, large solitary fibrous tumour and metastases. A subsequent contrast-enhanced computed tomography of the chest (CECT) (Figure 1) documented the lesion to be located in the paravertebral region, with heterogeneous densities inside, extending from the level of the 5^{th}–12^{th} thoracic vertebra indenting the posterio-superior surface of the right lobe of the liver with no underlying bone changes. A CT-guided biopsy revealed small blue round cells and trabeculae with areas of necrosis and haemorrhage which were CD-99 positive, establishing diagnosis of extraskeletal Ewing sarcoma (EES). On the multidisciplinary tumour board, thoracotomy was planned followed

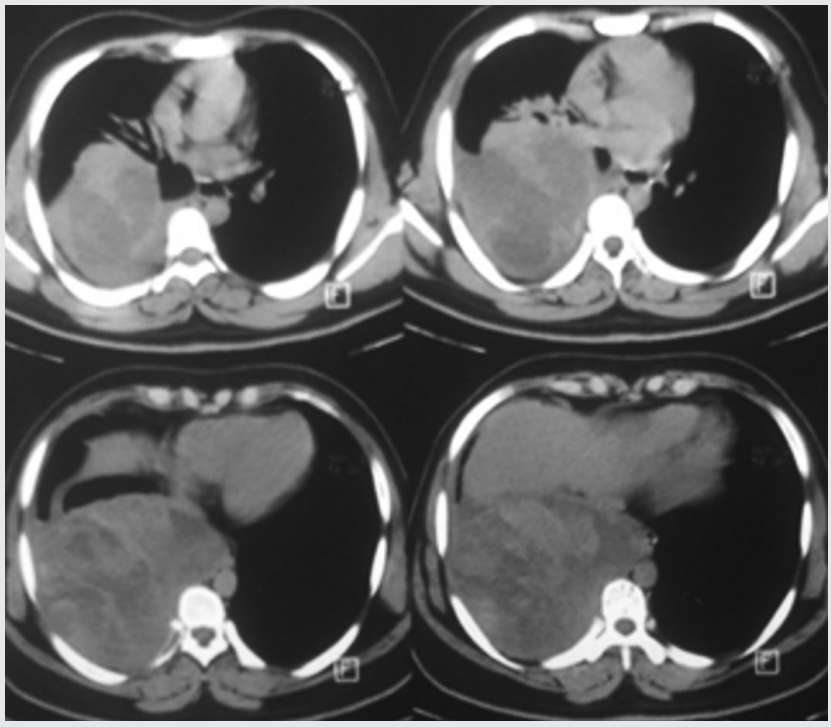

Figure 1. CT thorax showing a large heterogeneous lesion occupying the right hemithorax inferiorly having absent fat planes with the T5 to T12 vertebrae, overlying ribs and right hemidiaphragm. The right dome of liver is indented and pushed inferiorly. Internal areas of cystic attenuation are noted indicating necrosis

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by adjuvant chemotherapy (vincristine, doxorubicin, and cyclophosphamide, alternating with ifosfamide and etoposide).

The Ewing sarcoma family of tumours (EFT) consists of classic Ewing sarcoma of the bone, extraskeletal Ewing sarcoma (EES), malignant small-cell tumour of the thoracopulmonary region (Askin's tumour) [1] and primitive neuroectodermal tumours of the bone or soft tissues. EES has an uncommon incidence of around 1.1% of all malignant soft tissue tumours, predominantly in adolescents and young adults (10–30 years) [2]. EES commonly affects the extremities, soft tissues of the trunk (intercostal regions), head and neck, pelvis, and peritoneum. The tumour tends to spread locally, invading muscles or skeletal structures [3].

In the current case, however, the tumour was located in the paravertebral region and did not invade the adjacent soft tissue or bony structures. The surgery was uneventful, following which the patient was initiated on adjuvant chemotherapy and has no evidence of recurrence yet. EES has a favourable five-year survival (61%) in comparison to skeletal Ewing's Sarcoma (10%) depending on age, size and location of tumour along with response to therapy.

To conclude, EES should be considered as a differential for heterogeneous lesions of the chest wall on radiology.

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Enteroclysis in gastrointestinal tuberculosis: an overview

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42-year-old female, working on a cow farm, without known comorbidities, complained of intermittent bowel movements (2 stools/day) lasting over 8 weeks, weight loss (6 kg), enduring low-grade fever, recurrent dry cough, asthenia and occasionally pain in the right lower abdominal quadrant. Physical exam revealed superficial abdominal tenderness, painful at palpation, narrowed to the right lower quadrant. Lab results within normal range. Positive SARS-CoV-2 nasopharyngeal swab test (RT-PCR). Negative stool testing. Normal chest X-ray. HIV negative. Abdominal high resolution computed tomography scan: swelling of the terminal ileum, extended low-density nodes. Received [1–3]: Hydroxychloroquine 400 mg daily (5 days), Lopinavir/Ritonavir 800 mg/200 mg daily (7 days), Dexamethasone 20 mg tab daily (5 days), Azithromycin 500 mg tab daily (5 days), Heparin Sodium 5000 IU injection daily (5 days). Barium follow-through examination (Figure 1A) displayed the Fleischner sign [4]. Bronchoalveolar washing and gastric aspirate negative for acid-fast bacilli. The biopsy sample taken during colonoscopy (Figure 1B) revealed multiple tubercle bacilli (Ziehl-Neelsen staining) [5]. Based on national anti-tuberculosis regimen, successful treatment was initiated for 24 weeks. Gastrointestinal tract is the 6th site of extrapulmonary tuberculosis (TB). Enteroclysis followed by barium enema is the best protocol for evaluation of gastrointestinal TB. COVID-19 may determine an immunosuppressive reaction. This can activate latent TB!

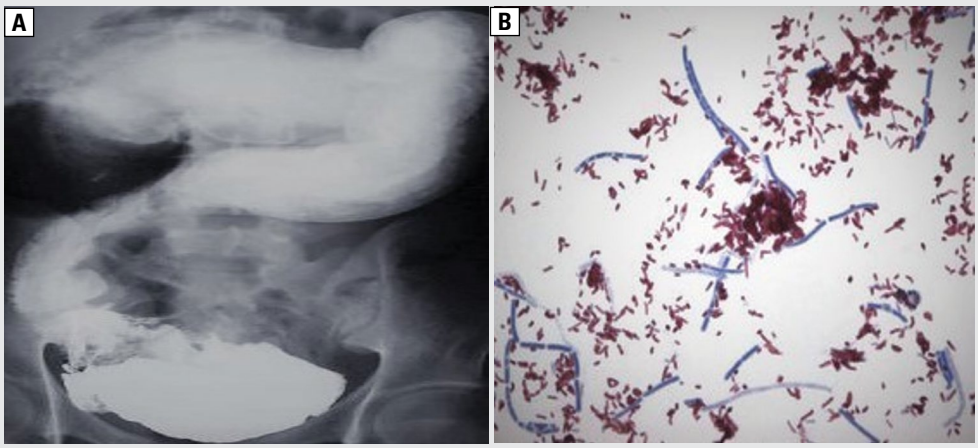

Figure 1. A. Barium enema revealing „Fleischner sign" (inverted umbrella defect). **B.** Histopathological exam of the intestinal mucosa seen through an optical microscope (oil immersion, 1000 × magnification), carbol fuchsin stain

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Management of non-COVID respiratory illnesses during the COVID-19 pandemic — a pulmonologist's perspective

To the Editor

The novel coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus continues to wreak havoc all over the world with approximately 57,714,184 infections and 1,373,065 deaths reported to date [1]. No definite therapy is available yet and vaccines are still under development. As the entire health-care system is entangled in tackling the pandemic, interim guidelines were formulated in order to organize optimal prioritization of health resources to avoid the compromise of non-COVID care. However, these guidelines were directed at generalized management of all non-COVID-19 related illness. Our letter focuses on the management of the common non-COVID respiratory illnesses, thereby aiming to provide clarity to treating physicians. Further, chronic lung disease is an independent risk factor for severe COVID-19 and mortality [2].

Bronchial asthma, defined by a history of variable respiratory symptoms (wheezing, shortness of breath, chest tightness, and cough) and variable expiratory airflow limitation, affects nearly 300 million people worldwide and leads to increased mortality in middle and low-income countries. The Global Initiative for Asthma (GINA) recommends continuation of asthma medications (particularly inhaled corticosteroids, oral corticosteroids, and biological therapy) as sudden discontinuation may lead to poor asthma control. Apart from pharmacotherapy, a written asthma plan aimed at recognizing exacerbations is advised. The main change in asthma care includes the avoidance of nebulizers and the preferential use of pressurised metered-dose inhalers (pMDI) with a spacer to avoid aerosol generation. The use of spirometry and peak-flow meters should be avoided unless compelling indications influencing clinical management arises. In the case of aerosol-generating procedures, strict infection control measures need to be adopted and personal protective equipment (PPE) should be used [3]. Until further evidence is available, it is safe to incorporate these measures into asthma management during the COVID-19 pandemic. Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the world and is a significant risk factor for poor patient outcome (ICU admission, invasive ventilation, or death) in COVID-19 [4]. The global initiative for obstructive lung disease (GOLD) interim guidelines advise for the continuation of maintenance therapy and adherence to the advice of local health teams in order to minimise the chance of disease spread [5]. Pulmonary rehabilitation (PR) is a key component of stable COPD management and can be performed via programs conducted online or by telephone [6]. In suspected COPD exacerbations, it is advised to rule out COVID-19 while effectively managing the exacerbation.

Pulmonary tuberculosis (PTB) continues to be one the leading cause of morbidity and mortality, especially in high-TB burden countries. Data regarding COVID-19 infection in PTB is limited and the World Health Organization (WHO) has issued an urgent message highlighting the need to maintain continuity of essential services for people affected with TB during the

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COVID-19 pandemic as it threatens to reverse the gains made in TB prevention and care. Patients with PTB and COVID-19 are expected to have poorer outcomes if anti-tuberculous therapy is interrupted. Early diagnosis, appropriate treatment, and preventive measures such as cough etiquette, personal hygiene, and social distancing need to be adopted as they help in reducing the transmission of TB and COVID-19. TB patients should be in constant communication with health care facilities to aid in the management of adverse drug reactions, comorbidities, nutritional and mental health support, and restocking of medical supplies. Steps have to be taken to ensure the timely availability of TB medicines and collection of sputum samples for follow-up testing. Until the pandemic subsides, a decentralized home-based treatment approach should be initiated for all TB patients. However, it is important to note that this may lead to an increased risk of household transmission of TB [7].

Lung cancer is the most common malignancy worldwide resulting in 2.09 million deaths in 2018. Lung cancer patients also have an increased probability of contracting SARS-CoV-2 infection due to immunosuppression from the malignancy, chemoradiation, advanced age, chronic lung disease, and other comorbidities. Diagnostic delay may occur in COVID-19 suspected/positive patients as elective/emergent bronchoscopy is postponed until 28-30 days after resolution of symptoms and viral shedding. Enrolment of new patients for chemotherapy has also been put on hold temporarily at various cancer centres. Diagnosis of lung cancer should be aimed at obtaining tissue with the least invasive method and to perform urgent bronchoscopies in a safe and timely manner after taking into consideration local availability of resources [8]. Apart from diagnosis and medical management, surgery in resectable lung cancer is also affected as elective surgeries are currently rescheduled. Enrolment in chemotherapy and radiotherapy should be initiated in a systemic manner after effectively ruling out SARS-CoV-2 infection. The decision for timing of surgery should be individualized on a case by case basis. In case of urgent surgeries, they should be preferentially done in negative pressure isolation rooms with full PPE [9].

Community acquired pneumonia (CAP) can be due to bacterial or viral pathogens. The most common organisms include streptococci, moraxella catarrhalis, haemophilus influenza B, chlamydia, and staph aureus. Viruses contribute to one-third of CAP. According to Medley *et al.* [10], empirical antibiotics are recommended against the most common CAP pathogens but are not routinely indicated in COVID-19 pneumonia. Cultures are not routinely advised and should be obtained only when multidrug resistant organisms are suspected. Measuring serum procalcitonin can help to prevent antibiotic overuse. Use of steroids or any immunomodulating agents are not indicated in bacterial CAP.

Apart from the above respiratory diseases, there are several other chronic lung diseases for which no definitive guidance is available yet and are beyond the scope of this article. The optimal management of these major non-COVID respiratory illnesses is paramount in reducing the burden of overwhelmed health care systems tackling the COVID-19 pandemic. Timely testing, ensuring adherence and compliance to therapy through social networking systems, telemedicine consultations, access to medication and hospital care in case of worsening symptoms, PR, and counselling counseling, and education social stigmas are the way forward in the new normal of learning to live with COVID-19 and will help recover lost ground on non-COVID respiratory care. As the saying goes, "the battle may have been lost, but the war is still there to be won".

Conflict of interest

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Potential effects of a flavonoid, hesperidin on SARS-CoV-2 disease

To the Editor

The novel coronavirus, COVID-19 or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in China in December 2019 [1].

SARS-CoV-2 uses the receptor angiotensin-converting enzyme 2 (ACE2) for infection by the transmembrane protease, serine 2 (TMPRSS2) on the surface of the host cell entry [2]. SARS-CoV-2 is not only rapidly spreading but has become a global pandemic that may challenge the economic, medical and public health of the world [3]. Following infection by SARS-CoV-2, cytokine storm is mediated by the release of large amounts of IFN-α, IL-1b, IL-6, IL-12, IL-18, IL-33, TNF-α, TGFβ, etc. by immune effector cells [4, 5]. Various biological compounds such as of flavonoids, have been showed as anti-asthmatic [6, 7], therapeutic, antioxidant, antiviral and with other properties in nature [8, 9]. Anti-SARS coronavirus 3C-like protease effects of plant-derived phenolic compounds were also reported [10]. Hesperidin is a common flavone glycoside found in citrus fruit such as lemons [11]. The virions load in hesperidin-treated Madin-Darby canine kidney (MDCK) cells were 148-fold less than that of the untreated MDCK cells infected by influenza virus. Hesperidin (100 μM) also decreased viral RNA level and enhanced antiviral state-associated genes expression in the uninfected A549 cells [12].

The inhibitory effect of hesperidin (0–25 mM) on influenza A virus (IAV) infected MDCK cells induced distinct reduction in IAV replication. Hesperidin had no cytotoxic effects on MDCK cells [13]. It is the compound that could target the binding interface between SARS-CoV-2 Spike and ACE2 human receptors [14]. It has been reported that hesperidin strongly binds to the active site of RNA dependent RNA polymerase (RdRp), which catalyzes SARS-CoV-2 RNA replication [15].

Hesperidin (2.0 mg/mL) significantly reduced expression of pro-inflammatory cytokines in human osteoarthritis (OA) chondrocytes [16].

The effects of hesperidin (5, 10, 50 and 100 μM) on hydrogen peroxide (H_2O_2) induced oxidative stress damages to chondrocytes, downregulated the mRNA levels of COX-2, IL-1β, TNF-α, MMP-3, MMP-9, and upregulated IL-10, TIMP-1, SOX9 [17]. Treatment of *Aeromonas hydrophila*-infected mice with hesperidin (250 mg/kg b.wt.), significantly suppressed inflammatory response through reduction of reactive oxygen species (ROS) production and adhesion molecules expression, as well as an increase of CD4+/CD8+ cell ratio [18]. Hesperidin (100 mg/kg. b.w) also reduced lipid peroxidation and inflammatory mediators (IL-1 β and TNF-α), while increased anti-inflammatory cytokines (IL-4 and IL-10) in induced Parkinson's disease in male C57BL/6 mice [19].

Nitric oxide (NO) has the potential therapeutic effects on acute respiratory distress syndrome in patients with COVID-19, and inhaled nitric oxide may become an alternate rescue therapy in patients with COVID-19 [20]. NO may inhibit the early stage in viral replication and could prevent viral spread, and recovery of patients [21]. Treatment of bovine aortic endothelial cells (BAEC) with hesperedin (10 μM for 5 h) stimulated production of NO [22]. The effect of hesperidin (15 and 30 mg/kg) on cardiovascular remodeling in rats significantly reduced oxidative stress markers, TNF-α, TGF-β1, and enhanced plasma

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nitric oxide metabolite (NOx) in L-NAME-induced hypertension in rats [23]. The results of a review of different studies in China showed that less than 10% of smokers infected with COVID-19 [24]. The intermittent bursts of high NO concentration in cigarette smoke may be a protective mechanism against SARS-CoV-2 [25].

Hesperidin may be used as a promising drug candidate for the prevention and treatment of SARS-CoV-2 due to antiviral, anti-inflammatory and antioxidant properties. Furthermore, hesperidin interferes with viral entry through ACE2 receptors, release of NO into the blood stream and improved immune system.

Conflict of interest

The author declares no conflicts of interest.

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Asthma control test to identify uncontrolled asthma in pediatric clinical practice

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To the Editor

Asthma management is a daily challenge in pediatric practice as many clinical and functional aspects continuously change over time. Asthma management should be oriented to attain and maintain asthma control [1]. The asthma control level drives the intensity of care. Different tools can measure asthma control. The Global Initiative for Asthma (GINA) classification and the Asthma Control Test (ACT), and its pediatric version (childhood-ACT, C-ACT) are the most commonly used in clinical practice [1–3]. These two ways are popular in daily practice. Other instruments also are available, including the Composite Asthma Severity Index (CASI), which measures asthma severity [4]. Asthma severity is another facet relevant in the management. However, asthma control and severity are closely connected, and the concept of control/severity practically combines the two measures.

In this regard, Mokhallati *et al.* [5] evaluated the predictive value of CASI in a cohort of 108 children with asthma. ACT and C-ACT were also used to obtain additional information for contextualizing CASI. This study showed that CASI actually predicted the physician assessments of asthma control and severity and confirmed that both were not distinct entities. Moreover, ACT/C-ACT was a reliable predictor of physician-assessed control. Based on this background, we would evaluate the role of ACT/C-ACT in a large multicenter nationwide study. For this purpose, the Italian Society of Paediatric Allergy and Immunology promoted a perspectival study ("ControL'Asma") to investigate asthma control in children and adolescents managed in clinical practice. The first outcomes showed that the type 2 high allergic phenotype was prevalent, and partly controlled-uncontrolled asthma affected about half of the participants [6]. Further, the project also demonstrated that rhinitis is frequent asthma comorbidity and rhinitis phenotyping is useful for adequately managing asthma [7].

Consequently, the present study aimed to thoroughly evaluate the asthma control assessment in 469 children and adolescents (69.3% males, mean age 11.2 years) consecutively visited across 10 Italian Paediatric Allergy centers. All patients were currently treated according to the GINA guidelines based on the asthma control level. The methodology has been reported in detail elsewhere [6].

The Ethics Committee of the Istituto Giannina Gaslini of Genoa initially approved the procedure (code number: 22253/2017; in the context of the Italian Project "ControL'Asma" promoted by the Italian Society of Paediatric Allergy and Immunology). All the other Review Ethics Committees further approved the study procedure and written informed consent was obtained from all parents. Clinical data were recorded by an electronic case report form designed expressly for this study.

Patient's characteristics at baseline were described as mean with standard deviation (SD), median with interquartile ranges (IQR), or count and percentage (%), as appropriate. Univariable and multivariate logistic regression models were performed to evaluate factors associated with uncontrolled asthma. Variables with $p < 0.20$ in the univariate analysis were candidates for subsequent multivariate analysis. Receiver operating characteristic curve analysis was graphed to calculate the Area Under the Curve (AUC) and identify the optimal cut-off score for ACT to

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discriminate uncontrolled from controlled asthma (by calculating Youden's index). Two-sided P-values ≤ 0.05 were considered statistically significant. The analyses were computed using SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

According to GINA classification, 261 (55.6%) subjects had well-controlled asthma, 152 (32.4%) partly-controlled, and 56 (12%) uncontrolled. At the multivariate analysis, only the adjusted age C-ACT/ACT (OR [odds ratio] = 0.66; CI [confidence interval] 0.59–0.75; p < 0.001) and forced volume vital capacity (OR = 0.95; CI 0.91–0.98; p = 0.002) were significantly associated with uncontrolled asthma. In particular, subjects with uncontrolled asthma had a C-ACT/ACT score of 17 (IQR 13–18), significantly (p < 0.001) lower than subjects with controlled/partly-controlled asthma (23; IQR 20–25). The receiver operating characteristic curve identified a value of 18.5 as a reliable cut-off with AUC 0.875 (CI 0.83–0.91; p < 0.001), 87% sensitivity, 78.6% specificity, and 0.66 Youden Index, as reported in the Figure 1.

These outcomes are consistent with previous studies that provided robust evidence for the ACT as a reliable questionnaire to assess asthma control. In particular, an 18-month follow-up study demonstrated that C-ACT use improved asthma care [8]. Moreover, the ATS/ERS Task Force on asthma control concluded that the assessment should be multicomponent and consider clinical aspects using adequate tools, including the ACT [9]. A systematic review exploring the diagnostic performance of asthma control questionnaires concluded that ACT had low accuracy in identifying patients with uncontrolled asthma as the AUC was 0.69 [10]. Our outcomes were inconsistent with these findings. Namely, the ACT performance was good to define uncontrolled asthma correctly.

ACT is, therefore, a reliable and practical tool to precisely assess asthma control. Other tools, including CASI and asthma control questionnaire, need the lung function measurement. It yields the tests unusable in primary care settings, where spirometry is frequently unavailable. Moreover, in the COVID-19 pandemic era, telemedicine represents a promising alternative to office visits. Thus, careful history and ACT could represent an optimal way to manage asthma at home realistically.

On the other hand, the current study had some limitations, including the cross-sectional design and the lack of biomarker evaluation.

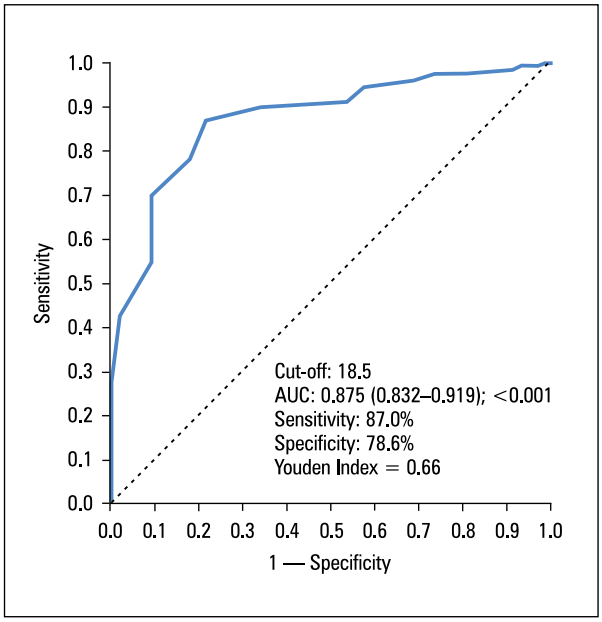

Figure 1. ROC curve for defining the optimal cut-off of C-ACT/ACT score in uncontrolled patients.

ACT — asthma control test; AUC — area under the curve; C-ACT — childhood-ACT; ROC — receiver operating characteristic

However, the study was conducted in a real-world setting, the number of participants was large, and the nationwide distribution of centers across Italy. These factors contributed to give outcomes that reflect what occurs in daily pediatric practice.

In conclusion, the ControL'Asma study showed that ACT is a suitable tool for identifying children and adolescents with uncontrolled asthma. As it is a questionnaire, ACT could be used in every setting, including primary care clinic and at home.

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Conflict of interest

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