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project manager: weronika.kordyka@viamedica.pl
Editorial Address: Biuro ZG PTChP, ul. Wronia 45, lok. 132, Warszawa, Poland
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LETTER TO THE EDITOR

Magdalena M Martusewicz-Boros, Wojciech J Piotrowski

[1]3rd Lung Diseases and Oncology Department, National Tuberculosis and Lung Diseases Research Institute in Warsaw, Poland
[2]Department of Pneumology and Allergy, Medical University of Lodz, Lodz, Poland

Diagnosis of sarcoidosis — the updated ATS 2020 recommendations through the prism of everyday clinical practice

Introduction

Sarcoidosis is a chronic multiorgan granulomatous disease of unknown etiology, which most often involves the lungs and intrathoracic lymph nodes [1]. The latest international guidelines for diagnosis and management of the disease were published in 1999 [1]. An update of the official position of the American Thoracic Society (ATS) expert regarding the Clinical Practice Guideline for the Diagnosis of Sarcoidosis was released in April, this year [2].

The document does not present a significantly changed approach to the diagnosis of the disease but presents an expert position on certain clinical situations that the practitioner meets when determining the diagnosis of sarcoidosis. The guidelines were developed according to the GRADE methodology (Grading of Recommendations Assessment, Development, and Evaluation), based on a systematic review of the literature and, where appropriate, meta-analysis, in order to summarize the best available evidence.

Diagnosis of sarcoidosis is not standardized, but there is a general consensus that it should be based on the following criteria: consistent, adequate clinical presentation, demonstration of the presence of granulomatous lesions in pathomorphological examination (in one or more tissue samples) and the exclusion of alternative causes of granulomatous disease [2].

In this article, the reader will find, briefly presented, the most important position of ATS experts regarding selected aspects of the diagnosis of sarcoidosis, with a comment from the authors of this editorial.

Summary of the ATS 2020 recommendations

Pathomorphological examination of lymph nodes

In patients who are highly likely to have a clinical diagnosis of sarcoidosis (e.g. Lofgren's syndrome, symptoms of "lupus pernio" or Heerfordt's syndrome), a panel of experts allows (suggests) NOT sampling lymph nodes (conditional recommendation, very low quality evidence). In asymptomatic patients with changes in the radiological image indicating bilateral symmetrical pulmonary lymphadenopathy, recommendations for or against a lymph node biopsy were not made, due to insufficient evidence, either for or against a routine lymph node biopsy. The need for further close clinical observation is emphasized in all patients whose biopsy was postponed.

In patients with suspected sarcoidosis, with hilar/mediastinal lymphadenopathy, in whom lymph node sampling is planned, endobronchial ultrasound (EBUS) bronchoscopy instead of mediastinoscopy is suggested as the first diagnostic tool (conditional recommendation, evidence of very low quality).

Screening for extrapulmonary sarcoidosis

In all patients diagnosed with sarcoidosis, screening for extrapulmonary lesions is suggested. Initial ophthalmological examination in all patients (regardless of the presence of ocular symptoms) is suggested as screening for ocular sarcoidosis (conditional recommendation, evidence of very low quality).

In the panel of initial laboratory tests, it is suggested that the concentration of serum creat-

Address for correspondence: Magdalena M Martusewicz-Boros, 3rd Lung Diseases and Oncology Department, National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland; e-mail: m.martusewicz@gmail.com
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inine for the assessment of kidney involvement is determined. Serum alkaline phosphatase should be always included in the list of laboratory examinations as the best marker of liver sarcoidosis (experts do not present their opinion for or against routine transaminases testing). Complete blood cell count as an initial screening for hematological abnormalities (conditional recommendations, evidence of very low quality) is suggested in all patients. The only one strong recommendation in the entire document concerns the examination of serum calcium levels for abnormal calcium metabolism, which is recommended to all patients with sarcoid diagnosis (including those who have no symptoms or other signs of hypercalcemia) (strong recommendation, evidence of very low quality).

If the assessment of vitamin D metabolism is considered necessary in a patient with sarcoidosis, for instance in patients with indications for supplementation therapy, testing of both: 25 and 1.25-OH vitamin D is suggested (conditional recommendation, evidence of very low quality).

To all patients with sarcoidosis who do not have cardiac symptoms, a resting electrocardiography (ECG) is recommended as a screening test for cardiac involvement (conditional recommendation, evidence of very low quality). If the patient has no cardiac symptoms, it is suggested that routine baseline echocardiography (ECHO) or outpatient 24-hour Holter ECG monitoring (conditional recommendation, evidence of very low quality) is NOT performed. The panel of experts concluded that the final decision whether or not to perform ECHO and Holter ECG should be considered individually in each case (conditional recommendation, evidence of very low quality).

Diagnostics in patients with sarcoidosis of suspected extrapulmonary disease

In patients diagnosed with sarcoidosis who are suspected of cardiac involvement — cardiac magnetic resonance imaging (cMRI) is suggested as a test of choice rather than cardiac positron emission tomography (cPET) or ECHO to obtain both, diagnostic and prognostic information. In cases where an MRI examination of the heart is not possible (due to lack of access to the equipment or contraindications to the procedure), it is suggested that cPET (conditional recommendations, evidence of very low quality) is performed.

To patients diagnosed with sarcoidosis who are suspected of having pulmonary hypertension (PH), an ECHO test is suggested (conditional recommendation, evidence of very low quality). The PH suspicion is made up of clinical symptoms, including exercise pain in the chest and/or fainting, shortened distance in a 6-minute walk test, desaturation during exercise, in addition, results of additional tests indicating reduced lung transfer factor for carbon monoxide ($T_{L,CO}$), enlarged pulmonary artery diameter relative to ascending aorta diameter assessed in chest computed tomography (CT), increased brain natriuretic peptide (BNP) and/or the presence of pulmonary fibrosis.

To definitively confirm or exclude PH in patients diagnosed with sarcoidosis in whom ECHO result is suggestive of PH, right heart catheterization (RHC) is suggested (conditional recommendation, very low-quality evidence). In the absence of suggestive echocardiographic changes for PH, individual indications for diagnostic RHC should be considered (recommendation based on best clinical practice).

Critical remarks and conclusion

The question whether a biopsy is necessary in all patients suspected of sarcoidosis has been discussed for many years among all experts in the field, and this discussion usually results in vast spectrum of diverse opinions. Although the majority admits that strongly suggestive clinical symptoms (i.e. Lofgren syndrome), in addition to clear and doubtless radiological signs of bilateral lymphadenopathy, firmly support the diagnosis, the recommendation does not give a "one-fits-all" answer to this question. Erythema nodosum is not a specific sign of sarcoidosis, and among a long list of potential causes, one can find lymphoma and tuberculosis — both possibly presenting with mediastinal lymphadenopathy. The value of proper evaluation of radiological signs has not been stressed in the document strongly enough. The recommendation to postpone a biopsy in a patient with bilateral hilar lymphadenopathy seems to be valid only in individuals with evidently predominant bilateral enlargement of hilar lymph nodes, and in case of atypical configuration (i.e. predominance of non-hilar lymphadenopathy or asymmetry), the decision of a biopsy should be sustained. The authors of the guidelines use the term "mediastinal lymphadenopathy" not assuming an attitude to the presence or absence of coexisting parenchymal disease. These axillary features may either increase or decrease the diagnostic anxiety. Clearly enough, we do not have much evidence to answer such a question. EBUS is recommended but the question arises whether the presence of few epithelioid cells in the cytological examination indeed allows for exclusion

of cancer-related sarcoid-like reaction or lymphoma? How to secure the diagnosis of sarcoidosis in view of the evidence that many patients suffering from tuberculosis and presenting with lymphadenopathy show non-caseating granulomas in the biopsy? The necessity of close monitoring (clearly emphasized by the guidelines' authors) and education of patients about the possible symptoms suggestive of alternative and much more dangerous diseases, possibly hiding under the mask of sarcoidosis, is of special importance. To conclude, individual approach is a key issue. Doctor's experience in the field is also extremely important, therefore, the best solution is to set up reference centers for sarcoidosis in order to minimize the risk of improper diagnosis.

The document does not discuss the importance of biopsies from bronchi or transbronchial biopsies (forceps or cryobiopsies). Obtaining confirmation of sarcoid granuloma from more than one organ significantly increases the probability of a correct diagnosis. Bronchoalveolar lavage (BAL), still routinely used in many centers worldwide, and a potential value of flow cytometry in the diagnosis of sarcoidosis have not been discussed either.

The recommendation concerning screening examinations does not cover all routine tests that are usually performed in sarcoid patients. There are several unanswered questions like the following: are spirometry and $T_{L,CO}$ necessary in all patients? Should abdominal ultrasonography be performed for screening of abdominal locations in all patients? What is the value of chest CT in the initial evaluation, and when should it be used for monitoring? And last but not least, have we forgotten about the assessment of disease activity? Is it still important? In which patients? What about biomarkers (ACE, sR-IL2, neopterin etc.) or imaging techniques like PET or scintigraphy? The list of unanswered questions is probably much longer, and problems may be different depending on the local conditions, related to disease population characteristics, health system resources and organization.

The authors' own experience is in line with the indication of the cMRI as the superior method in assessing the involvement of this organ, after confirming sarcoid changes in the pathomorphological examination of lymph nodes, the lung or other organs. MRI allows both morphological and functional assessment, providing it is conducted according to an adequate protocol. At this point, it is worth emphasizing that diagnostic success is not dependent on the possibility of performing the test, i.e. access to appropriate hardware and software, but primarily depends on the substantive preparation of the person who carries out and evaluates the test.

In conclusion, the ATS panel of experts adopted one strong recommendation regarding the evaluation of serum calcemia, 13 conditional recommendations and one recommendation resulting from clinical practice ("best practice statement"). The strength of the recommendation regarding the assessment of serum calcemia is of great practical importance as significant hypercalcemia may be a life-threatening condition. Therefore, such assessment should take place in every patient at the stage of initial diagnosis, as well as in further monitoring of disease course. One should always regard a biopsy confirmation of sarcoid granuloma as a diagnostic standard, considering not only mediastinal lymph nodes as a biopsy site but also other techniques like bronchial biopsy, transbronchial lung biopsy, and other easily accessible locations like peripheral lymph nodes. Only in patients with highly suggestive clinical symptoms and typical radiological features, the biopsy may be postponed under the condition that close monitoring of disease course may be secured. The lack of a recommendation regarding the necessity of performing lymph node biopsies in a patient with asymptomatic mediastinal lymphadenopathy reflects the lack of sufficiently valuable evidence to support its performance in all patients. Regression of changes in subsequent imaging exams and a stable radiological image over a 2-year period exempt from the obligation of biopsy. In a situation when we decide to discontinue a biopsy, it is necessary to inform the patient about the need of systematic monitoring of the disease course. The patient should receive information on the symptoms that may indicate the need for deepening and reevaluation of the diagnosis.

The authors agree with the position of ATS experts that there is an urgent need for higher quality evidence that would support clinical practice in the diagnosis of sarcoidosis, which would help to better understand and define the natural course of the disease. Nevertheless, the document published by ATS is a valuable proposition of the current standard of sarcoidosis diagnosis, based on an objective analysis and currently available data. Despite having ATS recommendations, local societies should undertake an effort of preparing similar documents, asking more detailed and specific questions that would consider local conditions.

Conflict of interest

None declared.

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Karina Jahnz-Różyk[1], Małgorzata Czajkowska-Malinowska[2], Katarzyna Krenke[3], Dorota Sands[4], Halina Batura Gabryel[5], Paweł Kabicz[6], Emilia Diaków[6], Barbara Więckowska[6]

[1]Military Institute of Medicine, Warsaw, Poland
[2]Department of Pulmonary Diseases and Respiratory Failure, Center of Pneumonology, Bydgoszcz, Poland
[3]Department of Pediatric Pneumonology and Allergy, Medical University of Warsaw, Poland
[4]Cystic Fibrosis Department Institute of Mother and Child, Warsaw, Poland
[5]Departemnt of Pulmonology, Allergology and Lung Oncology, Poznan University of Medical Sciences, Poznan, Poland
[6]Department of Analyses and Strategies, Ministry of Health, Poland

Analysis of hospital management of chronic respiratory diseases in light of the "Maps of Health Needs" project in Poland

Abstract

Introduction: The "Maps of Health Needs" project has been carried out in Poland since 2016 and its purpose is to implement quality-promoting and organisational solutions in the Polish healthcare system. This paper is the analysis of hospitalisations for chronic respiratory diseases recorded in Polish National Health Fund databases in 2014.

Material and methods: The study included 122,000 hospitalisations of adults and 22,000 hospitalisations of children. Epidemiological parameters (incidence and prevalence) and major hospitalisation parameters were determined through statistical analysis.

Results: The highest registered incidence was observed in asthma patients (548 per 100,000 inhabitants) followed by COPD patients (233 per 100,000 inhabitants). Asthma patients were also characterised by the highest prevalence, with lower values being observed in COPD patients. In the group of adults, patients aged 65 years or older and 80 years or older accounted for 44% and 14% of hospitalised adults respectively. The analysis also revealed that 66% of hospitalisations of adults included patients with asthma, COPD and respiratory failure. The development of respiratory failure prolongs hospitalisation and increases both in-hospital and post-discharge mortality. In children, 90% of the identified hospitalisations were for asthma, chronic inflammatory lung diseases and cystic fibrosis.

Conclusions: The results of the study demonstrate that pulmonary obstructive diseases are associated with a considerable burden. Therefore, corrective actions within the Polish healthcare system are required to decrease the number of hospitalisations for these diseases.

Key words: Maps of Health Needs, hospitalisations, chronic respiratory diseases

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Introduction

According to the "European Lung White Book" 600,000 people die from respiratory diseases in Europe each year, meaning that one in eight deaths is caused by them [1]. A total of 6 million hospitalisations due to respiratory diseases are recorded each year.

Epidemiological studies have shown that at least 1/3 of the population aged 5–80 years is at an increased risk of asthma, a lifetime risk for developing COPD in smokers is 40–50%, and lung cancer causes 20% of cancer-related deaths [1]. Until recently, cystic fibrosis (CF), a multi-organ genetic disease, was considered a childhood disease. Currently, however, as many as 42% of CF patients survive until the age of 18, and 5% — until 40 years of age.

The "Maps of Health Needs" project (www.mpz.mz.gov.pl/), co-financed by the European Social Fund (ESF) as part of the Operational Programme Knowledge Education Development, is a project whose goals to implement quality-promoting activities and organisational solutions in

Address for correspondence: Karina Jahnz-Różyk, Military Institute of Medicine, Warsaw, Poland; e-mail: kjrozyk@wim.mil.pl
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the Polish healthcare system are in line with the trend of health promotion, in accordance with the Ottawa Charter [2].

The project resulted in the creation of a system for mapping health needs within 30 groups of diseases and in the development of a base for systemic and implementation analyses within this scope.

The aim of this study was to analyse hospital management of selected chronic respiratory diseases.

Material and methods

Study population

The study population comprised all patients in Poland in whom a hospitalisation was reported in 2014 due to the following diagnoses (ICD-10) being part of chronic respiratory diseases:

1. Asthma (J45, J46);
2. COPD (J43, J44);
3. Cystic fibrosis (CF) (E84);
4. Interstitial lung disease (ILD) (D86, J60–J67, J70, J82, J84, J99);
5. Sleep-disordered breathing (SDB) (G47);
6. Chronic inflammatory lung diseases (J40–J42, J47);
7. Respiratory failure (J96).

Patients treated in the drug programme were excluded from the analysis of asthma. While an additional group of persons hospitalised for lung cancer was distinguished, it was not further analysed in detail since these patients often undergo diagnostic evaluation in respiratory wards, but in the Polish healthcare system they are treated in surgical and cancer wards.

Data collection

The data used in this study were derived from the payment reconciliation database of the public payer in the Polish healthcare system [NFZ (Narodowy Fundusz Zdrowia) — National Health Fund]. In Poland, payments for healthcare services are settled by the NFZ in accordance with the principles of Diagnosis-Related Groups (DRGs). In cases of non-surgical treatment, classifications into appropriate groups are primarily based on the ICD-10 diagnosis.

In addition to providing information about the diagnosis and the healthcare product paid for, the analysed database contained the following information used to create aggregated data concerning individual diseases of the respiratory system:

— Patient ID (enabling the same patient to be identified during different hospitalisations), including age, sex, and address;
— Type and date of admission; type and date of hospital discharge;
— Comorbidities;
— Healthcare provider's ID and geographical location;
— Type of ward providing hospitalisation;
— ICD-9 code procedure reported;
— Post-discharge mortality rate, which was determined using the database of deaths by the Ministry of Internal Affairs and Administration.

Statistical analysis

Statistical analysis was conducted using R software, version 0.99.902, and was mainly based on the data.table package.

Epidemiological statistics included the following parameters:

— Registered incidence — defined as the number of new patients with a specific diagnosis appearing in the public healthcare system. This parameter was calculated for 2014 on the basis of the NFZ data covering the period between 2009–2014;
— Registered prevalence — defined as the number of individuals with a specific disease, estimated as from 31 December 2014. Individuals with a specific disease were defined as all patients classified as new cases of that specific disease in the public healthcare system since 2009, who were still alive on 31 December 2014.

This publication also includes an analysis of hospitalisation-related parameters, such as:

— Number of hospitalisations;
— Cumulative percent of the number of hospitalisations;
— Number of patients;
— Number of hospitalisations per patient;
— ALOS (average length of stay);
— MLOS (median length of stay);
— Number of patient-days of hospitalisation;
— Average patient age;
— Percentage of adult patients aged 65 years or older and aged 80 years or older (in the case of children, it is the percentage of children aged up to 1 year)
— Percentage of in-hospital deaths;
— 30-day post-discharge mortality;
— 90-day post-discharge mortality.

Results

The population analysed in detail consisted of 122,000 adult patients [55,000 women and 67,000 men; mean age — 64 years; median

Table 1. Registered incidence and prevalence (2014)

	Registered incidence [thousand]	Registered incidence per 100,000 inhabitants	Registered prevalence [thousand]	Registered prevalence per 100,000 inhabitants
Asthma	209	548	1854	4878
COPD	89	233	730	1920
ILD	19	51	136	357
SDB	20	53	122	320
Chronic inflammatory lung diseases	73	191	612	1611
Respiratory failure	22	56	61	161

COPD — *chronic obstructive pulmonary disease;* ILD — interstitial lung disease; SDB — sleep-disordered breathing

age — 65 years; standard deviation (SD) — 15.3] and 22,000 children (aged up to 18 years; mean age — 6 years; median age — 5 years; SD — 4.6). The patients aged 65 years or older accounted for 44% (63,000) of the analysed group, and those aged 80 years or older — 14% (20,000).

Nearly half of the hospitalised patients (48.6%) were in lung disease and tuberculosis wards.

Incidence and prevalence

The highest registered incidence values were observed in patients with asthma, and the lowest ones in individuals with respiratory failure (Table 1). The highest registered prevalence values were also observed in patients with asthma, followed by patients with COPD and patients with chronic inflammatory lung diseases.

Hospitalisations of adults

The patients with COPD, respiratory failure and asthma accounted for 66% of the hospitalisations (Table 2). The subjects with COPD were characterised by the highest number of hospitalisations, the highest number of hospitalisations per patient (1.2) and the highest mean patient age, while as many as 29% of the hospitalised patients were those aged 85 years or older. The development of respiratory failure increased ALOS to 11.4 days.

The highest ALOS (12 days) was observed in the group of patients with CF, while the average number of hospitalisations per patient with CF was 2.2. This was the youngest and the smallest group among all hospitalised persons.

Hospitalisations of children

The cumulative percent of the number of hospitalisations showed that the reason for admission to hospital was asthma, chronic inflammatory lung diseases and CF in 90% of the hospitalised children (Table 3). The highest number of hospitalisations per patient was observed in patients with CF. The highest ALOS was found in patients with respiratory failure.

Deaths in adults

The development of respiratory failure led to 23% of in-hospital deaths, while 30% of the patients died within 30 days and 36% within 90 days after hospital discharge (Table 4). In the case of COPD patients, in-hospital deaths constituted 3%, whereas 6% of the patients died within 30 days and 10% within 90 days after hospital discharge (Table 4).

Deaths in children

The development of respiratory failure in children was also associated with the highest in-hospital mortality (5%), while 7% and 9% of them died within 30 and 90 days after hospital discharge, respectively. With regard to CF patients, 1% of deaths within 30 days after hospital discharge was observed.

Discussion

This publication discusses the burden of hospitalisations for chronic respiratory diseases on the Polish healthcare system.

The analysed data concerned hospitalisations for which the diagnosis of chronic respiratory disease was the first to be reported with an ICD-10 code.

It should, however, be emphasised that the method and order of ICD-10 coding may be the source of a reporting error [3] — an example can be the reporting of pneumonia in a patient with COPD using only one code.

Table 2. Hospitalisations in adults

Adults	Number of hospitalisations	Cumulative percent of the number of hospitalisations*	Number of patients	Number of hospitalisations per patient	Number of patient-days	ALOS	MLOS	Mean patient age	Percentage of patients aged 65+	Percentage of patients aged 80+
COPD (J43, J44)	43,134	28%	35,957	1.20	350,512	8.1	7	70.3	86%	29%
Respiratory failure (J96)	29,514	47%	25,004	1.18	335,893	11.4	8	69.1	81%	29%
Asthma (J45, J46)	28,870	66%	24,586	1.17	207,276	7.2	7	59.5	49%	13%
ILD (D86, J60–J67, J70, J82, J84, J99)	18,670	78%	13,751	1.36	126,242	6.8	5	56.8	46%	10%
SDB (G47)	17,816	90%	15,243	1.17	24,240	1.4	1	54.8	25%	1%
Chronic inflammatory lung diseases (J40–J42, J47)	14,425	99%	13,340	1.08	112,113	7.8	7	65.4	61%	19%
CF (E84)	838	100%	381	2.20	10,074	12.0	13	25.2	1%	0%

*Sum of the percentage of each category from the top of the table to the bottom, designed to total it up to 100 percent

CF — cystic fibrosis; COPD — chronic obstructive pulmonary disease; ILD — interstitial lung disease; SDB — sleep-disordered breathing

Table 3. Hospitalisations in children

Children	Number of hospitalisations	Cumulative percent of the number of hospitalisations*	Number of patients	Number of hospitalisations per patient	Number of patient-days	ALOS	MLOS	Mean patient age	Percentage of children aged up to 1 year
Asthma (J45, J46)	17,508	71%	15,760	1.11	63,922	3.7	3	7.2	8%
Chronic inflammatory lung diseases (J40–J42, J47)	2,705	82%	2,577	1.05	11,417	4.2	3	4.6	25%
CF (E84)	1,805	90%	1,055	1.71	10,342	5.7	2	8.1	29%
Respiratory failure (J96)	1,372	95%	1,163	1.18		13.1	3	4.3	47%
ILD (D86, J60–J67, J70, J82, J84, J99)	773	98%	665	1.16	5,162	6.7	6	4.5	39%
SDB (G47)	409	100%	386	1.06	616	1.5	1	7.6	6%

*Sum of the percentage of each category from the top of the table to the bottom, designed to total it up to 100 percent

CF — cystic fibrosis; COPD — chronic obstructive pulmonary disease; ILD — interstitial lung disease; SDB — sleep-disordered breathing

Table 4. Analysis of deaths in adults

Adults	Number of patients	Number of in-hospital deaths	In-hospital deaths as a percentage of all deaths	30-day post-discharge mortality	90-day post-discharge mortality
Asthma (J45, J46)	24,586	146	1%	1%	2%
COPD (J43, J44)	35,957	1,304	3%	6%	10%
CF (E84)	381	8	1%	2%	4%
ILD (D86, J60–J67, J70, J82, J84, J99)	13,751	366	2%	4%	6%
SDB (G47)	15,243	2	0%	0%	0%
Chronic inflammatory lung diseases (J40–J42, J47)	13,340	115	1%	3%	5%
Respiratory failure (J96)	25,004	6,684	23%	30%	36%

CF — cystic fibrosis; COPD — chronic obstructive pulmonary disease; ILD — interstitial lung disease; SDB — sleep-disordered breathing

The highest registered incidence was observed in patients with asthma, followed by subjects with COPD. Patients suffering from asthma were also characterised by the highest prevalence, while lower values were observed in individuals with COPD.

The cumulative percent of the number of hospitalisations showed that asthma, COPD and respiratory failure were responsible for 66% of hospitalisations of adults. The highest number of adult patients was hospitalised for COPD. Regardless of the underlying disease, the development of respiratory failure leads to the deterioration of the prognosis, prolongation of hospitalisation and increase in both in-hospital and post-discharge mortality.

Asthma, chronic inflammatory lung diseases and CF accounted for 90% of children's hospitalisations. Asthma was the reason for the highest number of hospitalisations. In children with CF, there was a relatively lower number of hospitalisations per patient. Respiratory failure prolonged the length of hospital stay significantly and was associated with higher mortality.

Respiratory system diseases are a complex, medical and economic problem in every country [1, 4–6]. The analysis of respiratory diseases, conducted in the United Kingdom, demonstrated that 12.7 million British individuals had a history of asthma, COPD or another chronic respiratory disease [6]. It is widely accepted that 50% of deaths and 25% of hospitalisations are caused by tobacco-related diseases, including lung cancer or COPD.

In 2011, a total of 694,000 hospitalisations were reported, 8% of which were for diseases of the respiratory system. Respiratory system diseases were the reason for 703,116 (8.3%) of hospitalisations, which translated into 6,167,509 patient days (10%).

The authors of the publication noted that the patient age significantly affected the percentage of hospitalisations in relation to a given diagnosis. Therefore, in children aged up to 14 years, 23.4% of the hospitalisations resulted from congenital anomalies and respiratory complications associated with them — 18% were for asthma and 1.9% were for CF.

At ages 15–64 years, asthma accounted for 15.5% of the hospitalisations, COPD — 18.2%, CF — 3.1%, and lung cancer — 7.1%. In patients aged 65 years or older, asthma accounted for as little as 2.2% of the hospitalisations, COPD — 26.5%, and lung cancer — 7.8%.

The number of hospitalisations for COPD was 43,134, which corresponded to 350,512 patient days, while ALOS was 8.1 days. In the UK analysis, there were 204,798 hospitalisations (2.4%), which corresponded to 2,346,324 (3.8%) patient days [6].

Many foreign analyses point to the burden of hospitalisations for COPD on healthcare systems even though incidence and hospital morbidity rates vary considerably. For instance, Oceania and, particularly, Australia and New Zealand, have a more considerable burden of hospitalisation than Poland [7, 8].

In New Zealand, the burden of hospitalisations for COPD varies by region and ranges from 410 per 100,000 inhabitants to 1,144 per 100,000 inhabitants [7].

In 2010, prevalence in Australia constituted 317 hospitalisations per 100,000 inhabitants [8].

In the Australian and New Zealand statistics alike, there was a significant impact of socioeconomic status on hospital morbidity, longer

hospitalisations and more frequent hospital readmissions [7, 8].

It should be emphasised that patients aged 65 years or older are a significant burden on hospitals. This paper reveals that the percentage of patients aged 65 years or older as well as patients reaching 80 years or older accounted for 86% and 29%, respectively.

In addition, this study showed that hospitalisations for COPD translated into 3% of in-hospital deaths, 6% of deaths within 30 days and 10% of deaths within 90 days after hospital discharge. The development of respiratory failure increases these percentages to 23%, 30% and 36%, respectively.

The issue of increased mortality after acute exacerbations of COPD was already considered several years ago [9–11]. A previous acute exacerbation requiring hospitalisation is known to be a significant risk factor for hospital readmission, whereas the risk of death increases seven-fold [11]. The study by Groenwegen et al. found that 8% of acute exacerbations of COPD led to in-hospital deaths and 23% of such patients died within one year after hospital discharge [9]. Respiratory failure leading to hospitalisation in an intensive care unit increases the death rate within one year after hospital discharge to 35%. The risk factors include: treatment with oral corticosteroids; hypercapnia; old age.

Even though a Danish study conducted between 2002–2008 demonstrated the reduction of the annual hospitalisation rate from 460 per 100,000 inhabitants in 2002 to 410 per 100,000 inhabitants in 2008 [10], the increase in in-hospital mortality [odds ratio (OR) — 1.16] and increased post-discharge mortality rate within one year (OR — 1.12) were also observed. Increased mortality was primarily found in patients with respiratory failure and multiple comorbidities; however, the distance to the hospital and treatment with oral corticosteroids — as in the case of the study by Groenwegen et al. — were also of relevance there. Bed occupancy also contributed to the adverse outcome of treatment. It was found that the decrease in the number of hospitals and beds resulted in the centralisation of hospitalisations for more severe cases of the disease. In the authors' opinion, this organisational change improved the prehospital care of patients with COPD.

The decrease in hospitalisations by 14% was also noted after a smoking ban had been introduced in Spain [12].

In 2014, a total of 46,000 hospitalisations for asthma were reported in Poland, including 29,000 of hospitalised adults and 17,600 of hospitalised children. Hospital morbidity rates constituted 92 per 100,000 inhabitants in adults and 253 per 100,000 inhabitants in children. In adults, the majority of the hospitalisations (18,000) were in lung disease and tuberculosis wards, followed by internal medicine wards (7,500) and allergy wards (3,500). The median lengths of stay varied by province and ranged from 3 to 6 days. Five percent of adult patients with asthma were hospitalised for more than 14 days. Acute severe asthma (status asthmaticus) (J46) was reported in the patients with asthma and ranged from 1% in the Kuyavian-Pomeranian Voivodeship to 13% in the Łódź Voivodeship.

This analysis merely shows the burden of asthma on the Polish healthcare system, which has been revealed by reporting activities, and may contain some errors due to incorrect diagnosis and incorrect reporting of an obstructive respiratory disease (asthma, COPD or coexistence of both). It is also important to differentiate between a hospitalisation for diagnostic evaluation of a disease and an acute exacerbation requiring hospitalisation. The unavailability of specialist outpatient care in the patients' local health centres often leads to unnecessary hospitalisations.

It should be noted that the number of patients registered in the NFZ databases has been relatively constant since 2009, with a predominance of women (115%) [13]. Approximately 55–57% of the registered patients live in urban areas, and 43–45% live in rural areas.

The GINA (Global Initiative for Asthma) document provides extensive information that suggests the possibility of controlling the disease outside the hospital setting (GINA) [5]. It is believed that the number of hospitalisations for asthma is proportionate to decreased disease control [14]. A considerable number of factors affects asthma control, including as follows: the lack of a correct diagnosis, undertreatment, patients' non-compliance, the lack of or non-adherence to a treatment plan.

In Australia, for instance, a 10% reduction in the number of hospitalisations was observed in 2015 when compared to 2010. In 2015, hospital morbidity averaged 1.85 per 1,000 inhabitants (1.93 per 1,000 inhabitants in urban areas and 1.64 per 1,000 inhabitants in rural areas). The authors point out to the fact that in epidemiological studies, optimisation of asthma treatment requires taking age, sex and place of residence under consideration. They stress the role of

primary care regarding the coordinated care of patients with asthma.

In Portugal, hospitalisations for asthma accounted for 2.5 per 1,000 hospitalisations, 28.1 per 100,000 inhabitants (66.6 per 100,000 individuals aged below 19 years) [16]. In-hospital deaths were estimated at 8 per 1,000 hospitalisations, corresponding to 2.4 per 1,000,000 inhabitants. Mortality statistics remain unchanged.

Hospitalisations of children with asthma merit a separate discussion. The diagnosis of asthma is more difficult to make in a child than in an adult for various reasons, which include the multiplicity of non-specific symptoms in the former. Many asthma phenotypes, including the wheezing phenotype, are particularly difficult to differentiate from asthma in this group of patients. Wheezing is a common symptom (especially in younger children) of many respiratory diseases, which causes that it is particularly difficult to differentiate it from asthma. It is often the case that children are hospitalised for bronchitis or bronchiolitis (the so-called wheezing babies), which is subsequently coded as asthma.

To sum up, the problem of hospitalisation for asthma in Poland includes:

— Conditions of the healthcare system;
— No asthma control, resulting from the lack of insufficient diagnosis and undertreatment of the disease;
— Differences in the diagnosis and treatment of asthma in children;
— Incorrect diagnosis (the lack of differentiation of asthma from COPD);
— A high proportion of smokers;
— Disease coding and reporting to the NFZ;
— Differences in coverage of medicines intended for the treatment of obstructive pulmonary diseases.

It is also important to consider statistical differences depending on the country in which the analysis is conducted: in Norway, the number of hospitalisations of children decreased by 51%, while it remained unchanged in Sweden [17].

According to the "European Lung White Book," the prevalence of asthma is estimated at 1.6–20% in Europe, whereas 60% of patients with allergies are mainly allergic to animal hair, dust and mould, including 10–20% of them have the wheezing phenotype that is difficult to differentiate [1].

Due to the complex clinical presentation of CF and multidirectional treatment, the quality of care provided to CF patients, as measured by lifespan, is considered one of the best indicators of healthcare quality in developed countries [18–20].

The most recent US studies have revealed that life expectancy of people born in 2010 is 40 years for females and 37 years for males [18].

In Poland, average lifespan of CF patients is shorter than that in Western Europe and the United States. Cystic fibrosis, a disease once considered a childhood one, currently affects adults as well (who only account for approx. 1/3 of all CF patients). Patients with CF account for 1% of hospitalisations of adults (with the mean age of 25 years) and 8% of hospitalisations of children (with the mean age of 8 years).

It is thus justified to follow the example of the rest of Europe by making attempts to establish CF treatment centres and to provide CF patients with comprehensive and multidisciplinary care.

Hospitalisations for CF occur at any age and the main reason is deterioration of lung function [20]. The most common management strategy involves intravenous antibiotic therapy, cleansing of the respiratory tract through appropriate inhalation and drainage therapy combined with an appropriate diet. Pulmonary rehabilitation is the key to the beneficial outcome of hospitalisation.

The registry of CF patients in the United States included 21,488 patients in 2003 and 28,134 in 2013, as well as collected data on hospitalisations [18]. The number of hospitalisations per 1,000 CF patients increased from 994 to 1,072 in 2013. During hospitalisation, 1.5% of the patients aged 27 years on average died (MLOS — 7 days). Between 2003 and 2013, mortality decreased from 1.9% to 1.2%. The risk factors included: chronic liver disease, acute kidney disease, and previous lung transplantation (6.5% of the hospitalisations). Keeping registries of CF patients contributes to improved patient care and to the pursuit of novel — often individualised — treatments.

Conclusions

This publication serves as evidence that hospitalisations for asthma and COPD, especially those complicated by respiratory failure, constitute a serious burden on the Polish healthcare system. In accordance with the project assumptions, organisational and implementation-related actions have to be taken to reduce this burden.

Conflict of interest

None declared.

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Mihan Pourabdollah Toutkaboni[1], Mehrdad Farahani[2], Abdolreza Sadegh[3], Arda Kiani[4], Makan Sadr[5], Kimia Taghavi[6], Atefeh Abedini[6]

[1]Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Interventional Pulmonology, Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]Virology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[6]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

The incidence of mTOR marker in tracheal adenoid cystic carcinoma by immunohistochemical staining

Abstract

Introduction: There is an association between the activation of mammalian target of rapamycin (mTOR) signaling and aggressive tumor growth in multiple forms of cancer, including *adenoid cystic carcinoma* (ACC). ACCs are uncommon yet a malignant form of neoplasms that arises within the secretory glands. Therefore, the aim of this study was to investigate the increase of mTOR in the ACC tumors in order to survey the possibility of treating these tumors with mTOR inhibitors.

Material and methods: Samples from known cases of the lung and tracheal ACC were retrieved from the archives of the pathology department of Masih Daneshvari hospital, and immunohistochemical (IHC) staining for mTOR was performed on them. After preparation of the blocks with specific antibodies, tumor cells with cytoplasmic and/or nuclear expression of mTOR were considered as positive cells by applying a specific scoring method introduced in this study.

Results: The paraffin blocks of 26 patients were surveyed and the IHC marker of mTOR was positive in the tumors of 10 patients (38.5%). Out of 10 mTOR positive cases, 5 were females and 5 were males. *The primary site* of the surveyed tumors was the trachea and bronchus in 12 cases (46%), salivary glands in 7 individuals (27%), and lung tissue in 7 cases (27%), and there was no significant correlation between *the primary site* of the ACC tumors and the existence of the mTOR markers in them ($P = 0.67$). From all cases, 13 patients (50%) had cribriform and tubular cells without solid components, 9 cases (34.6%) had cribriform and tubular with less than 30% of solid components, and 4 cases (15.4%) had cribriform and tubular cells with more than 30% of solid components. There was no significant difference between the morphologies and the existence of mTOR markers in them ($P = 0.741$).

Conclusions: As the incidence of mTOR markers is seen in patients with tracheal ACC, evaluation and scoring of mTOR in these persons can be helpful as further studies can distinguish the use of it in the treatment of the disease.

Key words: immunohistochemistry, adenoid cystic carcinoma, mTOR

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Introduction

The mammalian target of rapamycin (mTOR) as a serine/threonine kinase belonging to the phosphoinositide 3 kinase-related kinase (PIKK) family is expressed in most mammalian cells to control growth and metabolism [1–5]. The mTOR1 and mTOR2 complexes are involved in normal cell growth and developmental process and are crucial for its viability by regulating the

Address for correspondence: Atefeh Abedini, Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran; e-mail: dr.abedini110@sbmu.ac.ir
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kinases of the AGC family, protein synthesis and autophagy [6]; accordingly, their dysregulation is implicated in the pathophysiology of several diseases, including cancer, type 2 diabetes, and neurodegeneration [7].

There is a correlation between the activation of mTOR signaling and aggressive tumor growth in many cancers [8]. As a matter of fact, after the hyperactivation of mTOR signaling, the tumors grow at an increased rate and they are less likely to disappear because of an increased amount of protein synthesis and inhibition of autophagy [9, 10]. Also, the activated mTOR pathway helps proliferation, migration, and survival of tumor cells, which increases the tumor invasiveness [11]. In addition, this pathway reduces the tumor cells sensitivity to chemotherapy and hormonal treatment [12]. Hence, inhibition of its activity increases the chemotherapy effectiveness and improves the outcome of the treatment of these tumors [13]. The natural inhibitor of mTOR is rapamycin. Temsirolimus and everolimus are other important inhibitors whose effectiveness in the treatment of different cancers has been proven [13, 14].

Adenoid cystic carcinoma (ACC) as an aggressive neoplasm of salivary glands is the second most common primary malignancy of tracheal neoplasm, in whose pathophysiology the mTOR pathway may be involved [15]. Although the incidence of this tumor is low, about 90% of them are malignant [16]. The treatment of the patients with ACC includes surgical resection of the tumors with their margins and reconstruction, chemotherapy, and radiotherapy [17], and as mentioned, if the marker of the mTOR pathway is high in these tumors, using the mTOR inhibitors may increase the chemotherapy effectiveness. The aim of this study was to investigate the incidence of mTOR in the ACC tumors in order to survey the possibility of treating them with mTOR inhibitors.

Material and methods

The samples of the Pathology department of Masih Daneshvari hospital were searched for ACC tumors of the trachea with diagnosis confirmed between 2010 and 2014, with the approval of the institutional review board. Then, paraffin blocks of known cases of the ACC of the lung and trachea (primary and metastatic carcinoma) were retrieved from the archives of the department and reviewed by two different pathologists without knowledge of the initial diagnosis.

Immunohistochemistry

Formalin-fixed paraffin blocks of known cases of the ACC of the lung and trachea were used in our study. In brief, following dewaxing, washing and rehydration of the slides through xylene and graded alcohols, microwave heating in citrate buffer were used for antigen retrieval. Endogenous peroxidase was blocked in ChemMate peroxidase-blocking solution (Dako). Phospho-mTOR (Ser2448) — Cell Signalling #2976, monoclonal rabbit, dilution 1:50 was the main antibody and EnVision™ Dual Link System (Dako) was used as a detection system [18].

A positive immunohistochemical reaction was indicated by brown cytoplasmic and/or nuclear staining. Cases were scored on the basis of the visually estimated percentage of tumor cells with positive cytoplasmic and/or nuclear staining.

Scoring

After the staining, tumor cells with cytoplasmic and/or nuclear expression of mTOR were considered as positive cells according to the scoring method presented below:

1. In the regions with the highest density of positive cells for each slide, the percentage of positive cells in 5 separate fields which had ≥1000 adjacent cells were counted and scored as: percentage of positive cells less than 1%: 0, between 1% and 25%: 1, 26% to 50%: 2, 51% to 75%: 3, 76% to 100: 4 (Table 1).
2. The color intensity of the stained blocks was scored based on color intensity as follows: lack of brown 0, mild: 1, moderate: 2, severe: 3 (Table 2).
3. The two obtained points for each tissue were multiplied to calculate the final scores (maximum score: 12) and the final scores were categorized into: 0-1: negative for mTOR, 2-3: weakly positive for mTOR, 4-6: positive

Table 1. Scoring of the percentage of positive cells

The percentage of positive cells	Score
< 1%	0
1–25%	1
26–50%	2
51–75%	3
76–100%	4

for mTOR, 7–12: strongly positive for mTOR (Table 3).

4. For statistical analysis, the samples were divided into two groups: positive for mTOR marker (final score > 3) and negative for mTOR marker (final score ≤ 3).

Statistical analysis

The data was analyzed using SPSS V22 software and an ANOVA test followed by a Chi-square test, a T-test and a Fisher's exact test.

Table 2. Scoring of the color intensity of the tissue fragments

Color intensity	Score
Lack of brown	0
Mild	1
Moderate	2
Severe	3

Table 3. The existence of mTOR markers according to the final scores

Final score	The existence of mTOR
0, 1	Negative for mTOR
2, 3	Weakly positive for mTOR (+)
4–6	Positive for mTOR (++)
7–12	Strongly positive for mTOR (+++)

Results

In the study, a total number of 32 patients with adenoid cystic carcinoma were reviewed by pathologists. Six patients were excluded due to the prior history of radiotherapy. Finally, the paraffin blocks of 26 subjects were surveyed (Table 4) and the immunohistochemistry (IHC) marker of the mTOR expression was positive in the tumors of 10 patients (38.5%). From 10 mTOR positive cases in immunohistochemistry surveys, 5 were females and 5 were males. Also, negative cases for mTOR were seen equally among men and women. Therefore, there was no difference between men and women in the existence of mTOR markers in the ACC tumors (Table 4).

The mean age of patients was 53.12 ± 15.3 and 57.7% of them (n = 15) had a mean age of 50 years or older (Table 4). There was no significant correlation between the existence of mTOR markers in ACC tumors and the age of the patients (P = 0.48).

The primary site of the surveyed tumors was the trachea and bronchus in 12 cases (46%), salivary glands in 7 cases (27%), and lung tissue in 7 cases (27%) (Table 4). The frequency of mTOR positive patients based on *the primary site* of the tumors are summarized in Table 5. The percentage of mTOR positive cases in tracheal and bronchial tumors was 41.7%, in salivary gland tumors was 28.6%, and in lung tumors was 42.8%. In the study, there was no significant correlation between the primary site of the ACC tumors and the existence of mTOR markers (P = 0.67).

Table 4. Demographic information, tumor location, mTOR existence and morphology of tumor cells

Variable		Number of ACC	Percentage	P value*
Gender	Male	13	50	1
	Female	13	50	
Age	< 50 years old	11	42.3	0.48
	> 50 years old	15	57.7	
The primary site of the tumors	Trachea and bronchus	12	46	0.67
	Salivary glands	7	27	
	Lung	7	27	
Type of tumor	Primary	20	77	0.457
	Metastatic	6	23	
mTOR existence	Positive	10	38.5	N/A
	Negative	16	61.5	
Morphology	Morphology**	13	50	0.741
	Morphology***	9	34.6	
	Morphology****	4	15.4	

*P value for comparing variables based on the existence of mTOR marker; **cribriform and tubular cells without solid components; ***cribriform and tubular with less than 30% of solid components; ****cribriform and tubular cells with more than 30% of solid components

Table 5. The frequency of mTOR positive cases based on the primary site of the tumors

IHC survey	Trachea and bronchus	Percentage	Salivary glands	Percentage	Lung	Percentage	P value
Positive	5	41.7	2	28.6	3	42.8	0.67
Negative	7	58.3	5	71.4	4	57.2	
Total	12	100	7	100	7	100	

IHC — immunohistochemical

Table 6. The frequency of mTOR positive cases based on their type

IHC survey	Primary tumors	Percentage	Metastatic tumors	Percentage	P value
Positive	8	40	2	33.3	0.457
Negative	12	60	4	66.6	
Total	20	100	7	100	

IHC — immunohistochemical

Among these tumors, 20 tumors (77%) were the primary tumors and only 6 of them (23%) were metastases from other sites (4 cases with metastasis from salivary glands and 2 cases with metastasis from the trachea) (Table 4). From the mTOR positive cases, 8 of them (40%) were primary tumors and only 2 of them (33.3%) were metastatic tumors (Table 6). There was no significant difference between metastatic or primary tumors in the existence of mTOR markers (P = 0.457).

In terms of the morphology of tumor cells, 13 cases (50%) had cribriform and tubular cells without solid components (morphology 1), 9 cases (34.6%) had cribriform and tubular with less than 30% of solid components (morphology 2), and 4 cases (15.4%) had cribriform and tubular cells with more than 30% of solid components (morphology 3). There was no significant difference between different morphologies in the existence of mTOR markers (P = 0.741). Different morphologies of the ACC tumor cells are shown in Figure 1.

Also, in the study, we investigated the site of the staining (Figure 2) in the tumor cells (cytoplasmic, nuclear, or both cytoplasmic and nuclear) based on the three types of morphology - the results are summarized in Table 7. Out of 10 patients who were considered positive (3 < final score), 4 had nuclear staining, 4 had cytoplasmic staining and 2 had both nuclear and cytoplasmic staining. Also, out of the 16 patients who were considered negative (3 > final score), 8 had no staining, 7 had cytoplasmic staining, 1 had both nuclear and cytoplasmic staining, but none had nuclear staining.

Discussion

This study was conducted to investigate the incidence of mTOR in the paraffin blocks of the patients with ACC tumors using immunohistochemistry technique. According to our surveys, the IHC marker of mTOR expression was positive in the tumors of 10 cases (38.5%) out of the 26 surveyed. In a similar study by Wang Li *et al.*, the mTOR marker in non-small cell lung cancer in 43.5% of 78 patients was positive [19]. Also, in the study by Meiling Wen *et al*. about the importance of mTOR expression in patients with colon cancer, out of 106 subjects with this cancer, 80 patients (75.5%) were mTOR positive [20]. It seems that the differences can be related to the different sample sizes, which suggests the need for more studies in this regard with larger sample sizes.

In addition, out of 10 mTOR positive patients in this study, 5 were females and 5 were males, and there was no difference between men and women in the expression of mTOR. The mean age of the patients in the study was 53.12 years and there was no significant correlation between the existence of mTOR markers and the age of the patients (P = 0.48). These results are consistent with the outcomes of the study by Wang *et al*. on the expression of mTOR in 210 patients with lung cancer and the results of the study of Meiling Wen *et al*.; in both studies, it has been shown that there is no significant relationship between the expression of mTOR and age and sex [19, 20].

In our study, 20 patients with ACC (77%) had primary tumors and the mTOR marker was

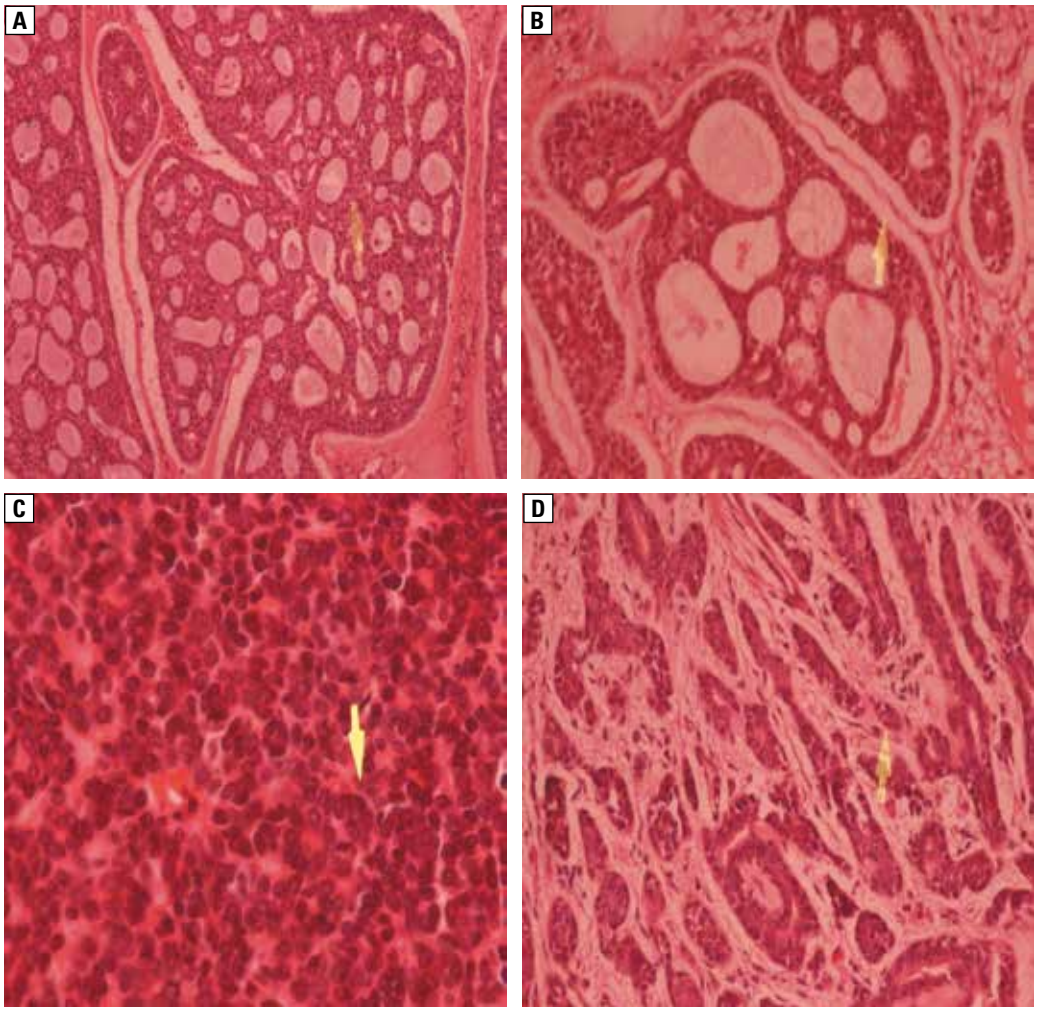

Figure 1. Different morphologies of the ACC tumor cells. **A.** and **B.** — cribriform; **C.** — solid; and **D.** — tubular

positive in 8 of them (40%), 6 patients had a metastatic tumor, and the mTOR marker was positive in 2 cases (33.3%). Hence, there was no significant difference between metastatic or primary tumors in the existence of mTOR markers (P = 0.457), although more studies with larger sample sizes are required.

The ACC tumors were divided into three groups according to the morphology: cribriform and tubular without solid components, cribriform and tubular with less than 30% of solid components, and cribriform and tubular with more than 30% of solid components [21]. Of the 26 patients, 13 cases were in the first group with 6 (42.8%) mTOR positive patients, 9 in the second group with 3 (37.5%) mTOR positive cases and 4 in the third group with 1 (25%) mTOR positive case. Although this difference is not statistically significant in our study, it seems that there is a reverse correlation between the percentage of solid components and the existence of mTOR markers, and according to the direct correlation between the high percentage of the solid component and the grade of cancer, it can be deduced that more invasive ACCs with higher grades have lower incidence of mTOR markers. This is consistent with the results of the study by Wang Li *et al*. that has shown that low-grade and moderate-grade neuroendocrine tumors have a higher incidence of mTOR markers than high-grade tumors [19].

Although the incidence of tracheal ACC tumors is low, about 90% of them are malignant [22], and their diagnosis is delayed due to the slow growth rate, and their atypical symptoms and patients are misdiagnosed with asthma or bronchitis [23]. The everolimus and temsirolimus are approved as anticancer drugs which targeted the inhibition of the mTOR pathway in the clinic [24].

The limitation to the study that is worth mentioning was the restricted number of cases that was due to the rare incidence of the disease. Also,

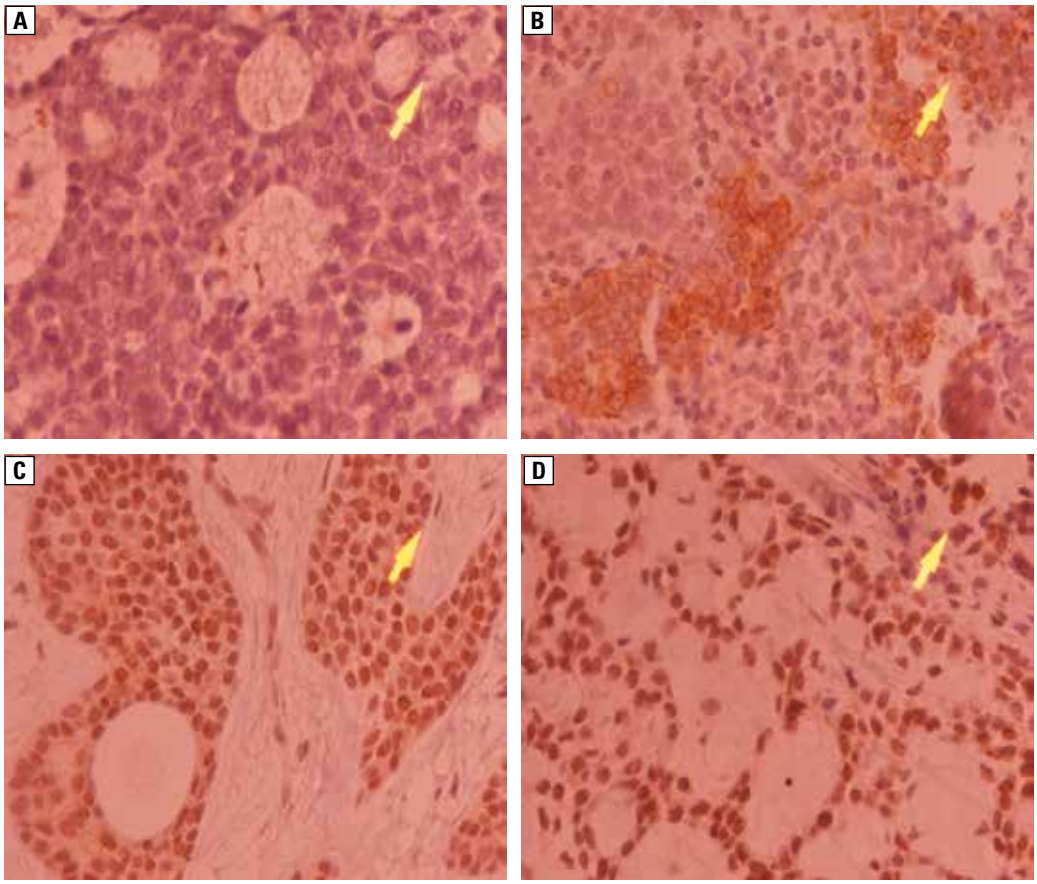

Figure 2. Different sites of the staining in the tumor cells. **A.** — negative; **B.** — mild cytoplasmic; **C.** — mild nuclear, and **D.** — moderate nuclear

Table 7. The site of the staining of tumor cells and the color intensity distinguishing between positive and negative cases

Variable		ACC			Total
		Morphology 1	Morphology 2	Morphology 3	26
The site of the staining in the positive cases	Negative	–	2	–	2
	Cytoplasmic	2	1	1	4
	Nuclear	2	–	–	2
	both cytoplasmic and nuclear	2			2
Total		6	3	1	10
The site of the staining in the negative cases	Negative	4	3	1	8
	Cytoplasmic	4	1	2	7
	Nuclear	–	–	–	–
	both cytoplasmic and nuclear	–	1	–	1
Total		8	5	3	16
Color intensity in the positive cases	Lack of brown	–	–	–	–
	Mild	–	–	–	–
	Moderate	5	4	1	10
	Severe	–	–	–	–
Total		5	4	1	10
Color intensity in the positive cases	Lack of brown	4	3	1	8
	Mild	6	–	1	7
	Moderate	–	1	–	1
	Severe	–	–	–	–
Total		10	4	2	16

ACC — adenoid cystic carcinoma

Table 8. The percentage of positive cells in slides distinguishing between positive and negative cases and final scores for all cases

Variable		ACC			Total
		Morphology 1	Morphology 2	Morphology 3	26
The percentage of positive cells in the slide for positive cases	< 1%	–	–	–	–
	1– 25%	–	–	–	–
	26– 50%	2	2	1	5
	51– 75%	1	2	–	3
	76– 100%	2	–	–	2
Total		5	4	1	10
The percentage of positive cells in the slide for negative cases	< 1%	4	3	1	8
	1–25%	4	1	1	6
	26–50%	2	–	–	2
	51–75%	–	–	–	–
	76–100%	–	–	–	–
Total		10	4	2	16
Final scores for all cases	0, 1 (–)	7	4	2	13
	2, 3 (+)	1	1	1	3
	4–6 (++)	3	4	1	8
	7–12 (+++)	2	–	–	2
Total		13	9	4	26

the study only surveyd patients of one ethnicity. Future research with larger and multiethnic data pool can better assess these findings.

Conclusion

For the first time in Iran, the IHC staining was used to investigate the mTOR pathway in the ACC tumors. The site of the staining in the tumor cells (nuclear, cytoplasmic, or both), the intensity of coloring, and the percentage of positive tumor cells were used to present a unique scoring system to be applied in the investigation of mTOR markers in ACC tumors. The mTOR can be used as a target for the treatment of the cancer, and in this study, the presence of mTOR marker in some of the ACC patients has been shown. Thus, further evaluation of using mTOR as suggestive indicator for targeted therapy of ACCs and the effectivity of mTOR inhibitors in the treatment for these tumors are recommended.

Conflict of interest

None declared.

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Stephen Allen[1], Alex Tanner[1], Ahmed Khattab[2]

[1]The Royal Bournemouth Hospital, Castle Lane East, Bournemouth, United Kingdom
[2]Bournemouth University, Fern Barrow, Poole, Bournemouth, United Kingdom

The threshold for detecting a rise in airflow resistance during tidal breathing is lower in older patients with COPD than in healthy people of similar age

Abstract

Introduction: To investigate whether or not the threshold for subjectively detecting an increase in the resistance to airflow (LDT) during tidal breathing at rest rises in older age in patients with COPD, as it does in healthy people and asthmatics in remission.
Material and methods: We conducted an open cross-sectional study of 31 older patients (age 55–89) with COPD and 60 healthy volunteers (age 55–86). Inspiratory and expiratory resistive load detection thresholds (ILDT and ELDT respectively) and spirometry were measured.
Results: The mean (SD) ILDT was 5.93 (7.02) kPa.s/L in COPD patients, compared to 11.11 (8.47) in healthy people ($P < 0.001$) in the same age range. There was no significant correlation between ILDT and age in the COPD group ($r = -0.182$, $P = 0.326$), though significant correlation with age was found in healthy people ($r = 0.591$, $P < 0.001$). ILDT and ELDT in COPD patients correlated significantly with the FEV1/FVC ratio ($r = 0.367$, $P = 0.048$ and $r = 0.481$, $P = 0.007$ respectively) but not with other spirometry indices, height, weight, BMI, oxygen saturation or smoking pack-years.
Conclusion: LDT during tidal breathing appears to be sensitized, and thereby lower, in older COPD patients, possibly due to altered central regulation of the threshold or as a consequence of the effect lung compliance, recoil and volume changes have on afferent input from mechano-receptors in COPD. Older COPD patients with good cognition are therefore likely to be as aware of changing airways resistance as younger patients and take appropriate therapeutic action.

Key words: airflow resistance sensing, ageing, spirometry, chronic obstructive pulmonary disease

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Introduction

In previous studies we showed that the threshold for the subjective detection of an increase in an incrementally applied external resistive load to airflow (LDT) during tidal breathing rises with age in a substantial proportion of healthy people and asthmatic patients in remission [1, 2]. We contended that this had implications for elderly patients who might consequently be less able to sense the early stages of a relapse in their asthma and therefore delay taking appropriate action. That argument was consistent with the commonplace clinical experience that older asthmatics tend to present later than younger patients when they are having an exacerbation. Following the same logic for the study reported in this paper we hypothesized that patients with chronic obstructive pulmonary disease (COPD) would be expected to exhibit a similar age effect unless the chronically high airflow resistance in patients with COPD results in an adaptive response leading to higher detection thresholds across the older (above 55 years) age range and thereby a loss of the progressive age-related rise seen in normal subjects and well-controlled asthmatics within the 55 to 80+ age band. This might in turn lead to a generally blunted sense of raised airflow resistance, one consequence of which could be a tendency to under-ventilate in response to rising resistance and could arguably contribute to carbon dioxide retention in some COPD patients

Address for correspondence: Stephen Allen, The Royal Bournemouth Hospital, Castle Lane East, Bournemouth, United Kingdom; e-mail: drscallen@aol.com
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[3]. There is some empirical evidence for this contention though almost all extant studies have been carried out in patients with COPD using sequential bronchoconstriction or under dynamic external airflow loading conditions [4–6]. During induced bronchoconstriction airflow resistance rises in relatively large and unpredictable increments so it is difficult to determine accurately the level of airways resistance at which a sensation of impaired airflow is first felt subjectively. Therefore, studies of that type might not provide the best insight into the influence of ageing and chronic airflow limitation on one of the most clinically relevant issues, namely whether the effect of age on airflow resistance sensing remains a factor in COPD, or is exaggerated, or is over-ridden by the neuro-physiological consequences of persistently high airflow resistance. Our method for measuring LDT was developed to address the limitations of the bronchoconstriction methods [1]. A full review of the mechanisms determining resistance sensing are outside the scope of this paper, though the background physiology and related hypotheses have been covered in depth in our previous publications [1, 2, 7]. In short, detecting subjectively an increase in airflow resistance is the result of sensing that a change has occurred in the relation between inspiratory effort and the resulting tidal volume, an example of length-tension sensing [8–10]. The dynamic central monitoring of such breath-by-breath lung volume movements must depend on sensory information arising in thoracic mechano-receptors, including those located in the diaphragm, chest wall, airways and lung parenchyma. The deterioration of proprioceptive acuity with age has been well documented for a number of functions such as postural sway, gaze stability and point relocation [11–16]. If an analogous change occurs in the accuracy and central processing of afferent mechano-receptor traffic from the diaphragm and chest wall it can be hypothesized that the resting LDT would rise progressively with age in patients above 55 years (the range within which most diagnosed COPD patients reside) and become more variable, as was observed in healthy people and asthmatics in remission [1, 2]. This was the primary hypothesis to be tested by the study presented in this paper. Therefore, we conducted an experiment to explore LDT in patients with COPD in relation to age and a number of other variables of relevance in COPD including spirometric indices and smoking exposure.

Material and methods

Study design and subject selection

We conducted a prospective open cross-sectional study. Patients with a diagnosis of COPD confirmed by pulmonologists using nationally agreed criteria [17] were invited to consent to participate. Potential participants were selected from a departmental diagnostic list. A preliminary powers calculation for comparing LDT between groups, based on a pilot study, indicated that from the 150 potential participants available for the study, and assuming a population proportion of 50% and margin of error of 20%, a sample of 30 would be the minimum for showing significant difference with 95% confidence. The selection was quasi-random as all patients with COPD on the list were initially considered before application of the inclusion and exclusion criteria and all those who accepted the invitation to participate entered the study. The inclusion criteria were: adults with a diagnosis of COPD, at least 6 weeks since any exacerbation requiring an intervention and currently back to clinical baseline, abbreviated mental test (AMT) [18] above 7, willing to consent to take part in the study. The exclusion criteria were: contra-indications to performing spirometry (such as recent eye surgery), history of significant cognitive impairment or overt dementia (unlikely to be able to perform the tests) [19], currently taking any medication that can alter respiratory sensory function or cause sedation (such as opioids or benzodiazepines), any known or apparent central or peripheral nervous system condition that might alter respiratory sensory function (such as stroke or sensory neuropathy), pulmonary co-morbidities that could alter the LDT (such as pulmonary fibrosis).

We also harvested LDT data from a study of normal people [1] to provide a comparison group across the same age range as the COPD study group and with approximately the same distribution of ages over the range. This was done after the COPD group had been recruited to enable the age range to be comparable. The normal group were all lifelong non-smokers, or ex-trivial smokers (< 1 pack-year), and all had normal spirometry indices and no history of respiratory disease or any other condition or medication that might alter their LDT measurements. They were all either university employees or staff and volunteers at our base hospital.

Measuring the LDT

We used exactly the same apparatus as in our previous studies of healthy people and asthmatic patients [1, 2], constructed with standard respiratory physiology equipment (Harvard Apparatus®). It was comprised of a flanged rubber mouthpiece connected to a unidirectional low resistance valve that was attached by a push-fit connector to an airflow resistor by a 90 cm section of low resistance respiratory tubing. The resistor could therefore be applied in either the inspiratory or expiratory mode. A disposable microbiological filter (Vitalograph®) was inserted between the mouthpiece and valve. The dead space was less than 50 ml and did not vary. In preparatory experiments [1] it was found that a resistor aperture of lentiform cross-sectional shape provided the most consistent and near-linear calibration curve over the flow range anticipated during tidal breathing. Calibration of the resistor through its operational range was carried out with the resistor incorporated in the apparatus and using pumped air at 5 litres per minute at room temperature (20–24 degrees centigrade) and ambient humidity, with flow determined by a bobbin flow meter (Rotameter®) and pressure by a water manometer using standard laboratory methods. In our previously published studies on volunteers willing to perform repeated LDT measurements we found a mean coefficient of variation of 4.3% in people under 65 years of age and 6.2% in those over 64 years of age (5 subjects performed 20 consecutive inspiratory LDT measurements in each group). We also found no significant differences between the LDT values of men and women when compared within young, middle aged and elderly age groups [1].

A detailed description of the LDT measurement procedure we developed has been published elsewhere [1, 2] and it is recommended that researchers wishing to construct a similar apparatus and to apply the technique should refer to those papers. In summary, LDT measurements were made in a quiet room with sources of distraction minimised. The patient was seated and requested to breathe tidally through the LDT apparatus wearing a nose clip and a pulse oximetry finger probe (Suaok® FS 10D). After a settling period of about 1 minute the resistor was closed, silently and out of sight, in half millimetre long-axis increments every 2 or 3 breath cycles until the subject indicated by raising a hand that they had reached the point at which they could first feel definite resistance to breathing. The aperture setting was recorded and later compared with the calibration curve to determine the measurement. Three such measurements were made in each of the inspiratory and expiratory modes. The means of the 3 readings were taken as the recorded inspiratory and expiratory LDTs (ILDT and ELDT respectively). No readings were discarded. Corrections for actual temperature, humidity and atmospheric pressure were not made on individual study days because they were found to be too small to be of significance.

Spirometry

Spirometry was performed on each participant using a desktop spirometer (NDD EasyOn-PC®). The ERS/ATS performance and interpretation standards [16] for forced spirometry were applied and recordings were made of peak expiratory flow rate (PEF), forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC). Subjects unable to have these indices measured to the required standard were not included in the data analysis. Height, weight, sex and age were recorded and used to calculate predicted values electronically using regional tables for Caucasians.

Study sequence

The order of data collection for each subject was: invitation and information, consent, AMT score, Medical Research Council Dyspnoea Scale (MRCDS) [20](used with permission of the Medical Research Council), record current medication, record clinical data (smoking history, sputum production and timing of last exacerbation), check inclusion and exclusion criteria, spirometry, LDT measurement.

Statistical testing

Some continuous data were found to be approximately normally distributed on visual assessment, though this was not clear in all cases and the Shapiro Wilk test rendered values of < 0.05 in every case. Because of the uncertain distribution and small samples, groups were compared by the Mann Whitney U test. To take account of some outlying LDT data points in the relatively small samples correlation coefficients were calculated by the Spearman method, with the Bonferroni correction for multiple testing. For categorical data Fisher's exact test was used to allow for the relatively small sample size. A significance level of 5% was adopted for group comparisons and correlations. All calculations were conducted using online software (socscistatistics.com).

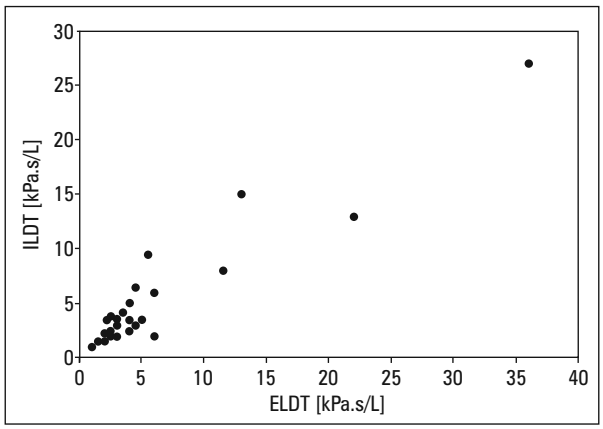

Figure 1. Correlation of ILDT with ELDT for COPD patients (N = 31, r = 0.959, P > 0.001, Spearman method). The line of identity between the axes is shown. ILDT — inspiratory airflow resistance load detection threshold; ELDT — expiratory airflow resistance load detection threshold

Results

We studied 31 COPD patients (25 men, all Caucasian) with a mean age of 72 years (median 77, range 55–89). All were smokers or ex-smokers with a mean exposure of 46 pack-years (range 15–94). They were all clinically stable and at their usual baseline at the time of the study. Their regular medication consisted of combined long-acting anti-muscarinic agent (LAMA), long-acting beta-adrenergic agonist (LABA) and corticosteroid (CS) by inhaler in 18 patients, LAMA plus LABA by inhaler in 3, CS plus either LAMA or LABA by inhaler in 6, LAMA alone by inhaler in 3 and no medication in 1. The mean AMTS was 9.5 (range 8–10), MRCDS was 2.7 (range 1–5), FEV_1 46% of predicted (range 23–59) and FEV1/FVC ratio 47.3% (range 25.5–68.3). It was confirmed that all met the spirometry criteria for COPD [17]. The mean oxygen saturation at rest breathing air was of 95% (range 88–97) and none had a fall in oxygen saturation when having their LDTs measured. Their mean body mass index (BMI) was 26 kg/m^2 (range 18.5–37.8).

The mean (SD) ILDT and ELDT values were 5.93 (7.02) kPa.s/L (range 1.5–36) and 5.19 (5.20) kPa.s/L (range 1–27) respectively, with no significant difference. Though the between-subject range was large there was close within-subject numerical concordance when comparing both indices (Figure 1) with a high degree of correlation (r = 0.959, p < 0.001) and the line of regression through the points was close to the line of identity between the axes, indicating an absence of tidal phase bias. Correlations between ILDT, ELDT and other variables are shown in Table 1. There was no significant correlation between ILDT and age (Figure 2), FEV_1, FVC, PEF, height, BMI, oxygen saturation and smoking pack-years. However, there was significant correlation between ILDT and FEV_1/FVC and between ELDT and FEV_1/FVC. No significant correlations with ILDT or ELDT emerged when FEV_1, FVC and PEF were expressed as percentages of the predicted values derived from age, height, BMI and sex. The mean (SD) ILDT of patients who produced sputum on most days (N = 18) was 5.64 (5.14) kPa.s/L and that of those

Table 1. Coefficients of correlation (r) and probability values (P) for ILDT and ELDT compared with a number of variables. (Spearman method, N=31).

	R	P	R	P
Age	-0.182	0.326	-0.164	0.377
FEV_1	0.072	0.710	0.088	0.690
FVC	0.154	0.410	0.161	0.440
PEF	0.004	0.989	0.008	0.991
FEV_1/FVC	0.367	0.048*	0.481	0.007*
Height	-0.322	0.130	-0.299	0.111
Weight	0.200	0.301	0.223	0.341
BMI	-0.099	0.616	-0.104	0.625
Oxygen saturation	0.232	0.294	0.244	0.317
Smoking pack-years	0.019	0.966	0.023	0.974

BMI — body mass index; ; ELDT — expiratory airflow resistance load detection threshold; FEV1 — forced expiratory volume in 1 second; FVC — forced vital capacity; ILDT — inspiratory airflow resistance load detection threshold; PEF — peak expiratory flow rate

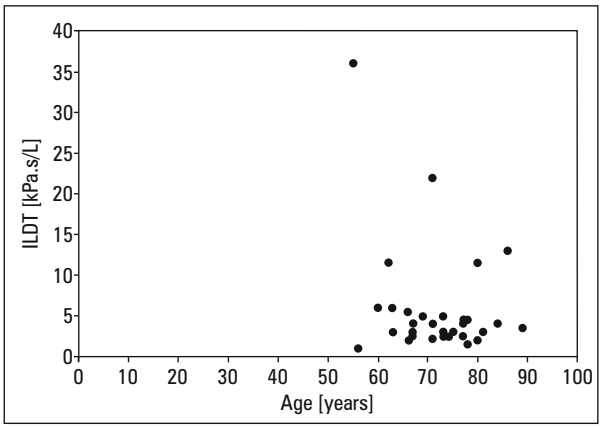

Figure 2. No significant correlation was found between ILDT and age in COPD patients (N = 31, r = -0.182, P = 0.326, Spearman method). ILDT — inspiratory airflow resistance load detection threshold

who produced sputum occasionally or never (N = 13) was 6.51 (9.32) kPa.s/L and there was no significant difference (p = 0.781) .

In the healthy comparison group (N = 60, 25 men, all Caucasian), the mean age was 70 years (median 74, range 55–86). The mean FEV_1 was 98% of predicted (range 86–117) and the mean FEV_1/FVC ratio was 77% (range 72 – 81) and was between 94 – 112% of predicted and thereby within the normal range for men and women. Their mean BMI was 27.0 kg/m^2 (range 21.0–36.5), all had an AMT score of 10 and MRCDS score of 0. There were no significant differences in the ages or BMIs of the COPD and healthy groups (P = 0.253 and P = 0.304 respectively), though there was a significantly higher proportion of women in the healthy group (P = 0.05). The mean (SD) ILDT was 11.11 (8.47) kPa.s/L which was significantly higher than in the COPD group (P = 0.005). In the healthy subjects a significant positive correlation was found between ILDT and age across the comparison age range (r = 0.589, P < 0.001)

The findings for ELDT were very similar for all comparisons.

Discussion

The findings of the study did not confirm the hypothesis we set out to test. On the contrary, we found that the mean LDTs in patients with COPD were lower rather than higher than those of the older healthy comparison group within the same age range in this study and very similar to the young and middle-aged healthy subjects in our previous study [1]. Further, there was no apparent tendency for LDTs to rise with age in the COPD group, unlike healthy control patients, across the 55 to 85 age range. There must be some caution in drawing any firm conclusions from these data, particularly the correlative relationships, because the sample was relatively small and the levels of statistical significance, or lack thereof, must be interpreted in the light of that limitation. Nevertheless, in group comparisons the clearly significantly lower mean LDT values in COPD patients compared to healthy people in the same age range is noteworthy. It appears that the physiological burden of chronically raised airways resistance does not have a blunting or down-regulatory effect on the ability to detect subjectively a rise in airflow resistance in COPD. Indeed, there appears to be preservation of that ability across an older age range in COPD in contrast to healthy people and asthmatics in remission [1, 2]. The reasons for this observation are not clear. It is known that people with COPD breathe with an exaggerated intrathoracic pressure swing between inspiration and expiration and at a higher and less mechanically advantageous lung volume [21] so it can be postulated that they could be predisposed to finer detection of perturbations in length-tension cycles during tidal breathing and thereby conserve LDT detection sensitivity into old age. If that were a contributary mechanism it might be expected that LDTs would rise in line with the FEV1/FVC ratio as those with higher ratios can arguably be considered to have less impairment of respiratory mechanics. We found such a rise that was of borderline statistical significance for ILDT though more clearly significant for ELDT. This supports the rationale described, though the number of participants was probably too low to comment on that aspect with certainty. Further, the unexpected negative correlation we found between LDTs and age might also be due to the deterioration in lung and chest wall mechanics over time in people with COPD, which might consequently eclipse the effects of ageing that we found in healthy subjects in this study and in previous work [1, 2]. Again, the small sample size, which was probably insufficiently powered for that particular part of the analysis precludes a firm conclusion. However, the trend makes physiological sense and needs to the specific focus of another study. The age and BMI characteristics of the groups were comparable so it is unlikely that those variables could account for the differences we observed. There was a significantly larger proportion of women in the healthy group. However, in our previous work [1, 2] we found no differences in the pattern of the relationship between ILDT and age between men and women, and that also appeared to be the case on scrutiny of the distribution of measurements in this study, though the samples were too small for confident statistical analysis.

In healthy people and patients with COPD the detection thresholds were below the resistance levels that we found to be distressing or uncomfortable in young and middle-aged healthy subjects who took part in our preliminary resistance ranging studies [1]. However, it is not known whether the afferent input from chest wall and diaphragmatic mechano-receptors has a linear influence on central length-tension processing. If not, it can be argued that the small additional resistances used when testing for LDT would have a proportionally larger central effect for COPD patients already tidally breathing at higher

lung volumes against chronically raised airflow resistance and greater recoil forces, thus allowing an alternative explanation for our observations to be posited. There is not complete agreement as to whether external airflow resistance loading is a valid analogue for intrinsic airways resistance, though it has been acknowledged that mechano-receptors must be involved [22]. The role of afferent sensory traffic from airway and lung parenchymal receptors [23] does not appear to be dominant in normal subjects, though could be more contributary to determining the sensitivity of central length-tension processing in those with lung disease, including COPD [24]. However, it has been observed that lung transplant patients, who have denervated lungs but intact chest wall and diaphragmatic sensation, retain the ability to judge lung volumes and have normal sensitivity to changes in external airflow resistance [25–27], which reinforces the key role of the mechano-receptors in the skeleto-muscular structures in airflow resistance sensing. The findings of our previous studies of healthy people and well-controlled asthmatics suggested that an age-related reduction in the sensitivity of airflow resistance sensing could be at least partly responsible for the clinically observed reduction in the ability of elderly people to detect early the changes in airflow resistance that occur, for example, in a worsening of asthma. However, the same does not seem to be the case for patients with COPD who appear to retain an acuity of airflow resistance sensing that is more typical of young adults.

There are clinical implications to the findings of this study. Our findings suggest that patients with COPD who are not undergoing an exacerbation are likely to be able to detect a rise in their airway resistance with a high degree of acuity at any age. Therefore, the concern that elderly COPD patients might delay taking reliever inhaled broncho-dilator therapy, or present later to medical services for help during an exacerbation due to ageing effects on resistance sensing is refuted, unlike some elderly asthmatics. It is apparently therefore not necessary to advocate that the COPD treatment guidelines [17] need take account of age for that specific reason. Of course, elderly patients with COPD do have a higher prevalence of other factors that complicate their management, such as dementia and heart failure, which must be acknowledged in their treatment plans.

Conflict of interest

None of the authors has any conflict of interest to declare.

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Nataliia Makieieva, Valeriia Malakhova, Yuliia Vasylchenko, Valentyn Tsymbal

Kharkiv National Medical University, Kharkiv, Ukraine

Are level of IL-13 and IL-4 predictive for formation of chronic inflammation in children with asthma?

Abstract

Introduction: Asthma diagnosis in young children may represent a clinical challenge. There are no standard prognostic and diagnostic methods. The aim of the study was to evaluate the clinical and prognostic assessment of IL-4 and IL-13 concentrations in children with recurrent wheezing.

Material and methods: The study included 96 children with recurrent wheezing. 81 patients were diagnosed as transient wheezing, 15 patients with asthma, and 25 healthy children were selected as controls. The concentrations of IL-4 and IL-13 were analyzed in exhaled breath condensate (EBC) using an enzyme-linked immunosorbent assay (ELISA). Data analysis was performed using Statsoft Statistica Version 8 (Tulsa, OK) and the statistical program MedCalc version 17.2.

Results: Both IL-4 and IL-13 concentrations were significantly higher in DDA (21.13 pg/mL, 26.13 pg/mL, respectively) and TW (13.86 pg/mL, 18.3 pg/mL, respectively) groups as compared to healthy controls (3.37 pg/mL, 16.35 pg/mL, respectively; $p < 0.001$), and the highest rates were observed in children with diagnosed asthma ($p < 0.001$, DDW vs TW, respectively). IL-4 concentration higher than 18.45 pg/mL (with sensitivity 86.7% and specificity 80%) and IL-13 concentration higher than 20.17 pg/mL (with sensitivity 100% and specificity 76.7%) in EBC in children with wheezing recurrence can be considered as a possible predictor of asthma development.

Conclusions: The concentration of the anti-inflammatory cytokines IL-4 and IL-13 were significantly increased in children with recurrent wheezing and the highest rates were found in asthma developing children. The concentrations of IL-4 and IL-13 in children with wheezing can be considered as a possible predictor of asthma development.

Key words: asthma, inflammation, IL-4, IL-13, children

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Introduction

Asthma is one of the most common chronic diseases in children and young adults [1–5]. The number of severe and uncontrolled forms of asthma has increased, leading to greater disability and mortality among patients [1–2]. The debut of the disease takes place in childhood [3–5]. Accurate diagnosis of asthma in this age cohort may represent a significant clinical challenge [1, 3, 6]. Firstly, the clinical signs of wheezing in children can be caused by many diseases, among which, besides asthma, there are hereditary and birth defects with metabolic processes, pathology of the gastroesophageal zone, congenital and acquired defects of the heart and main vessels and many others that should be included in the differential diagnostics [3–5, 7]. Secondly, in children under 6 years of age, there are limitations in the study of lung function. Therefore, in this cohort, diagnosis is based mainly on clinical and anamnestic data [6–8]. So, the global medical problem is to expand the diagnostic possibilities of early detection of asthma in children.

The pathogenesis of the disease, namely chronic inflammation, is a systemic process, but it damages mainly the respiratory tract [8, 9]. At the beginning of the formation of inflammation, the mechanisms do not depend on the type of the damage (allergic, infectious, etc.) and have general signs of action of cytokine complexes [10–13]. Several proinflammatory (IL1, IL-6, IL-8, TNF-α,

Address for correspondence: Valeriia Malakhova, Kharkiv National Medical University, Kharkiv, Ukraine; e-mail: malakhovavaleriia@gmail.com

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GM-CSF) and anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGF-b) are synthesized [11–13]. It is known that IL-4 function is to induce increased concentration of IgE, and IL-13 is a unique, Th2-linked, cytokine that interacts with B cells and thus regulates inflammatory and immune responses [14–16]. Recent studies have found an increase in serum concentration of these anti-inflammatory cytokines. They are not only able to induce the inflammatory process, but also support it, leading to a chronic activity. These interleukins that characterize chronic inflammation lead to airway remodeling and hyperplasia of the muscular system of the bronchopulmonary system [17]. Thus, it is advisable to study the concentration of these indicators directly in the inflammation locus.

The study of mediators and markers of inflammation released by damage of the respiratory system is possible due to the procedure of bronchoalveolar lavage or induced sputum [18]. However, because of the fact that these studies are related to invasive intervention, they cannot be repeated in a short period of time, especially in pediatric patients. Considering non-invasive methods that can be used in children, and to be able to reflect the state of local inflammation of the respiratory system, the exhaled breath condensate (EBC) seems to be optimal option [18]. It is a promising source of biomarkers of lung disease. EBC represents a matrix in which biomarkers can be identified [18, 19]. Till date interleukins' levels in EBC, especially in cohorts of young children have been rarely evaluated. In this study, we conducted clinical and prognostic evaluation of interleukin 4 and interleukin 13 concentration in EBC children with recurrent wheezing episodes.

Material and methods

Study design

This was a longitudinal cohort study of children with recurrent wheezing from February 2015 to November 2019 who were treated at a children's hospital. The study consisted of two stages. In the first phase (recruitment, February 2015 — May 2015), among the 305 children who were admitted to the hospital with wheezing, 126 children between the ages of 1.5 and 6, who met the inclusion criteria and did not fulfill exclusion criteria were included to the study. Inclusion criteria: signing informed consent by the patient's parents; patient's age from 1.5 to 6 years; current wheezing episode at recruitment is third and more. Exclusion criteria: congenital and chronic cardiopulmonary or neurological disease; hereditary diseases that lead to changes in the functioning of the respiratory tract, including cystic fibrosis; proven immune deficiency; proven or suspected acute or chronic bacterial infection, including infection of the oral cavity and respiratory tract; suspected or confirmed gastroesophageal diseases; previous treatment with antileukotriene drugs or systemic corticosteroids. The patients underwent specific treatment according to accepted recommendations [20]. All study subjects underwent clinical medical history review, physical examination and laboratory assessment. Children were also examined for IL-4 and IL-13 concentration in the exhaled breath condensate. The study of anti-inflammatory cytokines was conducted on the first day of the disease in the presence of clinical manifestations of wheezing.

The control group comprised 25 healthy children (of similar age/gender) without any signs of chronic or acute diseases for the previous three months, presented to Hospital for routine health control or vaccination. The parents of all patients and controls were informed about the study objectives and written informed consent was obtained before inclusion in the study.

Second stage (follow-up, May 2015—September 2019). The patients were called for re-examination at the age of 5–6 years. The inclusion and exclusion criteria in the study were re-evaluated. In the second stage, 96 children met the inclusion criteria and did not satisfy exclusion criteria. Anamnestic data for this period were studied. An examination was carried out to verify the diagnosis of asthma at the time of re-examination. The diagnosis was based on the GINA 2019 criteria and included symptoms (cough and wheezing) for more than 10 days during the upper respiratory tract infection, more than 3 episodes of wheezing per year, the child coughed between episodes, and there were present atopic dermatitis or food allergy history or family history of asthma [20]. An objective assessment of the severity of respiratory failure and differential diagnosis of obstructive and possible restrictive respiratory disorders were performed. The patients were divided into groups depending on the asthma diagnosed during this period or diagnosed during the visit. The first group included 81 transient wheezing (TW) children who stopped wheezing symptom at follow-up. The second group included 15 patients with doctor-diagnosed asthma (DDA).

Exhaled breath condensate: technical and diagnostic aspects

The device for EBC receiving was designed using a portable glass tube in accordance with material collection standards. The glass tube was cooled, surrounded by a mixture of refrigerants to obtain a temperature that reached from -5°C to -10°C. There, exhaled air in the form of droplets was converted to EBC [18, 21]. After collection, the glass tube was detached, and the sample was stored immediately at -70° C. The device has a manual control of condensing temperature and cleaning requirements of the tool between consequent trials. All collections were done between 8:30 and 09:30 a.m. The subjects wore a nose clip, which prevented contamination of the material with excretions from the nasal mucosa, and prevented the possibility of inhaling or exhaling through the nasal cavity. For children over three years of age, condensate was collected through systems with a single-use mouthpiece that was connected to a one-way exhalation valve to prevent the effects of inhaling the condensate. For subjects younger than two years, an exhalation valve with an inhalation mask was used.

Measurement of IL-4 and IL-13 in exhaled breath condensate

IL-4 in EBC were analyzed by the ELISA technique using commercial kits (Human IL-4, "Vector Best Ukraine", catalog number: A-8754), and IL-13 concentration were analyzed by the ELISA technique using commercial kits (Human IL-13, eBioscience (Bender MedSystems), catalog number: BMS231-3, USA) according to the manufacturer's instructions.

Statistical analysis

All statistical analyses were performed using StatSoft STATISTICA Version 8 package program (Tulsa, OK) and MedCalc statistical Software Version 17.2. Shapiro-Wilk's test was used, and histogram and q-q plots were examined to assess the normality. As the sample distribution was different from the normal, the median (Me) and the interquartile range were determined (Lq - lower quartile; Uq - upper quartile). Fisher's exact test, $\chi 2$ was used to calculate two relative indicators. Nonparametric Mann-Whitney test (MW) was applied to compare the two samples and Wilcoxon (T) nonparametric test was used to compare the two dependent samples. The difference between the two parameters was considered statistically significant at $p < 0.05$. The correlation between parameters was determined using the Spearman rank correlation analysis (r); $p < 0.05$ was considered to indicate a statistically significant difference. Receiver operating characteristic (ROC) curves were drawn for variables to determine the optimal «cut-off» values to predict an endpoint. The endpoint of this study is the formation of chronic inflammation in children with recurrent wheezing. The cut-off point of each variable and sensitivity, specificity, positive likelihood ratio (+LR), negative likelihood ratio (LR) of this "cut-off" point was obtained using the Youden index. To determine the most reliable screening tool among these four variables, pairwise comparison of these variables was performed by determining the differences between area under the curve using the Hanley and McNeil method.

Ethics approval and consent to participation

The planned clinical studies were carried out after receiving approval by the local ethics committee (date: February 1, 2015; number: 2015/01) and were conducted in accordance with the principles of the Helsinki Declaration, amended in October 2013.

Results

General information

Of the 96 children with recurrent wheezing enrolled to the study, the majority were diagnosed with transient wheezing (TW) (as they stopped wheezing at follow-up), and a minority developed asthma (DDA). These groups accounted for 84.38% and 15.62%, respectively. No significant difference was found when comparing age and gender groups. Anamnestic factors such as pregnancy, cesarean delivery, and frequent manifestations of viral infections of the upper respiratory tract, presence of pets, passive smoking were not statistically different between those groups. In regard of allergic history, atopic dermatitis, food allergy, allergic rhinitis, the presence of allergic diseases and asthma in relatives, wheezing in the first year of life were likely to be more frequent in patients with DDA. Elevated eosinophil level and high IgE level were also significantly increased in patients with DDA (Table 1).

IL-4 and IL-13 concentration

IL-4 concentrations were significantly higher in DDA (21.13 pg/mL) and TW (13.86 pg/mL) groups

Table 1. General information of anamnesis data [%(n)]

Sign	TW (n = 81)	DDA (n = 15)	P
Gender, M/F	48/33	10/5	p > 0.05
Age, years at recruitment Me (Lq; Uq)	2.58 (1.55; 4.10)	2.00 (1.50; 3.11)	p > 0.05
Age, years at follow-up Me (Lq; Uq)	7.09 (6.00; 9.90)	6.50 (6.00; 7.80)	p > 0.05
Caesarean section	42.2% (35/81)	33.3% (5/15)	p > 0.05
Gestation period	39 (35; 41)	38 (35; 40)	p > 0.05
Presence of atopic dermatitis in children	**39.5% (32/81)**	**73.3% (11/15)**	**p = 0.0227**
Presence of food allergy in children	**35.8% (29/81)**	**80.0% (12/15)**	**p = 0.0033**
Present of allergic rhinitis in children	**2.5% (2/81)**	**46.7% (7/15)**	**p < 0.001**
Present of allergic disease in relatives	**3.7% (3/81)**	**60.0% (9/15)**	**P < 0.001**
Presence of asthma in relatives	**2.5% (2/81)**	**53.3% (8/15)**	**P < 0.001**
Onset of wheezing of the first year of life	**48.1% (39/81)**	**80.0% (12/15)**	**p = 0.0267**
Current wheezing episode at recruitment	4 (3; 4)	5 (3;5)	p > 0.05
Current pet ownership at recruitment	27.2% (22/81)	26.7% (4/15)	p > 0.05
Repeated upper respiratory tract infection	85.2% (69/81)	80.0% (12/15)	p > 0.05
Passive smoking	27.2% (22/81)	26.7% (4/15)	p > 0.05
High eosinophil blood	**8.7% (7/81)**	**60.0% (9/15)**	**p < 0.001**
IgE increase, IU/ml	**25.9% (21/81)**	**86.7% (13/15)**	**p < 0.001**

DDA — doctor diagnosed asthma; TW — transient wheeze

as compared to healthy controls (3.37 pg/mL; $p < 0.001$), and the highest rates were observed in children with diagnosed asthma ($p < 0.001$, DDW *vs* TW, respectively) (Table 2). Similarly, IL-13 concentrations were significantly higher in DDA (26.13 pg/mL) and TW (18.3 pg/mL) groups as compared to healthy controls (16.35 pg/mL; $p < 0.001$), and the highest rates were observed in children with diagnosed asthma ($p < 0.001$, DDW *vs* TW, respectively).

Correlation between IL-4 and IL-13 parameters

The correlation between IL-4 and IL-13 concentration of all children with recurrent wheezing was found ($r = +0.37$, $p < 0.001$). The relationship between those biomarkers in children with DDA was $r = +0.74$, $p < 0.001$, and in TW cohort $r = +0.17$, $p < 0.001$.

Prognostic criteria for IL-4 and IL-13

To determine the prognostic significance of the concentration of IL-4 and IL-13, ROC curve analysis was performed. The relationship between cytokine level during the first episodes of wheezing and the development of asthma was determined. The cut-off value for the concentration of IL-4 higher than 18.45 pg/mL resulted in specificity of 80% and sensitivity of 86.7%, and for IL-13, concentration higher than 20.17 pg/mL resulted in specificity of 76.7% and sensitivity of 100% for the predefined endpoint, namely the formation of chronic asthma (Figure 1).

Discussion

This study found that among children with recurrent wheezing, a greater proportion of patients had transient wheezing and only 15.62% developed asthma during follow-up. There are many studies that describe the possible contributing factors in children with recurrent wheezing for formation of asthma. Several phenotypes are considered as the debut of the disease. The proportion of such children ranges from 13% to 16% according to various sources [6, 22–24]. This fact has been confirmed in our study.

In our study, an increased concentration of IL-4 in the exhaled breath condensate of children with recurrent wheezing in the onset of clinical manifestations was determined. The highest rates were found in children who developed asthma during the follow-up. IL-4 is known to stimulate IgE production, promote eosinophil accumulation in peripheral blood and tissues [14-17, 25–26].

Table 2. IL-4 and IL-13 levels in children with recurrent wheezing during the onset of clinical manifestations (Me [Lq; Uq])

	TW (n=81)	DDA (n=15)	Control
IL-4, Me (Lq; Uq)	13.86 pg/mL (10.16;16.48)	21.13 pg/mL (19.28;1.54)	3.37 pg/mL (2.95;3.62)
	KW by Ranks: H = 41.38; p < 0.001 MW: p1–2 < 0.001; p1-control < 0.001; p2- control < 0.001		
IL-13, Me (Lq; Uq)	18.30 pg/mL (16.11;20.17)	26.13 pg/mL (24.12;31.05)	16.35 pg/mL (14.86;17.45)
	KW by Ranks: H = 29.91; p < 0.001 MW: p1–2 < 0.001; p1- control = 0.0229; p2- control < 0.001.		

DDA — doctor diagnosed asthma; KW — Kruskal-Wallis test; Me (Lq; Uq) — median (lower quartile; upper quartile); MW— Mann-Whitney test; p significant with the Bonferroni correction; TW — transient wheeze

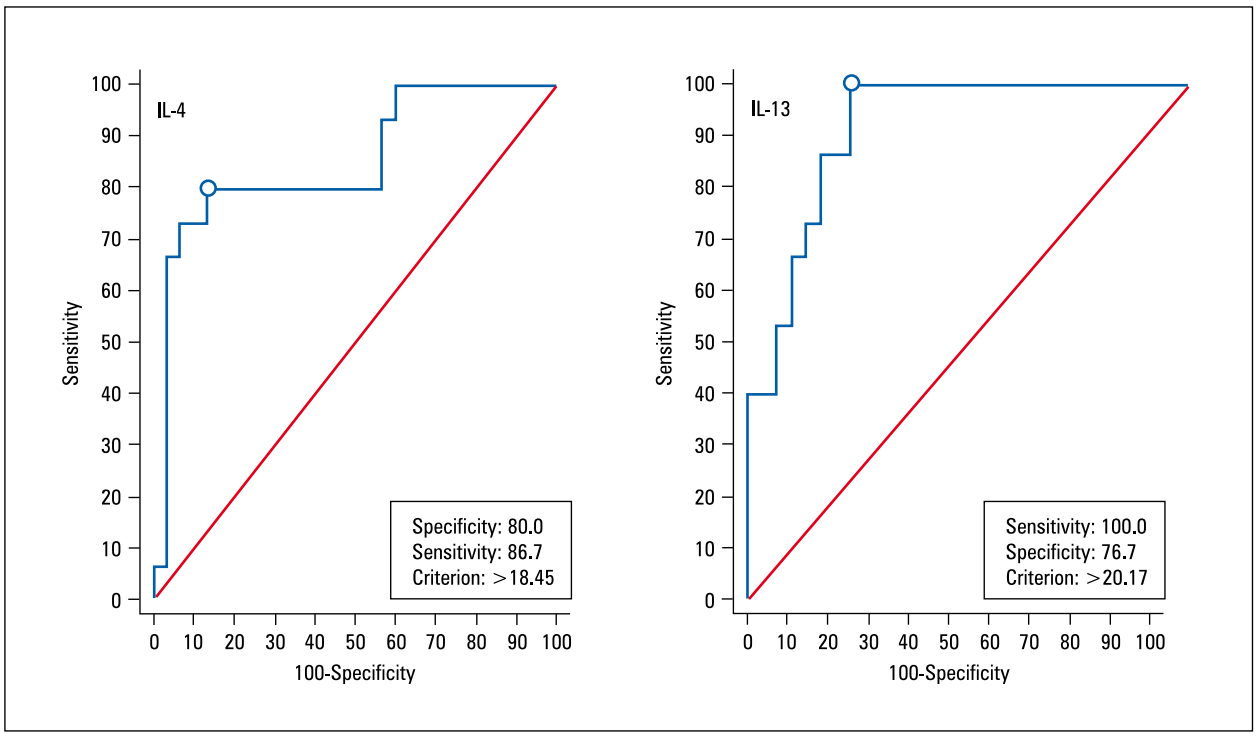

Figure 1. ROC curves for IL-4 and IL-13 as a biomarker of asthma development in children with wheezing episode

As a result, IL-4 is regarded as a cytokine that has a direct effect on the development of allergic inflammation [14–17]. An increased concentration of this cytokine has been shown to indicate the presence of allergic inflammation in patients. There are studies demonstrating changes in serum IL-4 concentration in atopic dermatitis in children [25], adults, and adolescents with asthma [14, 17]. Recent researches have proved that IL-4 concentration increase in the EBC of adult asthma patients, and IL-4 is considered as a marker of chronic allergic inflammation [26]. In our study, children up to 6 years of age were examined, but IL-4 was also elevated, which was likely to indicate an allergic nature of inflammation of the bronchopulmonary system. An increased IL-4 concentration in the exhaled breath condensate can be considered as a manifestation of an active inflammatory process, directly in the inflammation locus.

We found that in children with recurrent wheezing, in the onset of clinical manifestations, IL-13 concentration in exhaled breath condensate was also increased as compared to healthy controls. The highest levels were found in children who developed asthma. IL-13 is a Th2-linked cytokine and regulates the body's inflammatory and immune responses. It stimulates the production

of B cells and IgE, and inhibits the formation of inflammatory cytokines [14–17]. Recent studies have reported an increased IL-13 concentration in serum in atopic diseases in children [17, 25]. There are data that confirmed an elevated IL-13 concentration in the serum in adolescents and adults asthma [14, 17]. These studies suggest that changes in the concentration of this cytokine indicate the presence of allergic inflammation. We have also regarded it as a manifestation of allergic inflammation, as well as activity of the process in the respiratory system, taking into account the detection of indicators in the locus of damage.

Our study evaluated the relationship between IL-4 and IL-13 in EBC children less than 6 years of age and established direct correlation between indicators. Similarly, significant correlation was found in children who developed asthma. The correlations of IL-13 and IL-4 were made taking into account the presence of similar pathways that are involved in the synthesis of IgE, activation of eosinophils, mucus secretion [14-16]. Recent studies have demonstrated these relationships in adult patients with allergic airway disease [17, 25], where IL-4 has been identified as the first cytokine to be produced by mast cells and responsible for promoting the production of IL-13 of mast cells [15]. Our study confirms the relationship between these cytokines in the presence of allergic inflammation in children.

The ROC analysis determined the predictive concentrations of IL-4 and IL-13 for development of asthma in children with wheezes. The criterion of IL-4 > 18.45 pg/mL and IL-13 > 20.17 pg/mL were found to have prognostic significance, with high specificity and sensitivity rates in asthma formation in children with recurrent wheezing.

Non-invasive techniques are used to investigate the health status of adults and children. There are several non-invasive techniques for the determination of respiratory gases in exhaled air and compounds dissolved in exhaled lung secretion. There are studies using Electronic Noses (E-noses) that can play a potential role in the screening and analysis of various respiratory and systemic diseases [27–29]. The EBC was chosen for our study.

This study has several limitations. Firstly, about 45% of children were with comorbid diseases, such as atopic dermatitis or food allergy, which may result in higher concentration of IL-4 and IL-13 both in serum and EBC. Thus, the concentration of these cytokines obtained in children with asthma could have higher rates due to concomitant allergic diseases [14, 25]. However, there is no evidence-based data that concentration of IL-4 or IL-13 can increase in EBC in children with comorbid allergic diseases. Substances in the EBC are in large dilution, as the excretory fluid covers both the alveolar epithelial layer and the mucous layer of the respiratory tract. There is no existing model that allows collecting all the exhalation, since the loss of moisture depends on the humidity and temperature of the environment. These factors are likely to have an influence on the obtained results [18–19, 21, 26]. Another limitation of the study is that asthma is a heterogeneous disease and has many phenotypes [9, 22, 30–31]. There are types that have neutrophil and paucigranulocytic phenotype in their mechanism of development. The most common is the eosinophilic type of inflammation, which is associated with eosinophilic cellular infiltration and thickening of the basement membrane area. The performed studies and the selected cytokines reflect just this type of inflammation. This study was conducted taking into account only the eosinophilic phenotype, which is the most frequent in children.

In conclusion, among patients presenting with recurrent wheezing, approximately in every sixth child, the disease has been transformed into asthma. The concentrations of the IL-4 and IL-13 in exhaled breath condensate were significantly increased in children with manifestation of wheezing, and the highest rates were found in asthma developing children. Both cytokines are involved in the regulation of allergic inflammatory processes in the body, and have an influence on the respiratory tract, as it was detected basing on IL-4 and IL-13 concentration in the exhaled breath condensate. IL-4 concentration higher than 18.45 pg/mL and IL-13 concentration higher than 20.17 pg/mL in exhaled breath condensate in children with wheezing recurrence can be considered as a possible predictor of asthma development.

Conflict of interest

None declared.

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Nitin Joseph[1], Shreeshaina[2], Shivani Bhat Loliem[2], Vinod Kumar Gundi[2], Mrudula Bhargav Herambapura Subramanya[2], Shreyashree Belthur Shashidhar[2]

[1]Department of Community Medicine, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India
[2]MBBS student, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India

An assessment of risks associated with obstructive sleep apnea and its relationship with adverse health outcomes among pregnant women. A multi-hospital based study

Abstract

Introduction: Physiological changes in pregnancy increase the vulnerability of antenatal women to develop obstructive sleep apnoea (OSA). It is a known cause of several adverse health outcomes in pregnancy.

Objectives: To assess the risk status of OSA in pregnant women and to study its association with adverse maternal outcomes, fatigability, and daytime sleepiness.

Material and methods: Pregnant women were interviewed to assess for the risk of OSA, fatigability, and daytime sleepiness. STOP BANG, the fatigue severity scale, and the Epworth sleepiness scale were used to assess these parameters.

Results: The mean age of the 214 participants was 27.2 ± 4.7 years. 7 (3.3%) participants had a history of snoring louder than the volume of normal talking, or of being loud enough to be heard past closed doors. A moderate risk status of OSA was present among 3 (1.4%) participants. 45 (21.0%) pregnancies were high risk in nature. The risk status of OSA was associated with a high risk status of pregnancies among the participants ($p = 0.0088$). 41 (19.2%) participants had a history of significant fatigue over the previous week of the study. 7 (3.3%) participants reported mild to severe excessive daytime sleepiness. A history of snoring loudly ($p = 0.0179$) and OSA risk status ($p = 0.0027$) was associated with excessive daytime sleepiness.

Conclusions: A risk status for OSA was associated with a high risk pregnancy status and excessive daytime sleepiness among pregnant women in the current setting. Therefore, pregnant women with these conditions need to be evaluated for OSA. They also need to be suitably managed to ensure the healthy well-being of the mother and the baby.

Key words: pregnant women, obstructive sleep apnea, fatigue, daytime sleepiness

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Introduction

Pregnancy is a state associated with a number of physiological changes in the body. High levels of progesterone and estrogen during pregnancy cause fluid retention leading to swelling around the neck, mucus membranes, and nasal passages. These changes result in narrowing of the oropharyngeal diameter and increased upper airway resistance leading to the occurrence of snoring [1]. As many as 25% of pregnant women have been found to be first time snorers during the gestational period [1]. Heavy snoring accompanied by pauses in breathing lead to a state of obstructive sleep apnea (OSA) [1]. The state of reduced oxygenation during each episode of OSA can end with disastrous consequences both in the antenatal and in the intranatal period [2]. Prior studies have observed OSA complicating pregnancy and resulting in an increased risk of intensive care admission as well as an increased duration of time spent in the hospital [3].

Although OSA is a common complication in pregnancy, it unfortunately remains underdiagnosed due to reasons such as variable clinical presentations [4] and poor validity of certain questionnaire-based tools [5]. Overnight polysomnogram, which is the gold standard for its diagnosis, is an

Address for correspondence: Nitin Joseph, Department of Community Medicine, Kasturba Medical College, Mangalore, India; e-mail: drnitinjoseph@gmail.com
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expensive, time-consuming, and uncomfortable procedure which is not practical for application [6].

Surveys have reported that 90% of pregnant patients with OSA were unaware of OSA and its consequences [7]. Doctors may also misinterpret the excessive daytime sleepiness complaints among antenatal women as a normal symptom of pregnancy. This results in a further delay in its identification and management [8].

This study was therefore done to study the risk status of OSA among pregnant women at various tertiary care centres in an urban setting. Its association with adverse maternal outcomes, fatigability, and daytime sleepiness were also evaluated in this study.

Material and methods

This cross-sectional study was conducted among pregnant women, both admitted and those seeking outpatient services, at a private and a government tertiary care hospital in Mangalore, India. Ethical clearance was approved by the Institutional Ethics Committee. The reference number was IECKMCMLR/023/2019. Permission was granted by the medical superintendent of the respective hospitals. The study was conducted in the month of February 2019.

Consent was given in writing after clarifying the procedure and purpose of the study to each participant.

Assuming that 31.9% [9] of pregnant women are at a greater risk for developing OSA, the sample size was calculated as 214 at 95% confidence intervals and 80% power using the formula $n = 4pq/d^2$.

All participants were examined only once as a part of this study. They were enrolled using the convenience sampling method. Participants aged 18 years and above were included whereas those who refused to give consent for participation were excluded from this study. They were then interviewed using a structured interview schedule. The risk status for developing OSA was assessed using the STOP BANG questionnaire. It has eight items with responses for each in a "yes" and "no" format. The participant was categorized as having either a low, moderate, or high risk of OSA if they answered "yes" to < 3, 3–4, ≥ 5 items respectively. This tool has been validated to identify OSA in pregnancy and has been found to have the highest specificity among the various questionnaire-based OSA screening tools [10].

The current level of fatigue among participants was assessed using the fatigue severity scale (FSS) which consists of nine items. The responses to each item were "strongly disagree", "moderately disagree", "slightly disagree", "neutral", "slightly agree", "moderately agree", and "strongly agree" in a Likert scale with scores ranging from 1 to 7 respectively. A cumulative score of ≥ 36 was indicative of a significant level of fatigue among the participants.

Desire to sleep while engaging in different activities during the daytime in recent times was assessed using the Epworth sleepiness scale (ESS). The responses to the eight items in this scale were "would never nap", "slight chance of napping", "moderate chance of napping" and "high chance of napping", scored from 0 to 3 respectively. An ESS score over 10 was indicative of excess daytime sleepiness.

The internal consistency of the STOP BANG questionnaire, FSS and ESS used in this study had the Cronbach's alpha value of 0.724, 0.878 and 0.693 respectively, indicating good reliability.

Demographic details (age, occupation, education, and place of residence), obstetric details (obstetric score and gestational age), and the presence of risk factors (co-morbidities, history of tobacco usage, long standing medication history, family history of snoring, and known status of OSA) were enquired about among the respondents.

In addition, height, weight, neck circumference, and blood pressure were measured among all participants using instruments like stadiometers, measuring tapes, weighing scales and mercury sphygmomanometers as per standard guidelines.

Pre-pregnancy weight, recent haemoglobin values, and recent fasting blood sugar (FBS) values were noted from the antenatal records of the participants. BMI status was assessed using the Asian classification. Participants with haemoglobin values < 11 g/dL were categorized as anaemic and those with FBS ≥ 95 mg/dL of blood were interpreted as having increased blood sugar levels.

The interview schedule was content validated and was also language validated in the local language "Kannada" with the help of experts. The schedule was then pilot tested among 10 antenatal women who were not part of the main study.

IBM SPSS for Windows version 25.0, Armonk, New York was used for data entry and analysis. The Chi-square test and Fisher's exact test were used to test association. $p<0.05$ was considered a significant association.

Results

Out of the 214 pregnant women, 138 (64.5%) were from the government hospital and 76 (35.5%)

Table 1. Socio-demographic distribution of pregnant women

Characteristics	Number	Percentage
Age group (years)		
18–20	20	9.4
21–25	54	25.2
26–30	88	41.1
31–35	44	20.6
36–38	8	3.7
Educational status		
Graduate/postgraduate	24	11.2
Pre-university course/ diploma	48	22.4
High school	71	33.2
Middle school	34	15.9
Primary school	17	7.9
Illiterate	20	9.4
Occupational status		
Housewives	174	81.3
Semi-professionals	15	7.0
Semi-skilled workers	12	5.6
Unskilled workers	13	6.1
Place of residence		
Urban	169	79.0
Rural	45	21.0
Total	214	100.0

Table 2. Pregnancy-related characteristics among participants

Characteristics	Number	Percentage
Trimester		
First	13	6.1
Second	57	26.6
Third	144	67.3
Gravida		
Prima	118	55.1
Second	63	29.4
Third	25	11.7
Fourth	7	3.3
Seventh	1	0.5
Parity		
Nulliparous	125	58.4
First	60	28.0
Second	23	10.8
Three	6	2.8
Number of children		
None	129	60.3
One	63	29.5
Two	17	7.9
Three	5	2.3
Past history of abortion	27	12.6
High risk status of pregnancy	45	21.0
Recent haemoglobin value < 11 g/dL	43	20.1
Total	214	100.0

were from the private hospital. 27 (12.6%) were admitted while 187 (87.4%) had come for antenatal check-ups on an outpatient basis at these hospitals.

Reasons for admission included pregnancy induced hypertension (PIH) among 7 patients, abdominal pain and bleeding among 7, safe confinement among 2, oligohydramnios among 2, gestational diabetes mellitus (GDM) among 2, hypotension in 1, vomiting and dysentery in 1, renal calculi in 1, anaemia in 1, placenta praevia in 1, polyhydramnios in 1, and fetal microsomia in 1.

The mean age of the participants was 27.2 ± 4.7 years. The majority of participants were educated up to high school level [71 (33.2%)], were housewives [174 (81.3%)], and were from urban areas [169 (79%)] (Table 1).

The majority of pregnant women were in the third trimester [144 (67.3%)]. A past history of abortion was reported among 27 (12.6%) participants (Table 2). Out of the 214 participants, a history of a single episode of abortion was present among 23 (10.7%), two episodes among 2 (0.9%), and three and four episodes in 1 (0.5%) participant each. The current BMI status was underweight among 22 (10.3%), normal among 82 (38.3%), overweight among 52 (24.3%), and obese among 58 (27.1%) participants. A current body mass index (BMI) more than 35 kg/m^2 was present in only one participant.

45 (21.0%) of the total pregnancies were high risk in nature. The mean age of these women was 28.5 ± 4.5 years. Six (13.3%) were illiterates, 4 (8.9%) were educated up to primary school, 7 (15.6%) up to middle school, 10 (22.2%) up to high school, 12 (26.7%) completed a pre-university course/diploma, and 6 (13.3%) of them were educated at the undergraduate/postgraduate level. Six (13.3%) of them were coolie workers, 4 (8.9%) were beedi rollers, and the rest were house wives. Thirty two (71.1%) of them were from rural areas. Twenty nine (64.5%) were pri-

Table 3. Association between risk factors and OSA risk status among the participants

Characteristics	OSA risk status		
Trimester	**Moderate risk (%)**	**Low risk (%)**	**Total**
First	0 (0)	13 (100)	13
Second	1 (1.8)	56 (98.2)	57
Third	2 (1.4)	142 (98.6)	144
			X2 = 0.236, p = 0.889
Age group (years)			
≤ 30	1 (0.6)	161 (99.4)	162
> 30	2 (3.9)	50 (96.1)	52
			p = 0.1471
Total	3	211	214
BMI status before pregnancy			
Underweight/ normal	2 (1.8)	111 (98.2)	113
Overweight/obese	1 (2.7)	36 (97.3)	37
			p = 1
Total	3	147	150
Weight gain among women coming for ANC visit between 32 to 33 weeks in comparison to their pre-pregnancy weight			
< 11 kilograms	0 (0)	11 (100)	11
≥ 11 kilograms	1 (50)	1 (50)	2
			p = 0.1538
Total	1	12	13

ANC — antenatal care; BMI — body mass index; OSA — obstructive sleep apnoea

migravida, 13 (28.9%) were of second gravida, 2 (4.4%) were of third gravida, and 1 (2.2%) was of fourth gravida. Four (8.9%) of them were underweight, 17 (37.8%) were normal, 9 (20%) were overweight and 15 (33.3%) were obese as per their BMI status. The reasons for high risk pregnancies were PIH among 15, hypothyroidism among 6, GDM among 5, Rh negative status among 3, abdominal pain and bleeding among 2, hypotension among 2, oligohydramnios among 2, polyhydramnios in 1, threatened abortion in 1, fibroid in 1, ovarian cyst in 1, GDM and PIH in 1, GDM and asthma in 1, psoriasis in 1, placenta praevia in 1, pre-eclampsia in 1, and hyperthyroidism and PIH in 1 participant.

A history of the use of long-standing medications prior to pregnancy was present among 8 patients. The medications used by them were labetalol among 3, metformin among 3, and iron tablets among 2 patients. Current blood pressure readings were raised among 5 (2.3%), the most recent FBS levels were raised among 2 (0.9%) and the glucose challenge test result was on the higher side in 1 participant.

Seven (3.3%) participants had a history of snoring louder than the volume of normal talking or being loud enough to be heard through closed doors. Only 1 participant had a positive family history of snoring.

A history of snoring loudly was reported among 3 (6.7%) of the 45 participants with a high risk pregnancy status in comparison to 4 (2.4%) out of 169 participants with normal pregnancy status (p = 0.163). There was no association of having a history of snoring loudly as a result of gravida status >2 (p = 1), multigravida status (p = 1), parity > 2 (p = 1), number of living children > 2 (p = 1), past history of abortion (p = 0.5996), trimester status (p = 0.531), being a housewife by occupation (p = 1), being illiterate (p = 0.502), residential status (p = 0.349), recent FBS values (p = 1), haemoglobin < 11 g/dL (p = 0.34), a history of taking long standing medications (p = 0.237), family history of snoring (p = 1), age > 30 years (p = 0.3645), pre pregnancy BMI status (p = 0.6367), current BMI status (p = 0.715), and weight gain among women coming for ANC visit between 32 and 33 weeks (n = 13) in comparison

to their pre-pregnancy weight (p = 0.2949) among the participants.

Five (2.3%) participants had a positive history for someone having observed them stop breathing during sleep.

Moderate risk status for the development of OSA were present among 3 (1.4%) out of the total 214 participants.

There was no association between gravida status >2 (p = 0.3965), multigravida status (p = 0.589), parity > 2 (p = 1), number of living children >2 (p = 1), history of abortion (p = 1), third trimester status (p = 1), being a housewife by occupation (p = 0.4642), educational status (p = 1), place of residence (p = 1), family history of snoring (p = 1), haemoglobin < 11 g/dL (p = 0.571), history of taking long standing medications (p = 0.108), current BMI status (p = 1), weight gain among women coming for ANC visit between 22 to 23 weeks in comparison to their pre-pregnancy weight (n = 3; p = 1), weight gain among women coming for ANC visit between 39 to 40 weeks in comparison to their pre-pregnancy weight (n = 9; p = 0.4444) and OSA risk status.

A moderate risk status for OSA was present among 2 (3.9%) out of 52 women aged more than 30 years in comparison to 1 (0.6%) out of 162 women aged 30 years or below (p = 0.1471). (Table 3)

Risk status for OSA was associated with a high risk status of pregnancy among the participants (p = 0.0088) (Table 4).

Forty one (19.2%) participants were found to have a history of significant fatigue over the previous week of the study. Lower normal daytime sleepiness was present among 177 (82.7%), higher normal daytime sleepiness among 30 (14%), mild excessive daytime sleepiness among 3 (1.4%), moderate excessive daytime sleepiness among 3 (1.4%), and severe excessive daytime sleepiness in 1 (0.5%) participant in recent times.

A history of snoring loudly (p = 0.0179) and OSA risk status (p = 0.0027) was associated with excessive daytime sleepiness (Table 4).

Discussion

Early identification of OSA among pregnant women is essential for both maternal and fetal well-being.

A history of snoring loudly was present among 3.3% participants which was less than the findings of previous studies [21.2% and 35.3% (11, 12)]. The tendency to snore occurs as a result of the normal physiological changes during pregnancy which lead to narrowing of upper airways. A history of snoring and frequent snoring (≥ 3 nights/week) among pregnant women have also been linked to the surge of estrogen and progesterone levels during the antenatal period [13].

In the current study, 1.4% of the participants were found to have a greater risk for developing OSA which was again less than 13.4% [14], 18.4% [15], 26.4% [9], 26.7% [16] and 30.9% [17] reported in previous studies. The trimester wide distribution of greater risk status for OSA was none in the first, 1.8% in the second, and 1.4% in the third trimester among participants in this study. In previous studies, the risk of OSA in the first trimester was reported as 10.5% [18], 10.7% [2], and 30.4% [9]; in the second trimester 29% [19] and 33.3% [9]; in the third trimester 24.1% [2], 26.7% [18], 32% [9], and 34.7% [20] of all participants. The risk of OSA was therefore low in particular trimesters of pregnancy in this study compared to the results of other authors.

However, it is notable that the risk of developing OSA was observed to increase in the second and third trimesters as compared to the first trimester of pregnancy in this study. This can be explained due to the fact that the enlarging uterus elevates the diaphragm and alters respiration. These alterations during sleep increase the risk of upper airway collapse, and for OSA, as the pregnancy progresses. An increase in the levels of estrogen and progesterone as the pregnancy advances is also responsible. An increase in estrogen concentration increases mucosal edema and progesterone increases the respiratory centre's sensitivity to carbon dioxide and destabilizes the respiratory control mechanism [21]. Therefore, it is important to screen for OSA and monitor for its development during every trimester as the pregnancy advances. It has been recommended to screen for OSA between 12 and 18 weeks to allow adequate time for its evaluation and early management [22].

In a study done in Peru, overweight and obese pregnant women were more likely to report a history of snoring [23]. Snoring has been found to be a risk factor for PIH, pre-eclampsia and GDM [24]. However, these were different from our observations because no such association was seen.

No specific risk factors were associated with OSA risk status in this study. This was again different from findings of previous studies where age [2, 14, 18], tongue enlargement [2], pre-pregnancy BMI [9], first trimester BMI [18], current BMI [2, 9, 14, 13] and weight gain during pregnancy [9] were reported as potential risk factors for developing OSA.

Table 4. Association between a history of snoring loudly and OSA risk status with a high risk status of pregnancy, fatigue, and excessive daytime sleepiness among participants

	Fatigue status		
History of snoring loudly	**Present**	**Absent**	**Total**
Present	2 (28.6)	5 (71.4)	7
Absent	39 (18.8)	168 (81.2)	207
			p = 0.621
Total	41	173	214
	Daytime sleepiness status		
History of snoring loudly	**Excessive**	**Normal**	**Total**
Present	2 (28.6)	5 (71.4)	7
Absent	5 (2.4)	202 (97.6)	207
			p = 0.0179
Total	7	207	214
	Type of pregnancy		
History of snoring loudly	**High risk**	**Normal**	
Present	3 (42.9)	4 (57.1)	7
Absent	42 (20.3)	165 (79.7)	207
			p = 0.163
Total	45	169	214
	PIH/Pre-Eclampsia status		
History of snoring loudly	**Present**	**Absent**	
Present	1 (14.3)	6 (85.7)	7
Absent	17 (8.2)	190 (91.8)	207
			p = 0.464
Total	18	196	214
	Fatigue status		
OSA risk status	**Present**	**Absent**	**Total**
Moderate risk	2 (66.7)	1 (33.3)	3
Low risk	39 (18.5)	172 (81.5)	211
			p = 0.095
Total	41	173	214
	Daytime sleepiness status		
OSA risk status	**Excessive**	**Normal**	**Total**
Moderate risk	2 (66.7)	1 (33.3)	3
Low risk	5 (2.4)	206 (97.6)	211
			p = 0.0027
Total	7	207	214
	Type of pregnancy		
OSA risk status	**High risk**	**Normal**	
Moderate risk	3 (100)	0 (0)	3
Low risk	42 (19.9)	169 (80.1)	211
			p = 0.0088
Total	45	169	214
	PIH/Pre-Eclampsia status		
OSA risk status	**Present**	**Absent**	
Moderate risk	1 (33.3)	2 (66.7)	3
Low risk	17 (8.1)	194 (91.9)	211
			p = 0.233
Total	18	196	214
	GDM status		
OSA risk status	**Present**	**Absent**	
Moderate risk	1 (33.3)	2 (66.7)	3
Low risk	6 (2.8)	205 (97.2)	211
			p = 0.0954
Total	7	207	214

GDM — gestational diabetes mellitus; OSA — obstructive sleep apnoea; PIH — pregnancy-induced hypertension

Although OSA risk status was associated with a high risk status of pregnancy, it was not specifically associated with either hypertensive disorders or GDM among participants in this study. In other studies, OSA in pregnancy was associated with PIH [14, 24], preeclampsia [15, 24] and GDM [14, 24]. Various hypertensive disorders in pregnancy have been suggested to result from endothelial dysfunction as a consequence of OSA related intermittent hypoxemia [25]. Frequent arousals from sleep among OSA patients lead to a decrease in slow wave sleep. This causes sympathetic activation which disrupts the hypothalamic-pituitary-adrenal axis. Altered sleep also causes an alteration in cortisol synthesis and release. These mechanism induce insulin resistance and alter glucose homeostasis [26, 27]. Periodic hypoxia in OSA affects beta cell activity of the pancreas [28].

Pregnant women are therefore advised to sleep in a lateral position which keeps airways open and thereby minimizes the risk of hypoxia induced by OSA [25]. On top of this, continuous airway positive pressure (CPAP), if initiated early in pregnancy even in those with history of chronic snoring, would be beneficial in blood pressure control [4]. Tolerance to nasal CPAP has been found to be good during pregnancy without reports of any adverse effects [29].

There was no association between OSA risk status and fatigability among pregnant women in this study.

However, the present study found that a history of snoring loudly and OSA risk status were associated with excessive daytime sleepiness among pregnant women. This was in contrast to the findings of previous studies done in the USA [18] and in Thailand [9] where no such association was reported. Medical practitioners and patients themselves end up mistaking excessive daytime sleepiness as usual symptoms in pregnancy. This may further delay the diagnosis and management of OSA which needs to be taken care of [8]. Health care providers need to further take up the responsibility of bringing up the awareness about OSA and its consequences among pregnant women. This is important as pregnancy may be the only occasion during which the woman might seek medical attention.

Conclusions

OSA risk status was associated with a high risk pregnancy status and excessive daytime sleepiness among pregnant women in the current setting. Therefore, pregnant women with these conditions need to be evaluated for OSA. They also need to be suitably managed to ensure the healthy well-being of both the mother and the baby.

Limitations

Under reporting of information may be possible among participants with respect to snoring and other variables such as fatigability and daytime sleepiness. The cross-sectional design of this study is limited with regards to its ability to interpret temporality of the association. This could have been rectified had the same pregnant women been longitudinally followed up during the gestational period.

Conflict of interest

None declared.

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VIA MEDICA

REVIEW

Adam J Białas[1], Anna Kumor-Kisielewska[1], Paweł Górski[2]

[1]Department of Pathobiology of Respiratory Diseases, Medical University of Lodz, Lodz, Poland
[2]Department of Pneumology and Allergy, Medical University of Lodz, Lodz, Poland

Ageing, sex, obesity, smoking and COVID-19 — truths, myths and speculations

Abstract

In early December 2019, in the city of Wuhan in Hubei Province, China, the first infections by a novel coronavirus were reported. Since then, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been spreading to other cities and countries becoming the global emerging epidemiological issue and quickly reaching the status of a pandemic. Multiple risk factors of disease severity and mortality have been identified so far. These include old age, male sex, smoking, and obesity. This concise narrative review highlights the important role of these factors in the pathobiology and clinical landscape of Coronavirus Disease 2019 (COVID-19). We especially focused on their significant role in disease severity and mortality. However, in spite of intensive research, most of the presented pieces of evidence are weak and need further verification.

Key words: coronavirus infections, SARS-CoV-2, COVID-19, morbidity, mortality, risk factors

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Introduction

In early December 2019, in the city of Wuhan in Hubei Province, China, the first infections by a novel coronavirus were reported. The new pathogen was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease in which it is an etiological factor for was named coronavirus disease 2019 (COVID-19) [1]. Since then, SARS-CoV-2 has been spreading to other cities and countries becoming the global emerging epidemiological issue and quickly reaching the status of a pandemic. On the 4th of March, the first SARS-CoV-2 positive patient was officially confirmed by the Polish Ministry of Health [2]. Since that time, morbidity and mortality have been increasing across the country.

The key to effective treatment of these patients is to increase knowledge about the pathobiology of the disease which does not seem to be fully understood so far.

Multiple risk factors of disease severity and mortality have been identified so far. These include old age, male sex, smoking, and obesity [3–8]. Therefore, in our considerations, we decided to cover these issues in the broad and multifaceted context of interactions, mainly focusing on its interference with the immunopathological background of COVID-19. We attempted to summarize the current state of knowledge and draw some hypotheses for further research in order to raise questions and open up a debate about analyzed issues. Figure 1 summarizes the main points discussed in the manuscript.

Beyond age…

Ageing may be defined as a progressive decline in tissue homeostasis due, at least in part, to the accumulation of replicative, oxidative, and genotoxic stress over time [9]. An increasing body of evidence suggests that ageing is associated with chronic inflammation, both pathological and physiological.

Multiple causes may contribute to ageing-associated inflammation such as pro-inflammatory tissue damage, a dysfunctional immune system, proinflammatory cytokines secreted

Address for correspondence: Paweł Górski, Department of Pneumology and Allergy, Medical University of Lodz, Poland; e-mail: pawel.gorski@umed.lodz.pl
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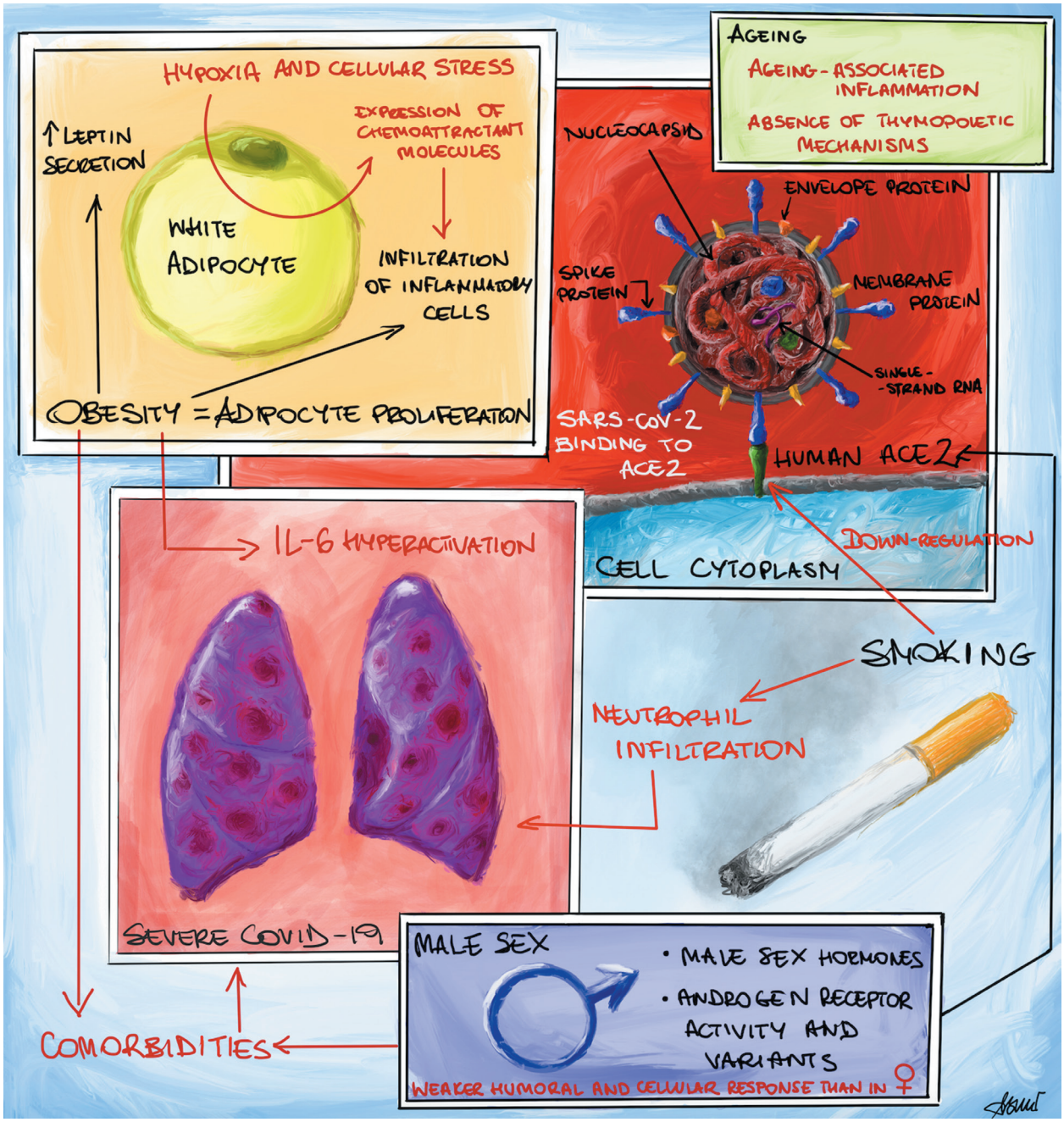

Figure 1. Graphical summary of the manuscript (author: AJ Białas)

by senescent cells, enhanced Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, and a defective autophagy response. These factors enhance the activation of inflammatory pathways (i.e. the nod-like receptor 3 (NLRP3) inflammasome) and then induce the production of cytokines such as interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), and interferons (IFNs). Long term inflammation could be a major cause of ageing-associated diseases [10]. In the SARS-CoV-2 infection, viroporin 3a has also been shown to trigger the activation of the inflammasome NOD 3-like receptor protein (NLRP3) and the secretion of IL-1β in bone marrow macrophages. This condition may result in an increased release of a large number of pro-inflammatory agents. Reports suggest that a storm of cytokines is released in patients with SARS-CoV-2 disease which is mainly manifested by increased IL-2, IL-7, granulocyte colony stimulating factor (GC-SF), monocyte chemo-attracting protein 1 (MCP1), and TNF-α. Of all the interleukins, IL-6 has been found to be associated with highly pathogenic SARS-CoV-2 infection due to

increased viral replication mainly in the lower respiratory tract [11, 12].

Telomere-controlled cellular ageing

The inflammatory process associated with telomere-controlled cellular ageing can lead to reduced tissue regenerative capacity. This is probably due to the impairment of stem and progenitor cell functions.

A telomere is a region of tandem repeats of short DNA sequences at the ends of chromosomes which are important for their stability and allow for the complete replication of the ends [13, 14]. The telomere proteome consists of more than 200 proteins that are associated with different aspects of telomere functioning including their protection, elongation, or telomeric DNA synthesis [13–16]. Telomere length homeostasis is essential for the proper functioning of the cell [14, 17]. Telomeres are also an important element in the ageing of cells. As well, they are involved in maintaining immune homeostasis. They are also the main indicator of biological age and are a measurable marker of both inflammation and oxidative stress [10].

Normal ageing is, by itself, associated with telomere shortening. A significant shortening of telomeres leads to cell death. The speed of shortening can be further increased by inflammation and oxidative stress and thus, affects the ageing process. In addition, cell telomere shortening may be associated with an increase in NF-κB transcription factor activity and overexpression of inflammatory cytokines such as TNF-α, IL-6, and IFN-γ in circulating macrophages [10, 18]. NF-κB is a family of seven transcription factors (p50, p52, p100, p105, RelA/p65, RelB, and C-Rel) and plays a central role in inflammation and cell response by controlling gene network expression [19, 20]. Activation of NF-κB is redox-sensitive and can be regulated by the changes in the oxidant/antioxidant balance [19]. The members of this family may activate expression of many pro-inflammatory genes which also play a role in lung inflammation [19, 21]. This evidence justifies the reports about upregulation of NF-κB activity in both naturally aged mice and multiple progeroid mouse models of accelerated ageing [9].

The generation gap?

The specific vulnerability of older people to severe COVID-19 disease is well documented. However, let us discuss why children seem to be more protected.

Scarpa *et al.* suggest that the essential role of the thymus could be crucial in the modulation of the immune response toward SARS-CoV-2 leading to a less severe phenotype in children when compared with adult COVID-19 patients. They identify inflammaging associated with the absence of thymopoietic mechanisms as a potentially predisposing condition that sustains the surge of proinflammatory cytokines that is specifically reported in older COVID-19 patients [22]. Indeed, severe adult COVID-19 pneumonia cases are associated with a decrease in regulatory T cells and CD4+/CD8+ T cells [23]. Since the thymus is the primary lymphoid organ essential for the development of T lymphocytes, its decreased functioning could lead to a drastic decrease in these T cells [24]. Moreover, one of the age-related changes in the immune system is the impaired generation of primary T cell responses against infection [24, 25].

Some facts about fats…

White adipose tissue is an active endocrine organ consisting of mature and developing adipocytes, endothelial cells, and immune cells such as adipose tissue macrophages, neutrophils, eosinophils, mast cells, and T and B cells. Thus, people with a normal body weight have a balance between adipocytes and immune cells which maintains tissue homeostasis. In particular, eosinophils and T-regulatory cells (Tregs) secrete anti-inflammatory cytokines (IL-10 and IL-4) that direct adipose tissue macrophages towards the anti-inflammatory phenotype, thereby maintaining a tolerogenic environment. This condition is also associated with the production of adiponectin, which increases insulin sensitivity [26, 27].

In turn, obesity causes adipocyte proliferation which leads to increased leptin secretion and infiltration of inflammatory cells. Adipocyte hypoxia and cellular stress were able to induce the expression of chemoattractant molecules with a consistent recruitment of macrophages, T cells, and B cells. When T cells are activated, the number of Treg cells is reduced [28]. The phenotype of macrophages changes from M2 to M1, which in turn accumulate around adipocytes and produce large amounts of proinflammatory cytokines such as TNF-α. In addition, obesity is characterized by a dysregulated secretion of adipokines such as leptin, adiponectin, LCN2, and PGRN which have become key regulators of the innate and adaptive immune systems [27, 28].

There is a lot of evidence showing a worse prognosis in obese patients infected with the

SARS-CoV-2 virus, especially among young people. It should also be mentioned that obesity is a well-known risk factor for respiratory diseases. Excessive growth of fat leads to hyperactivation of interleukin-6 and chronic inflammation which increases the risk of acquiring comorbidities such as diabetes and hypertension [27]. Again, both hypertension and diabetes are listed among the most prevalent risk factors for a worse prognosis in SARS-CoV-2 infection [12, 27].

When discussing the relationship between the inflammatory process and obesity in the SARS-COV-2 infection, we also have to discuss the role of mitochondria.

Generally speaking, mitochondria are multifunctional cellular organelles which play an important role in numerous aspects of cell morphology and physiology such as the synthesis of adenosine triphosphate (ATP), intracellular transfer of energy, redox homeostasis, regulation of cellular metabolism [29], cellular calcium homeostasis, synthesis of steroids [30], and apoptosis [31, 32]. However, mitochondria also play a key role in initiating inflammatory pathways. Mitochondrial dysfunction drives the ageing process by reducing cellular fitness, inflicting damage to other organelles, and/or causing mutations of the nuclear genome. One critical mechanism underlying the dysfunction of mitochondria is the accumulation of mutations and deletion of aged mitochondrial DNA (mtDNA), which is a consequence of the decline in mitochondrial autophagy (mitophagy). This process seems to decline in ageing individuals as the expression of several key components is decreased [33]. The dysfunction of mitophagy leads to the accumulation of damaged mitochondria, which then initiates inflammasomes and other inflammatory pathways. Mitochondria are essential for the NLRP3 inflammasome activation because they can activate NLRP3 inflammasomes by producing ROS. Furthermore, ROS-mediated mitochondrial permeability transition facilitates the release of mtDNA, which can stimulate pro-inflammatory signaling via the pattern recognition receptors RIG-I and MDA5, promoting the activation of NF-κB and interferon regulatory factors [33].

Based on the available data for SARS-CoV-2, its RNA is also located in mitochondria. It is postulated that SARS-CoV-2 transcripts found in mitochondria may affect mitochondrial function via ACE2 to avoid host cell immunity and to facilitate viral replication and COVID-19 disease [34]. Additionally, manipulation of host mitochondria by viral open-reading frames can release mitochondrial DNA (mtDNA) in the cytoplasm which can potentially serve as a potent danger-associated molecular pattern and can activate mtDNA-induced inflammasomes. This, in turn, can suppress both innate and adaptive immunity [33, 35].

Evil, damned evil and cigarette smoking…

The commonly accepted mechanism of SARS-CoV-2 cell entry is via use of the angiotensin converting enzyme 2 (ACE 2) receptor [36]. Some earlier papers suggested that smoking and nicotine are strong factors down-regulating ACE 2 expression [37]. Contrarily, results of current studies on airway epithelia are different [38, 39]. A potential adhesion site for SARS-CoV-2 is up-regulated in smokers independent of coexisting COPD. In the lung, SARS-Cov-2 infection and down-regulation of ACE2 receptors leads to increased angiotensin II activity that directly causes uncontrolled inflammation and lung damage [40, 41]. Although it has been suggested that an increase in the expression of ACE2 may contribute to the severe form of SARS-CoV-2-induced infection, it actually might be beneficial. Thus, looking for a prevalence of past or current smokers among COVID-19 patients might explain the important question both from a practical and theoretical point of view. Observational studies in China and in Italy have suggested that ARDS among smoking COVID patients is less prevalent than in patients who never smoked [42, 43]. The convincing meta-analysis of updated results from China confirms that active smoking is significantly associated with a risk of severe COVID-19 [44].

Smoking is strongly accompanied by neutrophilic airway inflammation [45]. SARS-CoV-2 infection induces biphasic immune responses. In the incubation stage, both innate and adaptive immunity are responsible for virus elimination. Therefore, inflammation can be limited, and the disease does not develop. Nearly 80% of infected people are only carriers of the infection [46].

In the second phase, when the protective response is diminished, the virus will propagate. Tissues damage develops following inflammation that is mostly mediated by neutrophils and macrophages and is especially noticeable in organs rich in ACE 2 receptors such as the lungs and kidneys. This cytokine storm seems to be mainly attributed to interleukin 6 [47].

There is no doubt that neutrophilic inflammation can be evoked and amplified by different factors. Smoking and other forms of indoor or

outdoor air pollution are two factors in the public space that can evoke neutrophilic infiltration of the lungs. Air pollutants have been shown to be important factors influencing both virus transmission and COVID development [48]. It is logical that the inflammatory process is amplified by smoking in severe stages of COVID. Controversies can arise, however, if nicotine, the stable constituent of cigarettes, is found to be protective against COVID progression. Nicotine has been found to be an agent that can prevent the onset of Acute Respiratory Distress Syndrome in animal models [49]. Nicotine has some anti-inflammatory properties due to agonistic cholinergic action [50]. Moreover, it inhibits the production of IL-6 and some other cytokines with no influence on interleukin-10 release [51]. Nicotine is approved by the FDA for medical use, but with many contraindications. Nicotine's mechanism of action might explain the controversies surrounding the influence of smoking on COVID initiation [52, 53]. However, emerging data indicate that both past and especially current smoking is an important determinant of worsening COVID-19 outcomes. This can have a positive effect on patients trying to quit smoking because the potential severity of the infection combined with the general knowledge about the harmful influence of smoking creates a situation where patients may consider smoking cessation. In fact, during the pandemic, many smokers tried to quit their smoking habit [54–56].

For individuals who use e-cigarettes, it is important to remember that they also have many harmful properties and can create a real hazard for future smoking addiction [57]. Heat-not-burn (HNB) tobacco products produce aerosol like traditional cigarettes, but with a lower level of harmful constituents, including carcinogens. It is important to remember that the impact of HNB products on population-level risk reduction will be maximized if smokers completely switch to the product (and abandon cigarettes). Individuals who use these products will find that their daily nicotine exposure remains stable or is reduced. That being said, the product will only reduce population-level risk if it does not attract non-smokers [58, 59]. To date, no study on vaping or HNB product use during COVID is available and discussing the possible result of replacing cigarette smoking with less harmful products is pure speculation. However, it seems logical that in smokers in whom all methods of smoking cessation were ineffective, new and different methods for combatting tobacco addiction should be explored.

To sum up this part of our considerations, it is evident that smoking promotes the progress of COVID-19. Paraphrasing Mark Twain, one can say that "there is evil, damned evil and cigarette smoking". Because of sociopsychological circumstances, the SARS-CoV-2 pandemic is an opportune time for smoking cessation in addicted people. The potential anti-inflammatory activity of nicotine does not seem to be significant seeing as the majority of studies show smoking as a promoting factor in the SARS-CoV-2 infection. However, nicotine replacement therapy (patches, gums) might be used during cessation therapy because of possible anti-inflammatory properties and a small risk of potential side effects. During smoking cessation, all known methods (i.e. psychosocial support, cytisine, varenicline) can be used. In smokers unable to quit smoking traditional cigarettes using these methods, using HNB products instead of traditional cigarettes may be recommended. According to a report by an independent study of Polish people trying to quit smoking, 50% of HNB product users achieved success. This is more than users of e-cigarettes, where only 25% of individuals succeeded. Moreover, in many young people, nicotine dependence starts from the use of e-cigarettes [23]. We do not recommend e-cigarettes since the balance of potential advantages and disadvantages from a public health point of view is negative.

When the lungs wear the pants…

Sex seems to be an interesting issue in the context of COVID-19. Notably, recent survival analysis performed by Li *et al.* revealed that male sex was one of the significant risk factors associated with death in patients with severe COVID-19 [6]. Chen *et al.*, in their early report from Wuhan, also reported that those who died in their cohort were more likely to be male [4]. Similarly, higher risks have been reported for men in the Italian population [7]. Another study by Jin *et al.* focused purely on gender differences in patients with COVID-19 in the context of severity and mortality and concluded that while males and females have the same prevalence, men infected with COVID-19 are at a greater risk for worse outcomes and death, independent of age [8].

In terms of analysis, we should initially examine differences in the expression of ACE2 by gender. Notably, it is activated and down-regulated by the spike protein of the virus which allows for the penetration of SARS-CoV-2 into epithelial cells and the myocardium. There is

evidence suggesting that hypertensive male mice have a spontaneously higher myocardial ACE2 expression than females, and that its levels decrease after orchiectomy [60]. This observation is concordant with the strong body of evidence indicating that ACE2 expression and activity may be influenced by sex hormones [61–65]. In light of this evidence, La Vignera *et al.* suggested that testosterone (or LH/hCG) administration could be temporarily discontinued or given at a lower posology in patients with hypogonadism, while estrogen replacement therapy may be considered in hypogonadal and postmenopausal women [60].

Male vulnerability to COVID-19 may be further enhanced by X-linked inheritance of genetic polymorphisms seeing as loci of both androgen receptors and ACE2 genes are located on the X chromosome [66].

On the other hand, ACE2 is also expressed in the testes (in spermatogonia, Leydig cells, and Sertoli cells) [67–69]. Ma *et al.* found that the level of serum luteinizing hormone (LH) was significantly increased, but that the ratio of testosterone to LH and the ratio of follicle stimulating hormone to LH was significantly decreased in males with COVID-19. This evidence, showing disturbances in male gonadal function, indicates the need for an increased focus on the evaluation of gonadal function among patients who have recovered from the SARS-CoV-2 infection, especially in reproductive-aged men [67].

Another interesting issue in the context of sex is transmembrane serine protease 2 (TMPRSS2) which is a critical factor in enabling cellular infection by coronaviruses, including SARS-CoV-2. Notably, androgen receptor activity seems to be required for the transcription of the *TMPRSS2* [66, 70].

In turn, McCoy *et al.* hypothesized that androgen receptor genetic variants associated with prostate cancer and androgenetic alopecia would be associated with racial variations in COVID-19 mortality. Authors also suggest that the use of anti-androgens like bicalutamide and enzalutamide or androgen modulators like finasteride and dutasteride may be beneficial [71].

Additionally, we have to emphasize that an increasing body of evidence indicates that there are dimorphic immune response differences to viral infections between males and females. These differences are unfavorable for males, who present with a higher mortality rate in epidemiological studies. Indeed, females mount a stronger immune response to viral infections due to more robust humoral and cellular immune responses. It is suggested that estrogen and testosterone differentially alter expression of genes involved in innate immunity (i.e. those encoding TLRs and interferons) thereby contributing to sexual dimorphism in the response to viral infections. These issues may also contribute to sex differences in the natural history of COVID-19.

However, most of the above-mentioned issues are just hypotheses and should be further verified. This is especially true in terms of the specific roles and levels of androgens in the pathobiology of COVID-19.

Conclusions

This review highlights the important role of ageing, sex, and smoking in the pathobiology and clinical landscape of COVID-19. We especially focused on their significant role in disease severity and mortality. However, in spite of intensive research, most of the presented pieces of evidence are weak and need further verification.

Conflict of interest

None declared.

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Mutlay Sayan[1], Swati Mamidanna[1], Mehmet Fuat Eren[2], Vasudev Daliparty[3], Teuta Zoto Mustafayev[4], Carl Nelson[5], Nisha Ohri[1], Salma K Jabbour[1], Aslihan Guven Mert[4], Banu Atalar[4]

[1]Rutgers Cancer Institute of New Jersey, New Brunswick, United States
[2]Radiation Oncology Clinic, Marmara University Istanbul Pendik Education and Research Hospital, Istanbul, Turkey
[3]Department of Internal Medicine, Raritan Bay Medical Center, Perth Amboy, New Jersey, USA
[4]Department of Medical Oncology, Mehmet Ali Aydınlar Acıbadem University, School of Medicine, Istanbul, Turkey
[5]Department of Radiation Oncology, University of Vermont Medical Center, Burlington, Vermont, USA

New horizons from novel therapies in malignant pleural mesothelioma

Abstract

Malignant pleural mesothelioma (MPM) is a relatively rare, but highly lethal cancer of the pleural mesothelial cells. Its pathogenesis is integrally linked to asbestos exposure. In spite of recent developments providing a more detailed understanding of the pathogenesis, the outcomes continue to be poor. To date, trimodality therapy involving surgery coupled with chemotherapy and/or radiotherapy remains the standard of therapy. The development of resistance of the tumor cells to radiation and several chemotherapeutic agents poses even greater challenges in the management of this cancer. Ionizing radiation damages cancer cell DNA and aids in therapeutic response, but it also activates cell survival signaling pathways that helps the tumor cells to overcome radiation-induced cytotoxicity. A careful evaluation of the biology involved in mesothelioma with an emphasis on the workings of pro-survival signaling pathways might offer some guidance for treatment options. This review focuses on the existing treatment options for MPM, novel treatment approaches based on recent studies combining the use of inhibitors which target different pro-survival pathways, and radiotherapy to optimize treatment.

Key words: mesothelioma, chemotherapy, radiotherapy, targeted therapy, immunotherapy

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Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer with a reported worldwide incidence of only 10 to 30 cases per million [1]. This malignancy involves the mesothelial cells of the pleura and its pathogenesis is attributed to a direct causal relationship with prolonged exposure to airborne asbestos particles. Chronic exposure to asbestos leads to inflammatory changes in the pleural mesothelium, subsequently leading to malignant transformation [2]. The most common sub-type of MPM is the epithelioid variety, followed by the sarcomatous and biphasic types [3].

The current standard treatment strategies for MPM include surgery for resectable tumors in combination with radiotherapy (RT) and chemotherapy. In spite of recent advances and research focused towards novel approaches to manage MPM, the median survival of patients with MPM is still estimated to be 8 to 14 months [4]. With the emerging resistance of the MPM tumor cells to RT and chemotherapy, there is an even greater need for developing new treatment approaches which can bypass and overcome these obstacles. Cancer immunotherapy has shown tremendous promise in providing solutions which can aid in improving the poor outcomes that continue to be associated with MPM. A more detailed understanding of the molecular biological mechanisms in MPM has provided hope that novel combinations of targeted therapies along with administration of radiation can be successfully developed to enhance the immune response against this malignancy. In view of this, we evaluate the available treatment

Address for correspondence: Mutlay Sayan, Rutgers Cancer Institute of New Jersey, New Brunswick, United States; e-mail: ms2641@cinj.rutgers.edu
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modalities for MPM, with a particular emphasis placed on the various cell survival pathways and the latest developments in the field of targeted chemotherapy and immunotherapy.

Current treatment approaches in MPM

The trimodality treatment involving surgery in combination with chemotherapy and/or radiotherapy continues to be the standard approach for management of MPM. Being an important and common treatment option in the treatment of MPM, surgery has been employed to either potentially cure the cancer or to provide palliation. The two main surgical methods include extra-pleural pneumonectomy (EPP) and pleurectomy with decortication (P/D). The preferred surgical of MPM continues to be widely debated with several studies showing conflicting results. Some retrospective studies have favored extended P/D, because it shows lower peri-operative mortality, morbidity, lower post-operative complications and better survival when compared to EPP (5, 6). Owing to the lack of randomized control trials, a clear conclusion of the optimal surgical procedure cannot yet be clearly determined. The National Comprehensive Cancer Network (NCCN) guidelines for MPM suggest that P/D is considered safer to EPP due to less post-operative complications and a higher quality of life.

RT is employed as an adjuvant or neoadjuvant treatment option in MPM and is mainly considered a palliative option [7]. Intensity modulated proton therapy has been suggested as a feasible option in the management of MPM in a recent study involving 7 patients [8]. Volumetric modulated arc therapy, a rotational form of intensity-modulated RT has also been considered in management of MPM [9]. Chemotherapy is considered a part of the trimodality regimen for MPM in patients, either before or after surgery and has shown to provide a median OS of 24 months [10]. In patients who refuse surgery and in medically inoperable patients, chemotherapy alone can also be considered [11]. The first-line treatment for MPM includes a combination of pemetrexed and cisplatin. Based on a recent study by Zalcman *et al.*, the NCCN guidelines recommended addition of bevacizumab to cisplatin-pemetrexed regimen for patients with unresectable MPM [12]. Pemetrexed-carboplatin and gemcitabine-cisplatin have also been recommended as acceptable first-line regimens based on relevant recent studies [13, 14].

Induction of cell survival pathways

Epidermal growth factor receptor (EGFR) pathway

EGFR is a transmembrane glycoprotein which belongs to the Her1 group of the ErbB family of tyrosine kinases [15]. This receptor forms an integral part of a complex signaling cascade which plays an important role in physiological pathways including controlling cell growth, proliferation and survival. Ligand binding to EGFR, phosphorylation of tyrosine residues, followed by receptor dimerization are the three steps which lead to cellular proliferation [16]. The overexpression of these receptors has been deemed significant in the pathogenesis of several cancers, which has led to the focus on development of targeted therapies towards these receptors [17]. Overexpression of EGFR is noted in about 70% of tissue specimens of MPM [18]. Asbestos, which is the major carcinogen associated with MPM, triggers aggregation of EGFR by forming reactive oxygen species, resulting in autophosphorylation and EGFR activation. This is subsequently followed by activation of the RAS/RAF/MAPK pathway leading to cellular proliferation and metastasis [19, 20]. There are several studies which have focused on EGFR gene mutations involved in MPM over the last few years. A study conducted in Japan demonstrated the presence of missense mutations of the EGFR gene in some cases of MPM [21]. Recent studies have suggested an epigenetic component involved in development of MPM. Moreover, oncogenic EGFR gene has been shown to cause downregulation and repression of Ten-eleven translocation enzymes (TET) DNA methylase, which leads to silencing of tumor suppressors [22].

The effect of radiation on the proliferation of tumor cells and association with EGFR has been studied extensively. The amplification of cellular proliferation after exposure to ionizing radiation is termed as accelerated repopulation and has been shown to be contributing partly to the development of radioresistance, especially in head and neck malignancies [23]. The possible mechanisms of EGFR phosphorylation and subsequent activation by radiation has been attributed to the release of TGF-alpha, which is an EGFR binding ligand [24]. A study detailing the effects of the combination of RT with ZD1839 (Iressa), which is a selective EGFR inhibitor in several cancers including mesotheliomas, conclusively showed that the combination arm yielded significant improvements in therapeutic index for radiation and enhanced tumor suppression compared to the radiation alone arm [25].

The two classes of drugs with established anti-EGFR targeted action are tyrosine kinase inhibitors (TKI) and monoclonal antibodies. TKIs such as gefitinib, afatinib, erlotinib, canertinib and lapertinib impart their action by acting as ATP analogues, competitively inhibiting the catalytic tyrosine kinase domain intracellularly. In-vitro studies have demonstrated that small molecule TKIs effectively decrease MPM cell proliferation [26]. However, initial clinical trials involving gefitinib showed no clinical efficacy in patients with MPM [27]. This contrast has been attributed to the theory that TKIs exert their action only in the presence of activating mutations of the EGFR gene, which are very rarely seen in MPM. As very few patients carry these mutations, the clinical efficacy of these drugs, when given individually, is lacking [28]. A Japanese study has been conducted in a patient harboring a rare EGFR mutation of G719C and S768I had been successfully treated with afatinib [29].

Monoclonal antibodies against the extracellular portion of EGFR such as cetuximab, nimotuzumab and panitumumab act by creating ligand inhibition, thereby blocking receptor dimerization and further downstream signaling. They also cause internalization and degradation of EGFR receptors leading to further downregulation [30]. The use of cetuximab, a chimeric mouse-human antibody, in rodent models has demonstrated significant tumor inhibition and improved survival [31]. Another monoclonal antibody, Nimotuzumab, approved for the treatment of colorectal and head and neck cancers, has demonstrated significant reduction in tumor volumes when compared to cisplatin-gemcitabine chemotherapy in animal models [32]. Novel EGFR targeted nanotechnology delivery techniques such as TargomiRs which are targeted minicells which are loaded with miR-16 mimic microRNA have also shown promise in preclinical models and clinical trials [33, 34].

Extracellular signal-regulated kinase (ERK) pathway

Mitogen-activated protein kinase (MAPK) pathway, which forms an integral link between upstream extracellular stimuli and downstream intracellular effectors, regulates cell differentiation, proliferation and death in both physiological and pathological milieus [35]. This cascade, which has been identified to be the most frequently mutated signaling pathway in humans, is composed of a RAS-RAF-MEK-ERK chain. Two types of distinct ERK proteins, ERK1 and ERK2, are activated by MEK through phosphorylation of their tyrosine and threonine residues, which results in the activation of transcription factors and kinases which predominantly orchestrates cellular proliferation. Activation of several feedback loops between substrates and ERK further amplifies cell differentiation [36]. The significance of ERK has been clearly demonstrated in studies related to epithelioid type of MPM. ERK2 was conclusively shown to be critical in the transformation and homeostasis of mesotheliomas by controlling gene expression in animal studies [37]. An in-depth analysis of asbestos-induced signaling pathways also reiterated the importance of ERK in the development of malignant mesothelioma. The transcription factor activator protein-1 controls proliferation of mesothelial cells by elevation of ERK-dependent Fos-related antigen (Fra)-1 [38]. Thus, downstream ERK, which controls a critical juncture in this pathway, has been targeted as a potential opportunity for treatment of cancers, especially those with MEK, RAF and RAS mutations.

Radiation leads to activation of the ERK pathway by causing tyrosine and threonine phosphorylation of ERK1 and ERK2 by MEK, which prolongs cell survival and proliferation [39]. This activation of ERK by radiation leads to expression of anti-apoptotic proteins such as Mcl-1, and Bcl-xL [40]. It also inhibits some pro-apoptotic proteins such as caspase 9 and Bim, leading to the inhibition of tumor cell suppression [41]. A link between radioresistance and ERK5 has been established in a study by Jiang *et al.* which showed that ERK5 overactivation was noted in lung cancer development and G2/M cycle transmission. ERK5 was also identified as a potential regulator of radiosensitivity in cancer cells and supported its use as a biomarker to predict radiosensitivity [42].

In therapeutic targeting of the MAPK pathway, the initial design and development of newer drugs was focused on RAF and MEK proteins. The inevitable drug resistance that develops against RAF and MEK inhibitors has shifted the focus to novel ERK inhibitors. Use of a specific ERK5 inhibitor XMD8-92 in human MPM cells conclusively showed inhibition of ERK5 phosphorylation, which eventually led to attenuation of MPM tumor growth by an inflammasome-mediated mechanism [43]. A recent study demonstrated that zoledronic acid can potentially aid in restoring immune reactivity and chemosensitivity in MPM by decreasing RAS/ERK activity [44]. A single-arm clinical trial carried out in

8 patients with advanced MPM showed modest clinical activity and no significant toxic effects in patients who received zoledronic acid [45]. An in vivo microenvironment study showed that Pirfenidone can decrease proliferation and migration of MPM cells by causing inhibition of ERK [46]. Arsenic trioxide (ATO), an inorganic compound used in traditional Chinese medicine, has been shown to induce apoptosis in MPM cell lines by affecting MAPK pathways such as ERK and c-Jun NH2-terminal kinase pathway [47]. In addition to these mechanisms, ATO has also demonstrated apoptosis in MPM cell lines by downregulating thymidylate synthase, Gli1 expression and E2F1 transcription factor [48, 49]. The potential benefits of these agents in all these studies provide a possible option in the future for repurposing them for use in MPM patients.

cAMP response element binding protein (CREB)

CREB, which belongs to the group of basic leucine zipper (bZIP) containing transcription factors, is a major regulator of basic cellular homeostasis, differentiation and growth [50]. After undergoing phosphorylation at its serine residues by other kinases, the transcriptional activity of CREB is activated [51]. Subsequently by regulating histone H3 and H4 methylation which controls chromatin recruitment, CREB modulates a number of physiological processes such as cell cycle, DNA repair, cell proliferation, angiogenesis, inflammation and immune responses [52, 53]. Overexpression of CREB has been implicated in many cancers such as glioblastoma, non-small cell lung carcinoma, breast carcinoma, hematopoietic malignancies and malignant mesothelioma, to name a few [54, 55]. CREB overexpression has also been shown to correlate with tumor recurrences, poorer prognoses and reduced survival in tumor patients [56, 57]. Several studies have emphasized the role of CREB in the pathogenesis of malignant mesothelioma. An in vitro study conducted on MPM cells showed that asbestos induced apoptosis also triggered the expression of several CREB target genes. It also demonstrated that doxorubicin increased the phosphorylation of CREB1 [58]. Another study using genetically CREB-silenced MPM cell lines and mouse xenograft models, conclusively proved that by regulating inflammatory signals, CREB plays a major role in controlling MPM tumor growth and development [59].

The activation of CREB following induction with radiation has been linked to radiosensitivity in several studies. A study by D'Auria *et al.* suggested that low dose radiation can trigger activation of CREB leading to cell survival. It also reviewed the pro-apoptotic role of CREB after exposure to ionizing radiation. This suggests that multiple mechanisms are involved in the radiation-CREB interaction and that future clinical trials involving this combination can provide a solution in cancer treatment [60]. Another study by Cataldi *et al.* showed that activation of CREB improves signal in leukemia cells which were exposed to ionizing radiation [61].

KG-501, a CREB inhibitor has been identified which can reversibly inhibit the interaction between CREB and CBP (CREB binding protein). The use of vandetanib, a tyrosine kinase inhibitor, along with doxorubicin in human MPM lines demonstrated that vandetanib alone decreased cell numbers in epithelioid cell lines and when used together synergistically resulted in increased doxorubicin toxicity in both epithelioid and sarcomatous cell lines [62]. This study suggested the combined use of these two drugs as a potential treatment option for MPM, owing to the impact on both ERK5 and CREB pathways. A new agent 666-15 has been identified as a CREB inhibitor with significant anti-tumor effects noted in both in vivo and in vitro studies and it holds promise as a future therapeutic option for MPM [63, 64].

Protein kinase B (AKT)

AKT is a serine/threonine kinase which exists as 3 isoforms and controls many cellular activities such as glucose metabolism, cell cycle progression and protein synthesis. It also blocks apoptosis by causing inactivation of several pro-apoptotic proteins [65, 66]. After being activated by phosphorylation, AKT induces a number of proteins located in the nucleus, cytosol and plasma membrane such as PRAS40, vimentin, palladin, p21 and p27 which enhance metastatic proliferation of cells [67, 68]. The system located upstream of AKT generates phosphatidylinositol triphosphate (PIP3) with the action of phosphoinositide 3-kinase (PI3K) [69]. AKT over activation has been commonly noted in several human malignancies including ovarian carcinoma, gastric carcinoma and pancreatic cancer [70]. Some proteins in the AKT pathway such as PI3K, periostin, eIF4E function as oncoproteins when they are overexpressed. On the other hand, inactivation of tumor suppressor genes in the AKT pathway such as PTEN, TSC and FOXO leads towards malignancy causing paths, with PTEN mutations being the highest frequency [71, 72].

The role of radiation on AKT pathway has been analyzed in many studies. Li *et al*. conclusively proved in a study involving 8 cell lines of glioblastoma multiforme that induction of AKT activation by ionizing radiation led to an increase in radioresistance of the cancer cells. They showed that a serum factor may be involved and EGFR inhibition by AG1478 and PI3K inhibition with LY294002 can help increase radiosensitivity in tumor cells [73]. Toulany *et al*. pointed out that understanding the specific dysregulations of AKT such as gene amplification, point mutations and overexpression, which eventually lead to AKT activation might result in a clearer estimate of the outcome to radiation administration [74]. They demonstrated that the dual inhibition of AKT and MEK increased radiosensitivity in k-RAS mutated non-small cell lung cancer. They also emphasized the control of DNA double strand repair by AKT activation might serve as a future target to enhance radiosensitivity.

Several studies have dealt with the association between abnormalities in AKT/PI3K pathway and its role in the pathogenesis of MPM. In a study conducted by Suzuki *et al.* in 21 MPM cell lines, downregulation of PTEN was most frequently identified as the cause for activation of AKT pathway [75]. A study by Varghese *et al*. on the molecular nature of MPM conclusively proved that activation of PI3K and mTOR resulted in shortened survival in patients with MPM [76]. Zhou *et al.* demonstrated that the PI3K/AKT/mTOR pathway is a crucial cascade downstream of multiple activated receptor tyrosine kinases (RTK) suggesting the future potential of multi-point targeting of PI3K/mTOR as a therapeutic consideration in mesothelioma. Dual targeting of PI3K/mTOR by BEZ235 had a more significant effect on MPM inhibition compared to individual inhibition [77]. AKT kinase interacting protein (Aki1) is a scaffold protein for the PI3K–PDK1–AKT module. A study by Yamada *et al*. in cell-based assays showed that Aki1 silencing affected the CREB pathway and led to decreased cell viability in MPM tumors [78].

Programmed death-ligand 1 (PD-L1)

Programmed cell death receptor 1 (PD-1) is expressed on activated T cells and with its ligands PD-L1 and PDL2, it controls T-cell effector functions [79]. Several studies have demonstrated that PD-L1 overexpression is noted in around 30–40% of MPM patients, with a relatively greater incidence in non-epithelioid subtypes [80]. Moreover, MPM with PD-L1 positivity has significantly been associated with a poorer prognosis than PD-L1 negative MPM (median survival of 4.8–5.0 months vs 14.5-16.3 months) [81, 82]. This finding led to the consideration that immune checkpoint inhibitors which affect PD-L1 can provide benefit in MPM.

Pembrolizumab, a humanized monoclonal antibody against PD-1, with a favorable safety profile and strong anti-tumor activity, has been approved for use in the management of several malignancies in more than 50 countries [83]. One of the first studies which involved pembrolizumab in MPM was the Keynote-028 Phase I trial conducted in 25 patients, and it showed a disease control rate (DCR) of 72%, a response rate of 20%, a median response duration of 12 months and was well tolerated [84]. Single arm phase II trials involving nivolumab, which also has PD-1 action showed objective response rates (ORR) between 15–29% and a median progression free survival (mPFS) between 2.6–6.1 months [85, 86]. Another agent avelumab, which has PD-L1 blocking activity, had a response rate of 9.4% in a study of 53 patients [87]. The CONFIRM trial, involving 336 patients with MPM randomized to nivolumab or placebo, which is ongoing in the UK hopefully will shed more light on this aspect [88].

Cytotoxic T lymphocyte-associated antigen (CTLA-4)

CTLA-4, an inhibitory receptor located on T lymphocytes, binds competitively to CD80 and CD86 ligands and attenuates CD28-mediated T cell activation. By inhibiting CTLA-4, there can be an increase in T cell activation which aids in mounting stronger anti-tumor immune responses. Ipilimumab and tremelimumab belong to the CTLA-4 family and have shown significant benefit in patients with advanced malignancies [89, 90]. Retrospective analysis of the phase II MESOT-TREM-2008 study revealed that the dosage of tremelimumab (15 mg/kg every 90 days) in chemotherapy-resistant advanced MPM was low [91]. This was followed by the MESOT-TREM-2012 trial with an increased dosage of tremelimumab (10 mg/kg every 4 weeks, and after 6 cycles every 12 weeks). Compared to 1 patient who achieved partial response in the 2008 study, 11 patients achieved disease control in the 2012 study [92]. Following the success of these 2 studies, the DETERMINE trial was conducted, a randomized controlled trial involving 571 patients who were randomized to tremelimumab or placebo arm. However, this study showed that tremelimumab did not significantly prolong survival or improve response in patients with previously treated MM [93].

A combination therapy of PD-L1 and CTLA-4 checkpoint inhibitors has also been studied with the aim to look for a more effective response in MPM patients. The MAPS-II trial, which included 125 patients with relapsed MPM across 21 hospitals in France, compared nivolumab (anti-PD1) with nivolumab plus ipilimumab (anti-CTLA4). It was concluded at the end of the study that nivolumab with or without ipilimumab showed an equally meaningful clinical response but higher drug-related adverse events where noted in the combination group (93% in combination vs 89% in monotherapy) [94]. A combination of tremelimumab and durvalumab in the NIBIT trial conducted in 40 patients with MPM showed comparable results to the MAPS-II trial [95]. The INITIATE trial, a single-arm phase II trial in 36 eligible patients with recurrent MPM, studied the combination of ipilimumab with nivolumab. A response rate of 38% and a DCR of 68% was noted at the study conclusion, but 94% reported experiencing an adverse event [96].

Dendritic cell (DC) therapy

DCs have often been referred to as "nature's adjuvants" owing to the important role they carry out in initiating an immune response by capturing antigens and efficiently presenting them to lymphoid T cells. DCs also modulate humoral immunity by directly interacting with B cells and indirectly with CD4+ T helper cells [98]. Over the last decade, DCs have become an integral target in cancer immunotherapy. A study by Cornelissen *et al*. in 10 patients with MPM, DCs were administered with cyclophosphamide showed a promising overall mean survival of 37 months (98). A recently performed clinical trial in which 9 patients with MPM were administered DCs pulsed with allogenic tumor lysate, 2 patients showed a partial response and a median OS higher than 22.8 months in all the patients [99].

Conclusion

MPM is an aggressive cancer of the pleural lining and continues to present challenges in its management. Radioresistance of the tumor cells as well as increasing resistance to chemotherapeutic agents, have made the achievement of optimal response rates difficult. A better understanding of the various cell survival and pro-apoptotic signal pathways might open up new avenues in the treatment of MPM. Employing inhibitors targeting EGFR, ERK, CREB and AKT pathways, in combination with radiotherapy, might help in overcoming the radiation resistance developed by tumor cells after administration of RT. Novel combinations of such small molecule inhibitors with existing approved chemotherapy regimens for MPM is also another possible alternative. Treatment options might also be available in the future in the domain of immunotherapy with several recent studies on inhibitors of PD-L1, CTLA-4 and dendritic cells showing promising results. There is a need to continue making efforts to further substantiate and deepen the understanding of the molecular mechanisms involved in MPM and to conduct clinical trials with the goal of optimizing treatment of MPM.

Conflict of interest

None declared.

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Nishant Chauhan, Aneesa Shahul S, Shahir Asfahan, Kunal Deokar, Ram Niwas

Department of Pulmonary Medicine, All India Institute of Medical Sciences Jodhpur, India

Cotrimoxazole-induced SIADH — a unique challenge during treatment of pulmonary nocardiosis

Abstract

A 62 year old male non-smoker diagnosed with pulmonary nocardiosis was initiated on Cotrimoxazole therapy at a dose of 20 mg/kg per day in three divided doses. He developed hyponatremia (serum sodium 105 mEq/L) on day 3 of therapy. The potential causes of hyponatremia were evaluated. After ruling out other causes, the cause was suspected to be Cotrimoxazole-induced syndrome of inappropriate anti-diuretic hormone secretion (SIADH). We subsequently re-initiated therapy with Cotrimoxazole and the hyponatremia (serum sodium 110 mEq/L) recurred. Upon discontinuation of therapy, serum sodium levels returned to normal. The patient was started on Amoxycillin-Clavulanic Acid as an alternative therapy for pulmonary nocardiosis which resulted in resolution of the hyponatremia. Cotrimoxazole-induced SIADH is a rare occurrence. This case is representative of a patient with Cotrimoxazole-induced SIADH and the causal relationship was confirmed once resumption of therapy with the offending medication resulted in hyponatremia.

Clinicians should be aware of this rare adverse effect of Cotrimoxazole and should monitor serum electrolytes during therapy, especially in the elderly and in those receiving high doses.

Key words: cotrimoxazole, SIADH, nocardiosis

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Introduction

Pulmonary nocardiosis, previously thought of as a disease that occurs mainly in the immunocompromised population, has become increasingly reported in individuals with structural lung diseases [1, 2]. Cotrimoxazole is the drug of choice for the treatment of nocardiosis. Although widely considered to be a drug with a decent safety profile, it has its own major and minor adverse effects [3, 4]. SIADH is a rare but serious complication associated with Cotrimoxazole therapy. Its occurrence is more frequently observed than previously recorded. SIADH is a disorder caused by the inability to suppress secretion of anti-diuretic hormone. There is impairment of water excretion and if water intake is in excess of urine output, water retention occurs leading to hyponatremia [5, 6]. The risk appears to be highest in the elderly population and is essentially dose-dependent [5, 7].

Case history

A 62-year-old male non-smoker was admitted due to complaints of acute worsening of breathlessness, fever, and cough with expectoration that had been present for 5 days. The fever was intermittent and high grade, and was associated with chills and rigor. Cough was productive with copious yellowish non-foul-smelling sputum. He had a history of sputum smear-positive pulmonary tuberculosis six years ago for which he received antitubercular therapy. He had developed obstructive airway disease as a sequela of pulmonary tuberculosis which was confirmed by spirometry. He was treated with inhaled bronchodilators for five years.

On admission he was severely ill, febrile (oral temperature 38.9°C), tachypneic, tachycardic, and normotensive with an oxygen saturation of 82% on room air.

Respiratory system examination revealed bilateral diffuse coarse crackles and rhonchi.

Address for correspondence: Aneesa Shahul S, Department of Pulmonary Medicine, All India Institute of Medical Sciences Jodhpur, India; e-mail: aneesambbs@gmail.com

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Other systemic examinations were unremarkable.

On evaluation, he had neutrophilic leukocytosis. The total count was 21,010 (89% neutrophils). Liver and renal function parameters were normal. Other unordinary lab results were documented as follows: serum sodium on admission was 134 mg/dL, the C-reactive protein level was 190 mg/L, and serum procalcitonin was 2.86 ng/mL. Chest X-ray showed bilateral non-homogenous opacities with haziness over the right upper zone (Figure 1).

He was started on empirical antibiotic therapy with IV Piperacillin-Tazobactam (4.5 g 4 ×/ /diem) and oral Azithromycin (500mg 1 ×/diem). Oxygen supplementation, nebulized bronchodilators, and other supportive therapy was provided. On day four, in view of inadequate clinical improvement, a contrast-enhanced computed tomogram of the thorax was completed which showed ground-glass opacities over the right upper lobe with multiple bilateral patchy areas of consolidation and centrilobular nodules (Figure 2).

Flexible fiberoptic bronchoscopy was planned and completed under continuous oxygen supplementation by nasal cannula with a target oxygen saturation of 90%. It showed purulent secretions from the right lobar and segmental bronchi. Bronchial anatomy was normal and aerobic cultures of bronchial secretions, Ziehl Nielsen staining, Gram-staining, Mycobacterium culture were sent. There was no bronchoscopy-related complication. An aerobic culture of bronchial fluids showed growth of Nocardia (the specific species were not identified). Modified acid-fast bacilli (AFB) stain after bronchial lavage showed filamentous branched acid-fast positive bacilli. Fungal stain and culture were negative. Mycobacterium culture was negative at the end of the required 42-day culture period.

As part of the Nocardia pneumonia treatment regimen, the patient was started on oral Cotrimoxazole at a dose of 20 mg/kg body weight. After 3 days of treatment, the patient developed asymptomatic hyponatremia. Sodium levels had fallen from 134 mEq/L to 129 mEq/L, and then subsequently to 105mEq/L. Even at this point, he did not have any symptoms. Causes of this electrolyte imbalance were investigated. Thyroid function tests were done and serum cortisol was measured and both results were found to be normal. A CT scan of the brain was normal. After ruling out other causes, the cause of the hyponatremia was suspected to be Cotrimoxazole-induced SIADH (serum sodium 105 mEq/L, urine osmolality 468 mOsm/kg, serum osmolality 250 mOsm/kg, and urine sodium 82 mmol/L). Fluid restriction was initiated, and hypertonic saline was administered. Cotrimoxazole was stopped and serum sodium levels returned to normal after continuing the above measures.

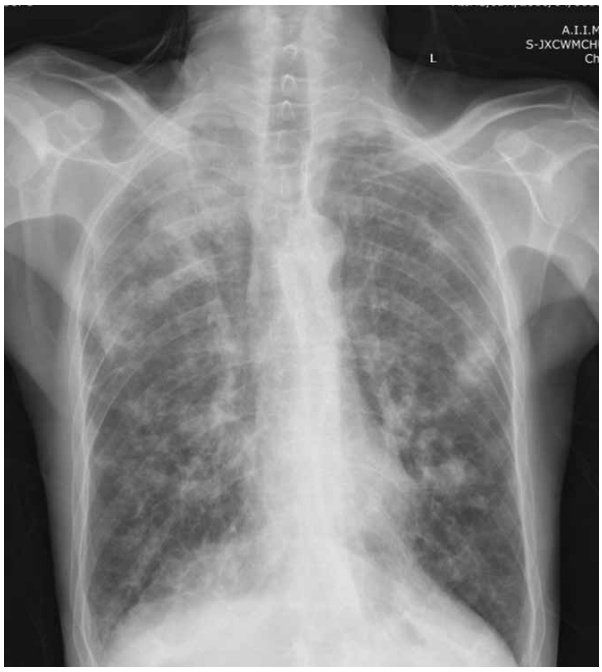

Figure 1. Chest radiograph PA view showing multiple bilateral air space opacities and bilateral flattened diaphragms

Since the treatment of Nocardia involves the use of long-term antibiotics, we decided to re-introduce Cotrimoxazole to see if it was the cause of the hyponatremia. On reintroduction of Cotrimoxazole, a fall in serum sodium levels was observed by day 3 (a level of 110 mEq/L). As a result, Cotrimoxazole was stopped and the patient was started on IV Amoxycillin-clavulanate (1.2 g, 3 ×/diem) as an alternative regimen. He received parenteral antibiotics for 10 days. There was a serial fall in total WBC count to 14,320, and serum procalcitonin had fallen from an initial 2.86 ng/mL to 0.11 ng/mL. The patient's clinical condition improved gradually and he was weaned off oxygen therapy while continuing therapy with Amoxycillin-clavulanate (625 mg 3×/diem) which was now administered orally. He was discharged in a stable condition and advised to continue oral Amoxycillin-clavulanate therapy at a dose 625 mg thrice daily for a duration of three months. Subsequent chest radiographs taken on follow up after 40 days of therapy showed significant clearance of radiological opacities (Figure 3).

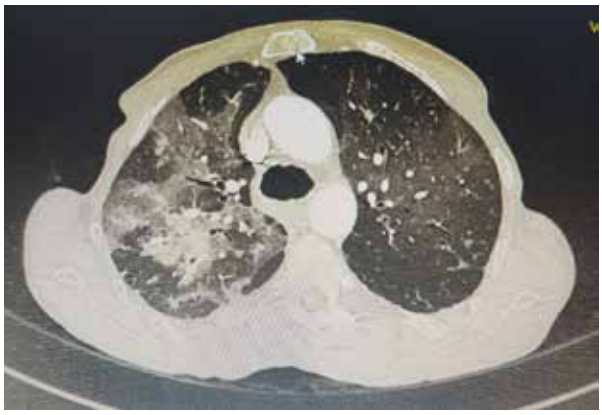

Figure 2. Contrast-enhanced tomogram of the thorax showing bilateral upper lobe ground-glass opacities and right upper lobe consolidation

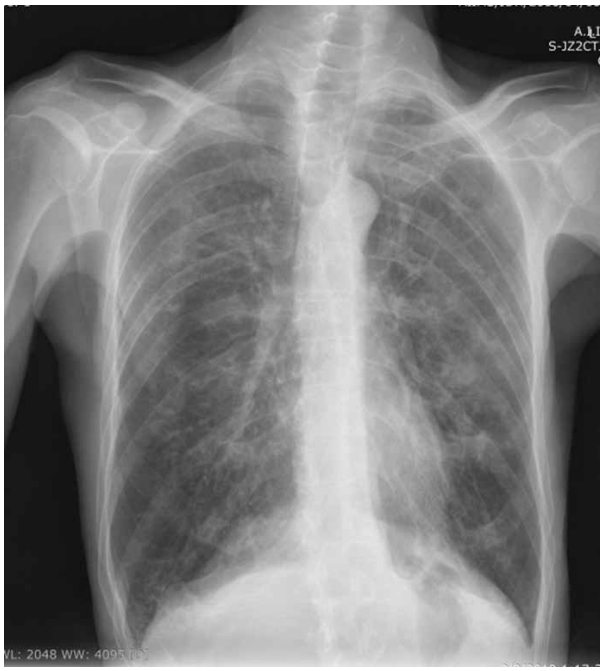

Figure 3. Chest radiograph showing clearance of previously noted opacities during follow-up

Discussion

Nocardiosis is classically known to be an opportunistic infection associated with impaired immunity, particularly cell-mediated immunity. However, up to one-third of patients have been found to be immunocompetent [2, 8].

Risk factors for Nocardiosis include infection with the Human Immunodeficiency Virus, transplants, cancers, rheumatic diseases, diabetes mellitus, steroid abuse, chronic obstructive pulmonary disease, and poor socio-economic conditions [8, 9].

Pulmonary involvement is the most common presentation of nocardiosis. Symptom onset may be acute, sub-acute or chronic and includes cough, dyspnea, chest pain, hemoptysis, fever, weight loss, and fatigue [2, 10]. Lobar consolidation is the most common radiographic presentation, followed by nodules/reticulations [10].

Although Trimethoprim-Sulfamethoxazole is the mainstay of treatment, the final drug regimen is decided on the basis of antimicrobial sensitivity, the severity of the disease process, and the organ of involvement [2, 10].

Cotrimoxazole is a sulfonamide antibiotic which contains Trimethoprim and Sulfamethoxazole. Trimethoprim is a heterocyclic weak base and is related in structure to the potassium-sparing diuretics amiloride and triamterene which act by blocking sodium reabsorption at the eNaC (epithelial sodium channel) in the distal nephron [10]. High doses of TMP has been shown to act as epithelial sodium channel inhibitors in the distal tubule.

SIADH can be caused by a variety of pulmonary and extra-pulmonary infections/malignancies, as well as by a range of medications [7]. SIADH is a common cause of hyponatremia. In a retrospective cohort study by Tsapepas et al, the incidence of hyponatremia was found to be 72.3% among those on high dose therapy with Cotrimoxazole [4]. Severe hyponatremia and SIADH is rare and described in only a few case-reports [11, 12].

In describing hyponatremia and its treatment, the cerebral effects are worth mentioning. The rate of sodium correction in hyponatremia of acute onset should be $\leq$ 10mEq/L in the first 24 hours and $\leq$ 8mEq/L for any 24-hour period thereafter. Effective serum osmolality is determined by serum sodium concentration. Hypotonic hyponatremia, if acute and severe, can lead to the entry of water into brain cells and ultimately, to cerebral edema [13, 14]. The brain adapts to such a level of hypotonicity by losing intracellular solutes, principally potassium and organic solutes called osmolytes. Hyponatremia, which develops within two or three days, is less likely to be complicated by cerebral edema because of this brain adaptation. Once the adaptation is complete, the rate of correction of hyponatremia is important. Overt rapid correction of hyponatremia can induce Osmotic Demyelination Syndrome (ODS) [13]. However, rapid correction in acute and severe hyponatremia does not induce ODS because the cerebral adaptation is still in its early stage [14].

In our case, the patient's thyroid and adrenal function, as well as brain imaging, were normal.

In view of no other plausible explanation for the hyponatremia, we considered Cotrimoxazole as the likely causative agent. The presence of low serum osmolality, high urine osmolality, and high urinary sodium in the absence of hypothyroidism and peripheral signs of hypovolemia pointed towards a potential iatrogenic cause. Once the hyponatremia was resolved with the cessation of Cotrimoxazole, and subsequently reappeared when therapy was re-started, the diagnosis of Cotrimoxazole induced-SIADH was evident.

Conclusion

This case report presents a rare but potentially fatal complication associated with Cotrimoxazole therapy: SIADH. Probable risk factors for this in our case are advanced age and a high dose of Cotrimoxazole. A high index of suspicion and close monitoring of serum electrolytes is necessary, especially in older patients.

Conflict of interest

None declared.

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Thomas William Lowder

University of Central Arkansas, Conway, United States

High-intensity exercise improves pulmonary function and exercise tolerance in a patient with TSC-LAM

Abstract

Introduction: While exercise has been shown to improve respiratory symptoms, exercise tolerance, and bone mineral density in many populations, no supervised exercise training interventions have been undertaken in patients with lymphangioleiomyomatosis (LAM).

Material and methods: One patient with TSC-LAM (tuberous sclerosis complex lymphangioleiomyomatosis) participated in two weekly sessions (50–60 min) of supervised aerobic exercise at 80–85% heart rate max for one year. Treadmill ergometry (VO_{2peak}), spirometry (FEV_1, FVC, FEV_1/FVC, peak flow), and bone mineral density testing were performed prior to every 3 months.

Results: After one year of supervised aerobic exercise training we saw dramatic increases in the patient's VO_{2max} (20%), FEV_1 (9.5%), FEV_1/FVC (9.1%) and peak flow (47%).

Conclusions: The results from this study indicate that supervised exercise training can improve exercise tolerance and pulmonary function in a patient with lymphangioleiomyomatosis. Further research is needed, including longitudinal studies with larger sample sizes, to determine long-term effects and consistency of these findings. Aerobic exercise may offer a viable alternative or compliment to pharmacological interventions in the treatment of lymphangioleiomyomatosis. We show that high-intensity exercise training can markedly and safely improve pulmonary function in a patient with TSC-LAM. While we did not record quality of life or mood states, our patient did report improved self-confidence as well as enhanced mood.

Key words: tuberous sclerosis, exercise, lymphangioleiomyomatosis

Adv Respir Med. 2020; 88: 356–359

Introduction

Approval was obtained from the Committee for Protection of Human Subjects at the University of Houston and the Institutional Review Board at the University of Texas Health Science Center (UTHSC) and at the University of Central Arkansas. Written informed consent was obtained from our patient prior to data collection. A 29-year-old female with tuberous sclerosis complex lymphangioleiomyomatosis (TSC-LAM; TSC diagnosed in 1988, age 5; LAM in 2004, age 21) with a history of related complications (more than twenty pneumothoraces, resulting in bilateral pleurodesis and pleurectomy; angiomyolipomas resulting in partial nephrecotmy) was referred from the UTHSC LAM Clinic by her LAM specialist. Her last pneumothorax was approximately five years prior to her inclusion in this trial (age 23), when she received her bilateral pleurectomy/pleurodesis. The psychological state of the patient was motivated but she was lacking in confidence in her ability to perform even moderate exercise (e.g., more than walking at a slow pace on a treadmill for more than a few minutes). The patient was taking sirolimus for 3 years, switching to afinitor 4 years ago due to attenuation (sirolimus benefits were plateauing) of sirolimus on disease. The patient did not have any brain lesions, nor had she ever experienced any episodes of epilepsy.

Material and methods

Pulmonary function tests (PFTs), a graded exercise test (GXT), and bone mineral density (BMD) were assessed prior to beginning exercise training

Address for correspondence: Thomas William Lowder, Conway, United States; e-mail: tlowder@uca.edu
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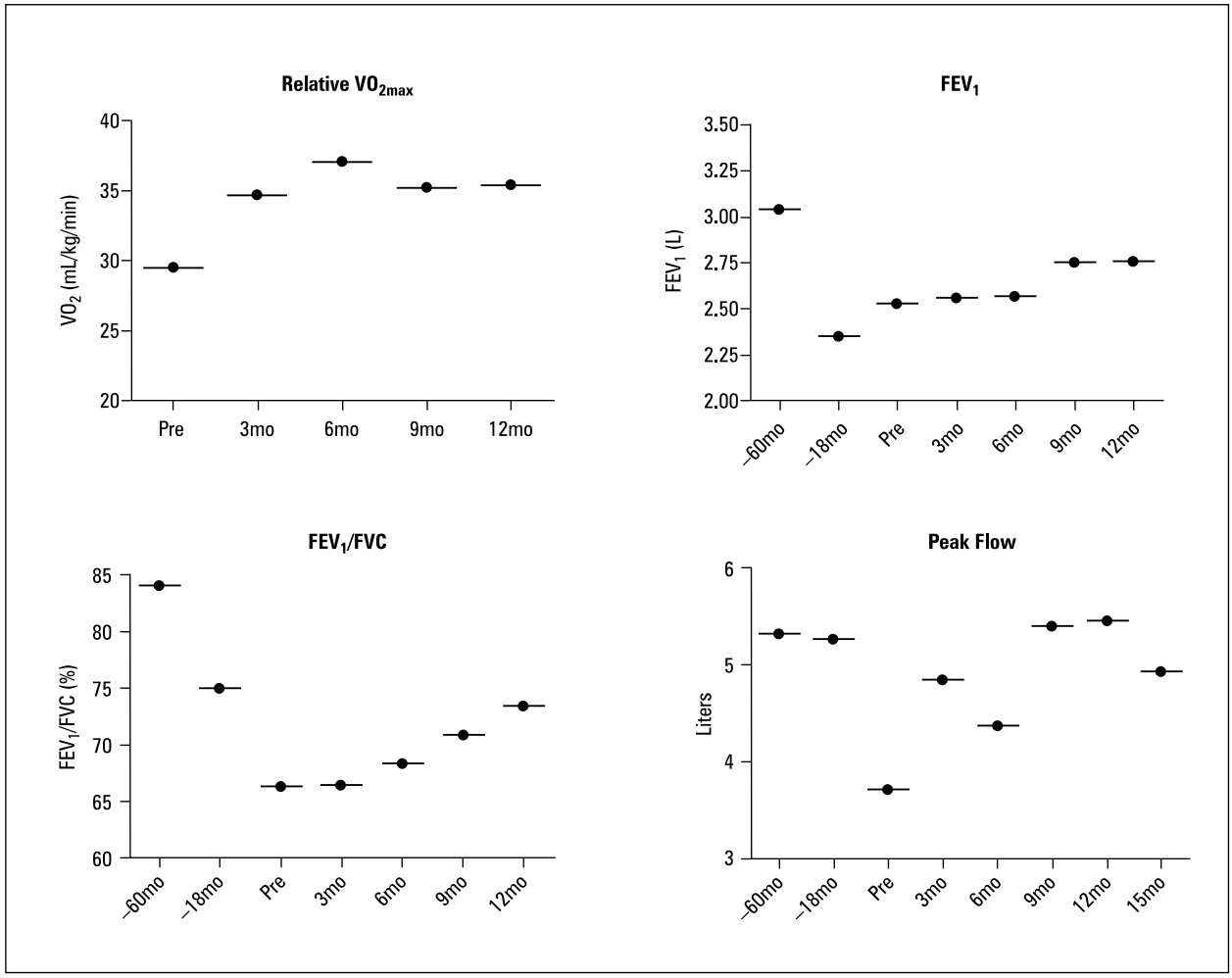

Figure 1. Oxygen uptake (VO_{2max}) and pulmonary function tests

(pre) and every three months. Twice weekly the patient reported for supervised exercise training (60 min/session), consisting of high-intensity aerobic (primarily treadmill/track running/sprinting) and resistance training. Patient performed a GXT to volitional fatigue to determine VO_{2max} using a Woodway Desmo treadmill (Waukesha, WI, USA) and a Quark CPET metabolic cart (Cosmed, Rome, Italy). PFTs were assessed using the Quark CPET metabolic cart. A Discovery QDR-4500 for Windows (Hologic, Inc., Bedford, MA, USA) dual X-ray absorptiometry (DXA) scanner was used to assess BMD. Tests were completed in room air without bronchodilator. PFTs prior to baseline (-60 months, -18 months) were taken from patient records.

Our patient demonstrated marked improvements in VO_{2max} (Figure 1A), FEV_1 (forced expiratory volume in 1 second) and FEV_1/FVC (forced vital capacity) % (Figure 1B and 1C), and peak flow (Figure 1D). Whole body and spine BMD also slightly improved or remained high despite already-high levels (Table 1). Forced vital capacity remained steady from baseline (Table 1). Patient maintained bodyweight (61.4–64 kg) and body fat (21.9–27.2%) pre to post. Peak flow improved by 47%; relative and absolute VO_{2max} improved by 20% and 23.4%, respectively. We do not have pulmonary function tests for the patient prior to her initial use of either sirolimus or following cessation of the drug. The baseline (-60 months) were taken approximately one year prior to the patient switching to Afinitor and the -18 months measures were the most recent PFT measures taken prior to her being seen in our laboratory.

Discussion

Lymphangioleiomyomatosis is a rare, multisystem disease affecting women of child-bearing age almost exclusively and can occur sporadically or as a manifestation of TSC-LAM [1]; indeed, LAM occurs in at least 40% of women with TSC [2]. Severe cases can lead to respiratory failure. Spontaneous pneumothoraces are common and

Table 1. Pulmonary function tests, VO_{2max}, and bone mineral density

Measure	-60 months	-18 months	Pre	3 months	6 months	9 months	12 months
FEV_1/FVC [%]	84.0	75.0	66.3	66.4	68.4	70.9	73.4
FVC [L]	4.33	3.71	3.82	3.88	3.80	3.97	3.82
FEV_1 [L]	3.04	2.35	2.53	2.56	2.57	2.75	2.76
Peak flow [L]	5.32	5.26	3.71	4.85	4.36	5.4	5.45
VO_{2max} [mL/kg/min]			29.43	34.66	37.02	35.23	35.34
VO_2 [L/min]			1833	2160	2351	2254	2262
DXA, whole body (g/cm^2)			1.3	1.277	1.31	1.275	1.308
DXA, lumbar spine (g/cm^2)			1.326	1.346	1.31	1.337	1.299

FEV_1 — *forced* expiratory volume in 1 second; FVC — forced vital capacity

are often the primary event leading to diagnosis and in most cases clinical events existed prior to diagnosis with one-third of patients exhibiting normal spirometry and obstruction evident in 57% of patients [3]. Despite great advances in diagnosis and patient management over the past two decades, treatment options are limited. As such, lifestyle choices and treatment options should be made as early as possible [2].

Sirolimus targets the mammalian target of rapamycin (mTOR) pathway and has been tremendously effective in improving pulmonary function in the majority of patients with LAM [4]. However, once treatment is stopped improvement disappears, and sirolimus appears to have a much greater effect in reducing extrapulmonary masses rather than pulmonary cysts [5]. Further, angiomyolipomas increase once therapy is stopped [6] and LAM is recurrent following lung transplant [7]. FEV_1 is considered the most clinically-relevant marker in determining disease severity [8]; patients with $FEV_1 < 30\%$ are often placed on transplant list. Improved FEV_1 could translate into reduced disease severity, potentially increasing time-to-transplant. A 100 mL increase in FEV_1 has been shown to be clinically important in COPD as this can be perceived by patients [9], and Westwood determined that a 100 mL increase in FEV_1 was associated with improved health status [10]. Our patient exhibited an elevated and sustained FEV_1 of 230 mL (10% increase, Table 1) beginning at 6 months. Exercise tolerance may be as clinically relevant as FEV_1 [11], as individuals able to perform even moderate physical activity would likely have a much-improved quality of life. Here we show an improvement in pulmonary function and exercise tolerance (VO_2).

Using exercise to treat women with LAM or TSC-LAM is novel. To date, only one study has examined the role of exercise in women with LAM [12]. While that study used exercise, only moderate exercise training was prescribed, and only 12 weeks (3 months) of exercise training was undertaken. Here we report on increased pulmonary function in a 29-year-old female with TSC-LAM following one year of intense exercise training. FEV_1 and peak flow improved at three and six months, with large increases after nine and twelve months (Figure 1A, D). VO_2 improved steadily, peaking at 6 months and remaining elevated. No decline in pulmonary function was seen after one year, unlike sirolimus [4]. These remarkable improvements demonstrate the potential of exercise to improve lung function in women with LAM. Increases were much more dramatic 9–12 months than 3–6 months after beginning the exercise intervention, with levels continuing to rise at one year. Our patient is continuing to exercise to determine if these measures remain elevated past one year. This is the first study in the literature that demonstrates the use of high-intensity exercise to treat LAM. This presents the possibility of extending time-to-transplant with minimal, non-invasive intervention. As VO_2 is correlated with disease severity [11], improved VO_2 translates clinically and physiologically for patients with LAM.

We are presently preparing a manuscript examining a larger (n = 8) group of LAM (seven sporadic LAM and one TSC-LAM) patients examining the positive benefits of exercise training on pulmonary function and bone mineral density at 3, 6, 9, and 12 month intervals. While the results of that study demonstrated marked improvements, this patient exhibited even greater results following high-intensity (as opposed to moderate-to moderately-high intensity) exercise training. Although quite deconditioned, the patient in this present study was remarkably healthy aside

from the pulmonary issues associated with TSC-LAM and was thus able to eventually participate in high-intensity exercise. Indeed, the patient's pulmonary function (FEV_1, peak flow) showed the greatest improvements after she had been training for more than six months, indicating that the patient needed to be conditioned before she could be trained using high-intensity exercise training. LAM and TSC-LAM patients undergoing pulmonary rehabilitation should consider longer-term training programs that will allow them to become conditioned to a level such that they can participate in rigorous (high-intensity) exercise programs to improve their gains made during exercise training.

Conflict of interest

The authors declare no conflicts of interest.

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Mediastinal germ cell tumour masquerading as loculated pleural effusion

Gopal Chawla[1], Pranav Ish[2], Avneet Garg[3], Kunal Deokar[4]

[1]All India Institute of Medical Sciences, Jodhpur, Basni , Jodhpur, India
[2]Vardhaman Mahavir Medical College and Safdarjung Hospital, Delhi, India
[3]All India Institute of Medical Sciences, Delhi, India
[4]All India Institute of Medical Sciences, Jodhpur, Basni , Jodhpur, India

We report a case of a large teratoma arising from the anterior mediastinum that presented as a confusing clinical picture of loculated pleural effusion which was successfully diagnosed and treated by surgical excision.

A 17-year-old girl with no comorbid conditions presented with trivial breathlessness and occasional cough. She was being treated for upper respiratory tract infection on and off for the past 6 months. Her breathlessness was greater while she played basketball at school and was associated with a cough which was dry in nature. She denied any fever, weight loss, previous disease, or any menstrual irregularities. Her physical examination revealed that she was apyrexial, normotensive, and eupnoeic. Her thyroid, lymph node, abdominal, and per-vaginal examinations were normal apart from decreased breath sounds in her right side. A chest X-ray revealed right-sided homogenous opacity with regular margins widening the cardiac shadow. There was no previous radiology for comparison. The suspicion of massive loculated effusion or some congenital cyst was made. USG of the chest showed the anechoic area with septations with isoechoic area making the possibility of loculated effusion or empyema more likely. Considering the patient's trivial symptoms and non-toxic nature which would have not been there in case of empyema, contrast-enhanced computerised tomogram was done. CT scan showed a large well-defined mass, which was low attenuating with focal areas of fat and calcification along with areas of cystic degeneration. Trucut biopsy was suggestive of mature cystic teratoma. The patient underwent open thoracotomy and enucleation of the tumour. Surgical and pathologic findings led to the diagnosis of a mature cystic teratoma with components of three germ layers. Gross examination showed a rounded tumour measuring 18 × 11.5 × 7.8 cm and weighing 300 g. The tumour was predominantly cystic, with thin, sharply delineated wall filled with sebaceous material and hair which mimicked loculated septated effusion on USG. Microscopically, the cyst wall was lined by the epidermis and some are lined by tall columnar with underlying sebaceous glands and hair follicles. The walls of the cyst showed pilosebaceous units and eccrine glands lobules of mature adipose tissue, hyaline cartilage, brain tissue and nerve twigs (Figure 1).

Germ cell tumours are the fourth most common mediastinal neoplasm, occurring almost always in the anterosuperior compartment. In this location, germ cell tumours are second only to thymomas and lymphomas. Five percent of germ cell tumours are extragonadal, the anterior mediastinum is the most common location followed by the retroperitoneum, pineal and suprasellar regions; and men are affected more often than women [1]. Microscopically, they are characterised by well-differentiated derivations from at least two of the three germ cell layers (ectoderm, mesoderm, and endoderm). Ectodermal elements may be represented by skin, teeth, and hair; mesodermal elements by bone, cartilage, and muscle; and endodermal elements by bronchial and gastrointestinal epithelium and pancreatic tissue. Cyst formation is typical, and at times they have raised protuberance projecting into the cyst cavity known as Rokitansky Nodule [2].

Most mediastinal teratomas like our case are asymptomatic or have trivial complaints unless they compress adjacent structures like the airways and vessels. Dull aching chest pain is often the most common presentation. At times some patients present with coughing up hair, i.e. trichoptysis indicating communication between the tracheobronchial tree and the teratoma. Physical examination is limited to chest dullness and diminished breath sounds. While some individuals have a bulging deformity of the chest wall, a draining fistula between the tumour and the skin, acanthosis nigricans, or superior vena cava obstruction [3].

Radiologically, teratomas are round or lobulated anterior mediastinal mass that has sharp margins and occupies one side of the thorax. Calcification, ossification, or even teeth may even be visible on chest radiographs. Chest CECT is the imaging modality of choice. Mediastinal mature teratomas contain soft tissue in virtually all cases followed by fluid in 88% and fat in 76%. All these elements are present in the same lesion

Address for correspondence: Gopal Chawla, All India Institute of Medical Sciences, Jodhpur, Basni , Jodhpur, India; e-mail: dr.gopalchawla@gmail.com
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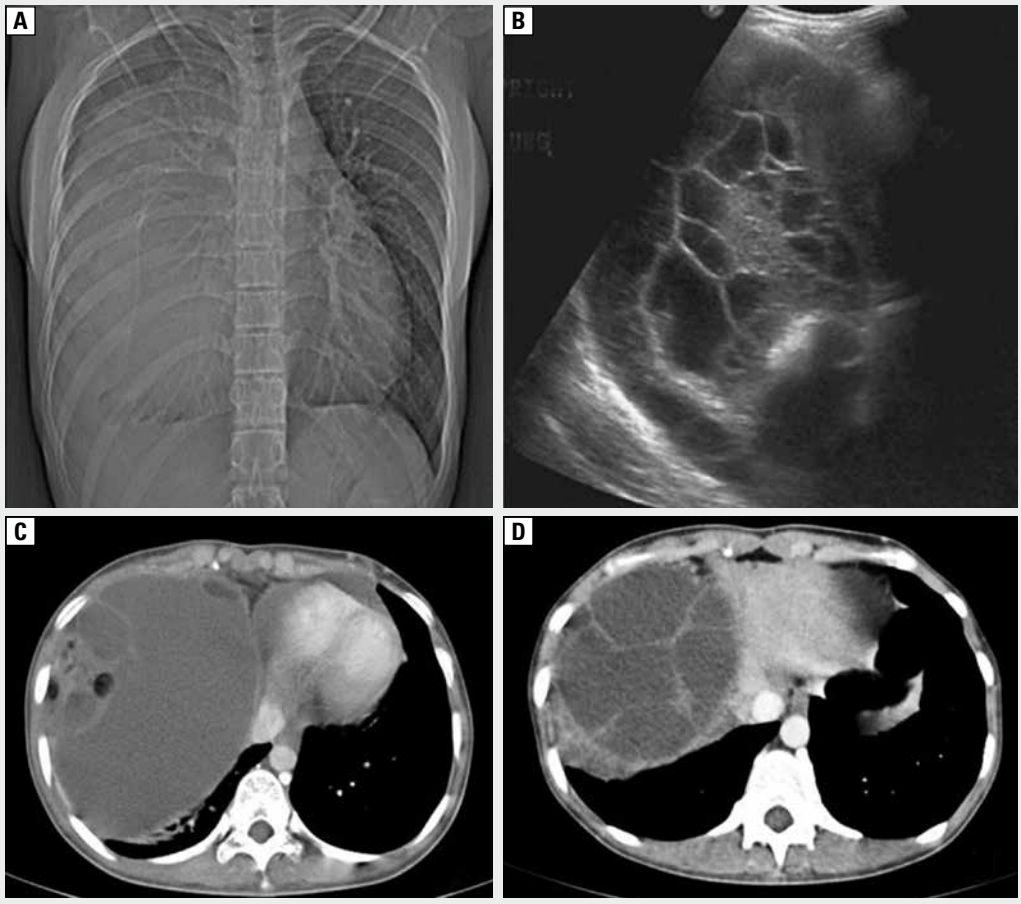

Figure 1. A. Chest X-ray showing homogenous opacity at the right side; **B.** USG of the chest showing effusion with multiple loculations; **C.** Chest CT showing large well-defined mass, which is low attenuating with focal areas of fat and calcification, areas of cystic degeneration; **D.** Anterior mediastinal cystic mass with solid enhancing septa

in 39% of cases. In 15% of patients, teratomas consist only of cystic lesions that contain neither fat nor calcification. A fat-fluid level within the mass is a highly specific finding but is seen less frequently. Teratoma may rupture at times, which radiologically is seen as inhomogeneity of the internal component of the mass or adjacent consolidation or pleural or pericardial effusion [4–5].

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A mediastinal mass with abdominal and pulmonary presentation

Pranav Ish, Abanti Das, Nitesh Gupta, Juvva Kishan Srikanth, Sakshi Batra, Shibdas Chakrabarti

Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi, India

A 60-year-old male presented with the acute onset of breathlessness and dry cough. He had a documented weight loss of 5 kgs with the lack of appetite. There was no history of fever, cough, sputum or haemoptysis. There was history of smoking with 50 pack-years and no history of alcohol intake. On examination, the patient had icterus. There was no palpable lymphadenopathy, clubbing or anasarca. On the respiratory system examination, right infrascapular air entry was reduced and hepatomegaly was present. Frontal chest radiograph (Figure 1A) and contrast enhanced CT (CECT) chest were done (Figure 1 B–D).

Considering the history and CT findings of a vertically oriented, fusiform-shaped posterior mediastinal mass extending to the both sides, partially encasing aorta and the oesophagus with areas of coarse calcifications with no vertebral erosion or extension to intervertebral neural foramen, a differential diagnosis of inflammatory pseudotumour, neurogenic tumour and lung adenocarcinoma were kept.

The patient underwent ultrasound-guided corebiopsy which revealed a poorly differentiated malignant tumour with tumour cells arranged in diffuse sheets (Figure 1 E–F).

The slides were subjected to immunohistochemistry stains. The tumour cells were positive for Vimentin and CD34 and negative for S-100, SMA, Desmin, Calretinin, TTF-1, EMA, Bcl-2 (Figure 1 G–I). Thus, a final diagnosis of spindle-cell sarcoma was made.

Due to limited published literature, spindle-cell sarcoma treatment guidelines remain unexplored [1]. Treatment options include surgery, chemotherapy and radiotherapy. Surgery has shown a modest improvement in mortality and hence is the first line therapy [1]. The use of adjuvant radiotherapy for SCS remains controversial, and the sensitivity of SCS to chemotherapy in the metastatic setting is highly variable [2]. Chemotherapeutic agents are also multiple - doxorubicin for metastatic spindle-cell carcinoma, dacarbazine, gemcitabine and docetaxel being the other agents used [3].

Our patient had poor performance status with severe airflow obstruction on spirometry. He was advised metered dose inhalers; nutritional buildup and counselled for chemotherapy. However, the man succumbed to his illness before any specific therapy could be initiated.

Thus, a high index of suspicion must be kept for uncommon malignancies, primarily sarcomas such as spindle-cell sarcoma as a differential for large mediastinal masses extending to multiple compartments without any specific imaging features. Diagnosis needs aggressive interdepartmental collaboration with invasive tissue sampling and advanced immunohistochemistry for making an early diagnosis.

Address for correspondence: Pranav Ish, Department of Pulmonary, Critical Care And Sleep Medicine, Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi, India; e-mail: pranavish2512@gmail.com

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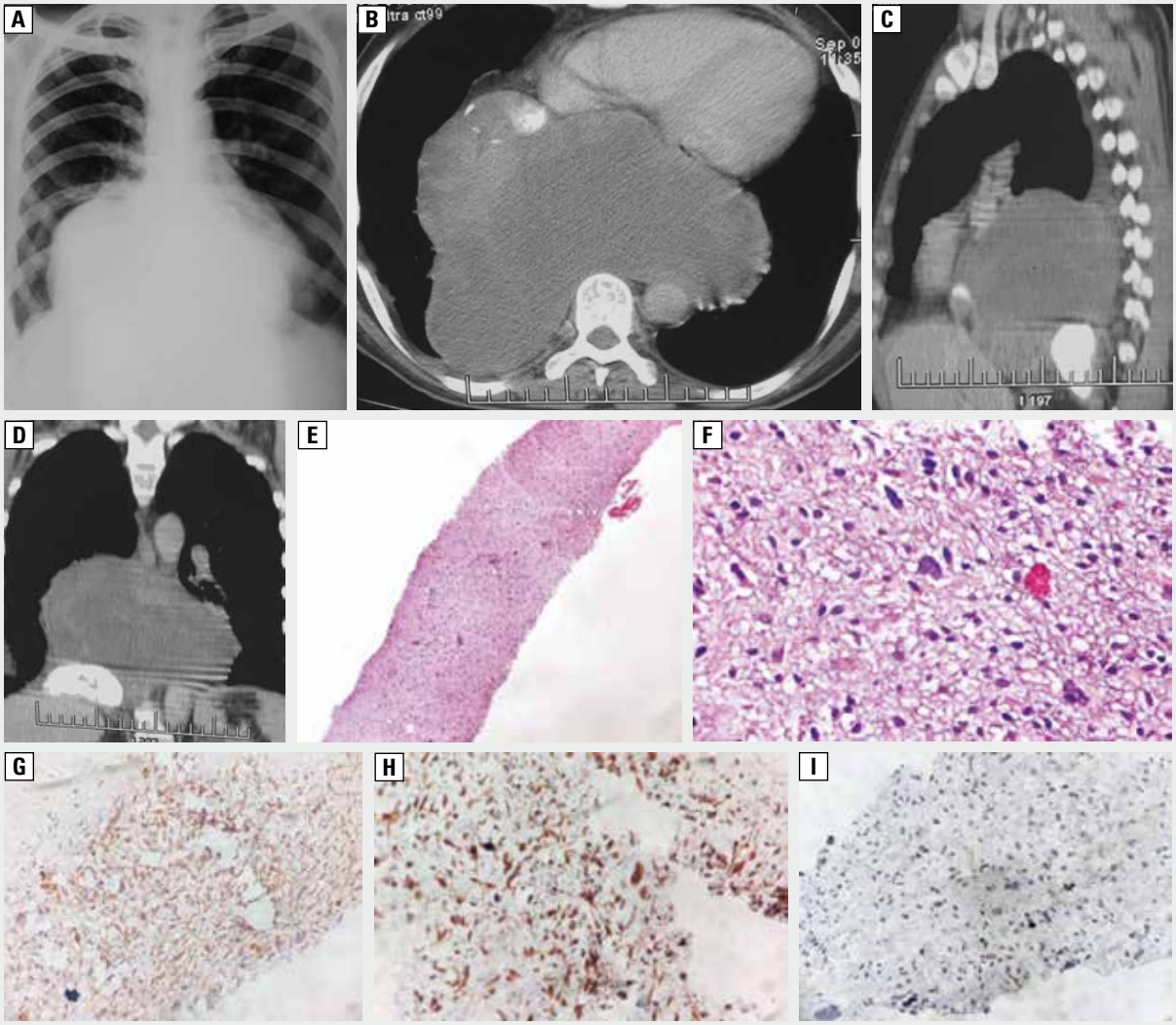

Figure 1. A. Frontal chest radiograph showing a mediastinal mass extending to the both sides of the midline, not silhouetting the cardiac outline. However, diaphragmatic outline is silhouetted, suggestive of likely posterior mediastinal origin; **B.** Axial CECT image shows heterogeneously enhancing solid posterior mediastinal mass extending to both sides of the midline showing areas of coarse calcification. Descending thoracic aorta is partially encased while the esophagus is not visualised,likely completely encased by the mass. No extension to neural foramen; **C and D.** Sagittal and coronal reformatted images reveal the vertically oriented fusiform configuration of the mass which is limited to the posterior mediastinum, not extending to the upper retroperitoneum. Note coarse calcification in its caudal aspect and mild right pleural effusion; **E.** Core biopsy from the soft tissue in low (10 X) magnification mass showing tumour cells; **F.** Higher magnification (40 X) shows the tumour cells are arranged in diffuse sheets. These cells are pleomorphic, spindle-shaped to elongated, have eosinophilic cytoplasm and hyperchromatic irregular nuclei. Interspersed tumour giant cell can also be seen; **G.** The tumour cells show vimentin positivity; **H.** The tumour cells show CD34 positivity; **I.** The tumour cells are negative for S-100, SMA, Desmin, Calretinin, TTF-1, EMA, Bcl-2

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Malek Chaabouni[1, 2], Walid Feki[1], Khansa Chaabouni[3], Samy Kammoun[1]

[1]Department of Pulmonology, Hedi Chaker Hospital, Sfax, Tunisia
[2]Department of Family Medicine, Faculty of Medicine, University of Sfax, Sfax, Tunisia
[3]Department of Biochemistry, Habib Bourguiba Hospital, Sfax, Tunisia

Vitamin D supplementation to prevent COVID-19 in patients with COPD: a research perspective

To the Editor

The Coronavirus disease 2019 (COVID-19) is a global pandemic that has caused now more than 200000 deaths. Its mortality is increased in people with comorbidities, such as chronic obstructive pulmonary disease (COPD) [1, 2]. There is increased evidence that the massive release of pro-inflammatory cytokines leading to the cytokine storm syndrome shapes the evolution of COVID-19 and is responsible of the severity of COVID-19 in some patients [3]. A recent review argued that vitamin D deficiency could have increased the COVID-19 outbreak and suggested vitamin D supplementation as a preventive action [4]. In fact, many factors seem to be correlated both to low vitamin D levels and the importance of COVID-19 spreading and severity. It is also important to highlight that the lockdown, implemented in many countries, prevents people to go out and then increases the risk of vitamin D deficiency.

Vitamin D receptor is widely present in different tissues of the organism. Therefore, vitamin D is involved in multiple biological metabolisms. It was proven to have immunomodulatory functions, and particularly suppresses pro-inflammatory cytokines [5]. Very few dietary sources are known to contain significant quantities of vitamin D, such as cod liver oil, oily fish and fortified food in some countries. This vitamin is mainly synthesized in the skin when exposed to ultra-violet (UV) radiation. Its synthesis is thus influenced by different factors such as the season, the time of the exposure during the day and the latitude. The zenith angle influences vitamin D production by modulating the path of UV radiation to the skin. That is why, latitude affects vitamin D levels, and vitamin D can hardly be produced neither above (to the north) and below (to the south) 33° latitude during winter months, nor in the early morning and late afternoon (approximately before 10 a.m. and after 3 p.m.). Vitamin D status changes depending on the period of the year. In fact, they reach their peak at the end of the summer, while the lowest levels are observed at the end of the winter. Besides, air pollution absorbs UV and so reduces vitamin D synthesis [6]. The facts that the outbreak is taking place when vitamin D levels are at their lowest, that the most affected countries by COVID-19 are northern countries and that polluted cities were more hit [7] suggested that it could be linked to sun exposure and vitamin D. Different factors may interfere with these variables such as the ability of the virus to resist to high temperatures as well as the effects of air pollution on health. It is still early to conclude about the spreading and severity of the disease in southern countries which differ in the time of the beginning, the governmental measures, the strength of the health system, and possible genetic and social factors.

Vitamin D deficiency represents a common public health issue in many countries worldwide. COPD patients are particularly at risk to have low levels of vitamin D due to multiple risk factors associated to the disease, such as aging, reduced outdoors activity, poor diet, and the use of corticosteroids [8]. COPD is more than just an airways disease. In fact, it may generate a systemic

Address for correspondence: Malek Chaabouni, Department of Family Medicine, Faculty of Medicine, University of Sfax, Sfax, Tunisia; e-mail: chaabouni.malek@gmail.com
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inflammatory process responsible of secondary extra-pulmonary impairments. Although COPD patients share certain risk factors that may explain some of the comorbidities, such as smoking history, physical inactivity and aging, other frequently observed comorbidities cannot be easily attributed to them. There is increasing evidence that COPD is just a part from a chronic inflammation linking these comorbidities and explaining why they develop together [9]. Vitamin D deficiency could sustain and aggravate the systemic inflammation associated to COPD. Reports have also shown that vitamin D deficiency was associated to exacerbations and hospital admissions, as well as lung function [5]. That may be explained by the immunomodulatory effects of vitamin D. Recent research showed that vitamin D supplementation significantly reduced COPD exacerbations [10]. These encouraging results are a further argument to test this option.

Although vitamin D deficiency was not proved to be neither a risk factor of COVID-19, nor a determinant of its severity, it represents a preventive perspective that needs to be further studied. Future studies should precise the relation between stages of COPD and COVID-19, and test the effectiveness of a preventive vitamin D supplementation.

Conflicts of interest

The authors declare no conflict of interest.

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Fabio Davoli[1], Venerino Poletti[2], Franco Stella[3]

[1]Department of Thoracic Surgery, Santa Maria delle Croci Teaching Hospital of Ravenna, AUSL Romagna, Ravenna, Italy
[2]Department of Diseases of the Thorax, Azienda USL Romagna, Morgagni Hospital, Forlì, Italy
[3]University of Bologna, Department of Thoracic Surgery, Azienda USL Romagna, Morgagni Hospital, Forlì, Italy

Three-chamber chest drain system in the COVID-19 era: is there a risk of further transmission?

To the Editor

Once a chest tube is placed, during an emergency or after thoracic surgery, a Chest Drain System (CDS) is attached. The most commonly used CDSs are the analogic three-chamber plastic units, consisting respectively in a drainage, a water seal and a suction chamber [1].

The three chambers are in communication and the water seal prevents the air returning to the pleural space during inspiration. As a CDS is set up, the water seal is filled with standard saline solution or water. If your choice is a wet CDS the suction column also has to be filled up to the desired level.

The dramatic outspread of coronavirus disease 2019 (COVID-19) has changed the management of patients undergoing thoracic surgery and, consequently, the habits of a Thoracic Surgery ward and operating theatre. Unfortunately, COVID-19 transmission to patients and health care staff is well reported [2]. We would like to arise concerns about the chances of infection for both nurses and surgeons when using standard CDS in COVID-19 patients.

This is mainly for three reasons: firstly, in the case of a broken or overturned CDS there could be a direct contamination by fluids from the chest cavity. Secondly, we cannot exclude that aerosol transmission occurs from within the CDS to the surrounding atmosphere [3]. Finally, there can be contamination while dealing with the suction system, because the CDS may be connected to the external wall vacuum by a plastic tube. As previously described, a CDS is a system of three plastic chambers and viable coronavirus has been detected on plastic surfaces up to 72 hours after application [4].

For all these reasons the safety of a standard three plastic chamber CDS in a COVID-19 patient has to be questioned in its present form, without appropriate precautions. The best means of protecting physicians and nurses from COVID-19 infection during daily ward activities shouldn't be the cleaning of the CDS. Once connected to the chest tube(s) the CDS is placed on the floor and periodical cleaning with alcoholic solutions (e.g. twice a day) exposes the operators to further contamination.

A reasonable way to reduce the possibility of contamination is to substitute the fluids commonly used to fill the water-seal chamber (for both dry and wet CDS) and the suction chamber (for the wet CDS only). We advocate the use of an alcohol-based solution (ethanol 62–71%) instead of water/saline solution to fill both the water-seal and suction chamber in order to minimize the risk of aerosol diffusion.

Ethanol-based solutions are found to be more effective in the prevention of further spread of COVID-19 on inanimate surfaces if compared to chlorhexidine and other biocidal agents [5]. The solution has to maintain the fluid properties of water to ensure the correct functioning of the CDS. Finally, the risk of alcohol evaporation and direct inhalation by the patient into the chest

Address for correspondence: Fabio Davoli, Department of Thoracic Surgery, Santa Maria delle Croci Teaching Hospital of Ravenna, AUSL Romagna, Ravenna, Italy; e-mail: fabio.davoli78@gmail.com
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cavity during inspiration is negated by the unidirectionality of the system. However, further studies are needed to confirm our advice.

Conflict of interest

The authors have nothing to disclose.

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