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New spirometry? The 2019 update of the test standardization

Introduction

Spirometry is a commonly performed assessment of lung function for diagnostic purposes as well as for monitoring of chronic lung diseases. The last international standardization of this study was published in 2005 [1], and the Polish version of the recommendations appeared a year later [2]. After 14 years, a group of experts from two leading scientific societies, ATS (American Thoracic Society) and ERS (European Respiratory Society), published a joint position that updated the standardization of spirometry [3]. There are essentially no significant changes to the test technique, but there have been some modifications regarding the circumstances surrounding the test and the assessment of the maneuvers. Due to the short nature of this article, the reader will find below information on selected and, in the authors' opinion, the most important key changes compared to the standards being in use for the last 14 years.

Contraindications for spirometry

Spirometry is generally a safe procedure, as demonstrated by many years of experience and publications in the literature. Adverse events occur at a frequency of 5/10,000 and are usually mild (syncope was most commonly observed). The risk of adverse events in spirometry is primarily associated with significant fluctuations in chest pressures and their impact on the abdominal and thoracic organs, venous return and systemic blood pressure. Therefore, caution must be used for patients with medical conditions that could be adversely affected by these physiological consequences. The ATS/ERS recommendations on spirometry published at the end of 2019 give all contraindications with the status of "relative" and list the following situations requiring special attention (consider the appropriateness of performing the test):

— Due to increases in myocardial demand or changes in blood pressure
  - Acute myocardial infarction within 1 wk
  - Systemic hypotension or severe hypertension
  - Significant atrial/ventricular arrhythmia
  - Noncompensated heart failure
  - Uncontrolled pulmonary hypertension
  - Acute cor pulmonale
  - Clinically unstable pulmonary embolism
  - History of syncope related to forced expiration/cough
— Due to increases in intracranial/intraocular pressure
  - Cerebral aneurysm
  - Brain surgery within 4 wk
  - Recent concussion with continuing symptoms
  - Eye surgery within 1 wk
— Due to increases in sinus and middle ear pressures
  - Sinus surgery or middle ear surgery or infection within 1 wk
— Due to increases in intrathoracic and intraabdominal pressure
  - Presence of pneumothorax
  - Thoracic surgery within 4 wk
  - Abdominal surgery within 4 wk
  - Late-term pregnancy

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— Infection control issues
  • Active or suspected transmissible respiratory or systemic infection, including tuberculosis
  • Physical conditions predisposing to transmission of infections, such as hemoptysis, significant secretions, oral lesions or oral bleeding.

It is worth noting that the presence of aortic aneurysms has ceased to be a contraindication. Spirometry should always be interrupted if pain occurs during maneuvers. Relative contraindications do not exclude the possibility of spirometry, but they should be taken into account when referring the patient to this examination. Individuals with potential contraindications that would prevent an examination in primary care settings may be examined in more experienced centers and with the access to emergency care. The decision to perform spirometry is determined by the referring physician based on a risk and benefit assessment for the individual patient. Potential contraindications should be provided on the referral form.

Equipment requirements

The most important change was adapting the standards to the requirements of the International Organization for Standardization (ISO) standard number 26782 dated 2009, replacing the ATS standard curves with curves according to the mentioned requirements and narrowing the accuracy range to ± 2.5% in both: testing the spirometer using standard curves as well as checking calibration using a 3-liter calibration syringe. Information was also added about the required accuracy of the 3 L calibration syringe, which should be within ± 0.5%, what in practice gives the accuracy of the entire calibration process at the current recommended level of ± 3%. Recommendations for daily checking/performing calibration indicate the need for maneuvers in a wide range of flows (from 0.5 to 12 L/s, i.e. piston movement lasting from 0.5 to 6 sec), which in practice also means checking the so-called linearity. It is also recommended to periodically check the calibration syringe for accuracy and monthly leak test.

Personnel qualifications

The relationship between the commitment and skills of the researcher and the quality of spirometry are emphasized. It is the responsibility of the person performing the study to observe the patient and interact with him in order to achieve optimal results. This requires a combination of skills acquired during training and experience that comes with the time and number of performed tests.

Patient's data

It is obvious that the correct person's anthropometric data should be obtained and, for this purpose, appropriate height and weight measurements taken. Current standards specify the degree of accuracy for individual values (age and height to 1 decimal place, weight to the nearest 0.5 kg). Gender and belonging to an ethnic group at birth, not declared by the patient, should be used to calculate predicted values. In the absence of these data, the person interpreting the result should be notified. The list of behaviors to be avoided before spirometry testing was modified: smoking electronic cigarettes (vaping) and consumption of intoxicants (to avoid problems with coordination, understanding and physical performance) were added.

Spirometry measurements

Values of FEV_1 (forced expiratory volume at the first second of exhalation), FVC (forced vital capacity) and their ratio FEV_1/FVC expressed as a fraction (or less correctly as a percentage) called the Tiffeneau index still remain crucial for the spirometry test result. However, it is worth noting that the current standards also indicate an important role (and recommend performing) of the measurement of the FIVC (forced inspiratory vital capacity) maneuver after the FVC maneuver in order to verify the correctness of the inspiration preceding the forced exhalation. For this reason, spirometers measuring only expiratory volumes (in practice no longer employed) should not be used.

An important change is the redefinition of a technically correct (acceptable) forced expiration maneuver and the associated introduction of the concept of a maneuver technically unacceptable but clinically useful.

A forced expiratory maneuver is considered as technically correct if the following criteria are met:
— sufficiently fast onset of exhalation as indicated by BEV (back extrapolated volume) < 5% of FVC or < 100 mL, whichever is greater;
— no artifacts (e.g. coughing, closing the glottis, incomplete breathing effort);
— one of the criteria for successful completion of the maneuver was achieved in the following hierarchy:

Table 1. Grading System for FEV_1 and FVC (graded separately)

Grade	Number of measurements	Repeatability: Age > 6 years	Repeatability: Age ≤ 6 years
A	≥ 3 acceptable	≤ 0.15 L	≤0.1 L*
B	2 acceptable	≤ 0.15 L	≤0.1 L*
C	≥ 2 acceptable	≤ 0.2 L	≤0.15 L*
D	≥ 2 acceptable	≤ 0.25 L	≤0.2 L*
E	≥ 2 acceptable OR 1 acceptable	> 0.25 L N/A	>0.2 L* N/A
U	0 acceptable AND ≥ 1 usable	N/A	N/A
F	0 acceptable AND 0 usable	N/A	N/A

*Or 10% of the highest value, whichever is greater; applies for age 6 years or younger only. FEV_1 — forced expiratory volume at the first second of exhalation; FVC — forced vital capacity; N/A — not applicable

- a plateau was reached on the volume-time curve (volume change at last second exhalation < 25 mL);
- exhalation continuously lasted 15 seconds;
- satisfactory FVC repeatability has been obtained or the FVC measured last is bigger than the best so far (in the same session).

If FIVC is correctly recorded, then the inspiratory — expiratory differences (FIVC — FVC) should not be greater than 100 mL or 5% of FVC. If satisfactory reproducibility is not achieved (the differences between the 2 best FVC and FEV_1 results exceed 150 ml), it is recommended to repeat maneuvers, but no more than 8 forced exhalations should be performed during one measurement session.

It is worth noting that there is no longer striving for a minimum of 6 seconds of exhalation in cases where a plateau of the volume-time expiratory curve is previously reached. The recommendations also mention about the time to reach PEF (peak expiratory flow), which should be as short as possible and usually should not exceed 0.15 sec, but this is not a mandatory criterion.

The "U" category has been added to the previously slightly modified thresholds of acceptable repeatability [4] — a useful result but not meeting the technical acceptability criteria. To simplify, these are maneuvers during which there is no doubt about the correct start, but none of the conditions for successful completion can be achieved. Interpretation of such results is possible but has limited value, especially in the case of results in the abnormal range. A new scale was introduced regarding the quality of the spirometry test from the point of view of the degree of repeatability of results (A, B, C, D, E, U, F), in which FEV_1 and FVC measurements are assessed separately (Table 1) [3].

Bronchodilator responsiveness testing

The need to distinguish the concepts of reversibility of obstruction (defined as normalization of the FEV_1/FVC ratio) from the significance of improvement (assessed as response in the FEV_1 or FVC) and the necessity to unify the nomenclature in this area were pointed out. The list of drugs and the recommended time periods for which they should be discontinued before the test to check the reactivity of the airways to the bronchodilator have been updated. For drugs from the ultra-long-acting beta2-agonist group (e.g. indacaterol, vilanterol, olodaterol), the recommended time since the last dose is 36 hours, for long-acting muscarinic receptor antagonist (e.g. tiotropium, umeclidinium, aclidinium or glycopyronium), delay should be a bit longer, approx. 36–48 hours. It is worth noting that these periods are shorter than when preparing for methacholine challenge test because the protective effect of these drugs lasts longer than the bronchodilation effect.

There is also a recommendation that the first spirometry test performed for diagnostic purposes should always be accompanied by bronchodilator responsiveness testing because the lack of airway obstruction in the baseline does not exclude possible significant improvement after bronchodilators. The recommendation seems to be justified from a purely substantive point of view, but the authors' experience shows that the "reversibility test" should not be the first spirometry in the subject's life because the effect of the drug may be superimposed on the learning effect and the improvement observed may be the result of a better technique rather than the effect of the real bronchodilation.

Conflict of interest

The authors have no conflict of interest related to the subject matter.

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The effects of mold sensitivity on the clinical characteristics of adult asthmatic patients

Abstract

Introduction: The effects of mold sensitivity on the development and course of asthma have been researched previously, although study results vary. We sought to evaluate the characteristics of our mold-sensitive patients in comparison with those of other adult asthmatic patients.

Materials and methods: Data were collected retrospectively from adult asthmatic patients who underwent regular follow-ups at our tertiary care outpatient clinic for immunology and allergic diseases. Patients were grouped and compared according to three categories of aeroallergen sensitivity status determined via a skin prick test. The study variables were demographic data, asthma-onset age, comorbid conditions, asthma-related emergency department visits and hospitalizations, systemic corticosteroid burst, asthma control assessment tests, and pulmonary function tests.

Results: In total, 242 patients' data were evaluated. Their mean age was 48.6 ± 15.4 years, with female predominance (81.4%). Mold-sensitive asthmatics composed 34.7%, while the aeroallergen-sensitive group without molds (33.1%) and the non-sensitized group (32.2%) composed the rest. The mold-sensitive group had a higher rate of polysensitization (92.8%) than the sensitized group without molds. In multinomial logistic regression analysis, mold sensitivity was positively associated with shorter asthma duration, absence of sinonasal polyposis, presence of allergic rhinitis, and generally well-controlled asthma compared to the non-sensitized group. Also, mold sensitivity was positively associated with shorter asthma duration, drug allergy, and absence of systemic corticosteroid bursts compared to the sensitized group without molds in logistic regression analysis.

Conclusion: Our mold-sensitive asthmatic patients demonstrated better asthma symptom control. It should be considered that mold sensitization in adult asthmatics is not always a poor prognostic factor.

Key words: adult asthma, mold sensitivity, asthma control

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Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation [1]. There are many factors that have a role in the development and control of asthma [1]. Mold exposure and sensitization to molds constitute a significant trigger factor. Mold sensitizations and their role in asthma have been previously evaluated in many studies [2]. Most of these investigations have found that mold sensitivity has an effect not only on the development of asthma, but also in the success of asthma control efforts and the severity of asthma [2] by way of inducing type I allergic reactions in susceptible individuals [3].

Direct associations between increased fungal exposure and a loss of asthma control are numerous [4]. Previous studies have suggested an increased risk of asthma development after mold exposure at an early age [2, 5]. McSharry *et al.* found that high environmental mold exposure was associated with poor lung function [6]. Besides the impact on asthma development, fungal sensitization was also found to have an effect on the persistence and activation of asthma symptoms, and on the severity of asthma [2, 7].

Mold species including *Penicillium*, *Aspergillus* and *Cladosporium* have been studied in conjunction with asthma. *Alternaria* species have been found to increase the risk of asthma symp-

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tom exacerbation [8]. In previous research, sensitization to *Alternaria* and *Cladosporium* had been associated with severe asthma [4]. The European Community Respiratory Health Survey also showed that *Alternaria* and *Cladosporium* were significantly associated with asthma severity [9]. In one study, patients admitted to the intensive care unit for a severe asthma attack were found to have a positive skin prick test for *A. alternata* and *C. herbarum* [10].

Notably, climate change has been labelled as a potential contributing factor in aeroallergens due to the risk of accelerated mold sporulation in environments with increased CO_2 [11, 12]. The effects of the existing climate on fungal sensitization also impact characteristics of various aeroallergens, including mold sporulation patterns [11, 12]. Turkey's diverse regions have significantly different climates because of the country's irregular topography. For example, the coastal areas of Turkey bordering the Black Sea have a temperate oceanic climate with warm, wet summers and cool-to-cold, wet winters. The Turkish Black Sea coast is the only region of Turkey that receives such high precipitation throughout the year. Thus, inhabitants in this region face the possibility of indoor and outdoor mold exposure throughout the year more so than their neighbors.

The effects of mold sensitization have been thoroughly reported in severe asthmatics [2]; however, in general asthmatic patients, the link is not as definite. Although mold sensitization and asthma outcomes are generally associated with poor prognoses [2], research from various geographical areas has indicated the opposite [13]. Therefore, in this study, we sought to explore the percentage and pattern of mold sensitization among adult asthmatics and to reveal the similarities and/or differences between mold-sensitized asthmatics and non-sensitized patients. To our knowledge, this study is the first to evaluate the clinical characteristics of adult asthmatics according to their mold sensitivity status in Turkey.

Materials and methods

Study design

A retrospective case–control study was conducted after obtaining local ethics committee approval on June 20, 2018 (approval no. 40465587-120).

Setting and participants

Patients meeting relevant criteria who were admitted to the department of immunology and allergic diseases in the outpatient clinic between June 2013 and June 2018, and having an asthma diagnosis according to Global Initiative for Asthma (GINA) guidelines [1] were retrospectively evaluated from their paper files. The hospital has electronic records for all patients. In this department, doctors also collected and filed separate paper files for each patient. These files contained demographic characteristics, past medical history, comorbid conditions, anamnesis regarding asthma-related admissions, and patient symptoms. Laboratory data, skin prick test (SPT) and acceptable pulmonary function test (PFT) results of the patients were also recorded to that file by the doctors, along with records of follow-up evaluations. In the follow-ups, which were made at 3 or 6 month intervals, patients were questioned about symptoms, exacerbations, and all prescribed medications. Physical examinations and required test results, such as PFT, were also recorded at that time.

All data regarding the allergic status of patients was pulled from tests administered in our hospital. Previous records or patients' anamnesis regarding allergic diseases were confirmed by test results. In patients with symptoms of allergic rhinitis (AR), the diagnosis was confirmed by nasal endoscopic examination by ear-nose-throat experts, regardless of patient SPT results. Sinonasal polyposis (SNP) diagnoses were also confirmed by nasal endoscopic examination.

Among all relevant patients, 242 adult asthmatics met the inclusion criteria and were therefore included in this study. Inclusion criteria were as follows: asthmatics older than 18 years of age who participated in regular follow-up visits at least once a year who had SPT results and acceptable PFT results (14). Exclusion criteria were being younger than 18 years of age, having irregular follow-ups or follow-up periods being under a year, missing patient file information, and patients who had respiratory comorbidities such as bronchiectasis and/or allergic bronchopulmonary aspergillosis, among others.

Data collection

Patients' demographics, smoking history, body mass index (BMI), asthma history, asthma duration, age at asthma diagnosis, comorbidities, presence of allergic rhinitis, sinonasal polyposis, drug allergies, systemic corticosteroid use, and lifetime hospitalization/emergency department visits were obtained from manually filled records. PFT and SPT results performed concurrently on the same day were recorded.

Records of SPTs were obtained from patient follow-up records. For each patient, an SPT had

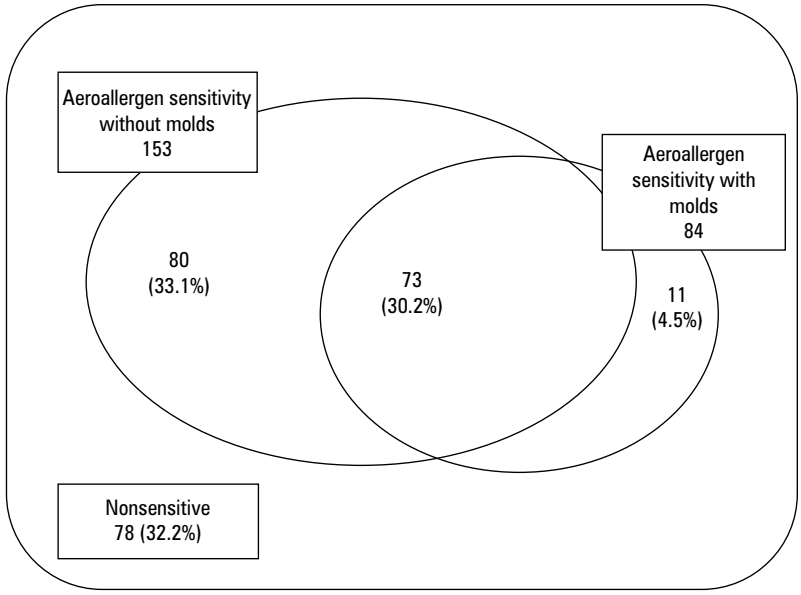

Figure 1. Aeroallergen sensitization pattern of the patients

been performed involving common inhalant allergens including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (dust mites); *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium* (molds); cat and dog dander; latex; pollens from grass, trees, and weeds; and cockroaches (Allergopharma, Reinbek, Germany). SPTs were performed on the volar forearm and were read after 20 minutes. A wheal reaction with a mean diameter of 3 mm greater than the negative control was considered to indicate allergen positivity.

PFT was performed according to recommendations (14) using a pulmonary spirometer (CareFusion, Germany, 234 GmbH). The best of three attempts was recorded. Postbronchodilator forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), and forced expiratory flow at 25–75% of FVC ($FEF_{25–75}$) values were obtained from the study participants' files. Our procedures for SPT and PFT are thoroughly described in our previous studies' methods section [14, 15].

For evaluating asthma symptom control, the GINA assessment of asthma control in adults was used [1, 15].

Statistical analysis

Patients were grouped into three categories according to SPT results: non-sensitized patients, sensitized patients without molds, and sensitized patients with molds (Figure 1).

For data analysis, the Statistical Package for the Social Sciences version 22 (IBM Corp., Armonk, NY, USA) was used. Categorical variables are expressed as absolute and relative frequencies, whereas quantitative variables are expressed as means and standard deviations. To evaluate the relationship between independent variables (i.e. demographic variables, asthma history, asthma clinical course, and PFT) and dependent variables, Pearson's chi-squared test was used. For categorical variables and numerical variables, one-way analysis of a variance test was used with a post-hoc Tukey test. Associations were considered significant at $p < 0.05$. Multinomial logistic regression was used to find differences among the three groups. A multivariate logistic regression analysis was performed by the backward likelihood ratio test to find differences between the mold-sensitive group and the sensitized group without molds.

Results

In total, 242 adult asthmatic patients' data were evaluated. The mean age of the study participants was 48.6 ± 15.4 years. Most study subjects were female (81.4%), homemakers (68.6%), had never smoked (81.8%), had comorbid diseases (56.6%), had AR (81.4%), and were on less than five medications for asthma (81.4%) (Table 1).

Figure shows the sensitization patterns of patients according to SPTs. Out of 242, the non-sensitized patient rate was 32.2%. Of them, 33.1% had aeroallergen sensitivity without molds, 30.2% had mold sensitivity plus at least one other aeroallergen sensitivity, and 4.5% had only mold sensitivity. Therefore, most of the patients in the mold-sensitive group were also sensitized to other aeroallergens.

Table 1. Demographic characteristics of patients and comparison of them among groups

Variables	Total (242)	SPT negative (n = 78, 32.2%)	SPT positivity without mold sensitization (n = 80, 33.1%)	SPT positivity with mold sensitization (n = 84, 34.7%)
Age (mean ± SD)	48.6 ± 15.4	59.9 ± 14.1[#¶]	45.8 ± 14.9	44.5 ± 14.7
Gender				
Female	197 (81.4)	65 (33.0)	64 (32.5)	68 (34.5)
Male	45 (18.6)	13 (28.9)	16 (35.6)	16 (35.6)
Job**				
Housewife	166 (68.6)	63 (38.0)*	49 (29.5)	54 (32.5)
Occupational risk	52 (21.5)	3 (12.5)	13 (54.2)	8 (33.3)
No occupational risk	24 (9.9)	12 (23.1)	18 (34.6)	22 (42.3)
Smoking status				
Non-smoker	198 (81.8)	65 (32.8)	64 (32.3)	69 (34.8)
Former smoker	22 (9.1)	9 (40.9)	9 (40.9)	4 (18.2)
Current smoker	22 (9.1)	4 (18.2)	7 (31.8)	11 (50.0)
Smoking (pack/year)	3.69 ± 8.4	3.37 ± 7.71	4.75 ± 10.5	3.1 ± 7.1
BMI (kg/m^2)	30.0 ± 6.4	32.1 ± 7.5[#¶]	29.5 ± 5.4	28.6 ± 5.6
Comorbid disease				
Present	137 (56.6)	52 (38.0)[#]	48 (35.0)[#]	37 (27.0)
Absent	105 (43.4)	26 (24.8)	32 (30.5)	47 (44.8)
AR				
Present	197 (81.4)	52 (26.4)[#¶]	68 (34.5)	77 (39.1)
Absent	45 (18.6)	26 (57.8)	12 (26.7)	7 (15.6)
SNP				
Present	36 (14.9)	18 (50.0)[#]	11 (30.6)	7 (19.4)
Absent	206 (85.1)	60 (29.1)	69 (33.5)	77 (37.4)
Drug allergy				
Present	18 (7.4)	10 (55.6)[¶]	2 (11.1)	6 (33.3)
Absent	224 (92.6)	68 (30.4)	78 (34.8)	78 (34.8)
Number of medications used for asthma and/or rhinitis symptoms				
≥ 5 [n%]	45 (18.6)	9 (20.0)[#¶]	22 (48.9)	14 (31.1)
≤ 4 [n%]	197 (81.4)	69 (35.0)	58 (29.4)	70 (35.5)
Mean (± SD)	2.93 ± 1.32	2.74 ± 1.19	3.20 ± 1.42	2.85 ± 1.30
Omalizumab use				
Present	21 (8.7)	5 (23.8)***	9 (42.9)	7 (33.3)
Absent	221 (91.3)	73 (33.0)	71 (32.1)	77 (34.8)
FEV_1%	88.0 ± 13.8	86.4 ± 15.3[¶]	94.3 ± 16.0	90.0 ± 14.9
$FEV_{25–75}$	68.3 ± 30.1	64.2 ± 30.2	74.0 ± 28.9	71.1 ± 30.6
FEV_1/FVC	76.4 ± 8.77	75.5 ± 9.99	76.9 ± 7.82	77.0 ± 9.45

[#]Compared to the group that had have prick test positivity with mold sensitization; [¶]Compared to the group that had prick test positivity without mold sensitization; *Among housewives, normal prick test was significantly higher than the group with occupational risk, Patients that have jobs with occupational risk factor; [*, #, ¶]Show the statistical significance between groups, $p < 0.05$; **Job was classified according to presence of occupational risk factors for asthma; ***5 patients who used omalizumab and had a negative skin prick test were found to have specific IgE positivitiy for perennial allergens.
AR — allergic rhinitis; BMI — body mass index; FEV_1 — forced expiratory volume in first second; FEF_{25-75} — forced vital capacity and forced expiratory flow at 25–75% of forced vital capacity; SPT — skin prick test

Table 2 shows data relating to the asthma characteristics of the included patients. The mean age at asthma diagnosis was 39.3 ± 15.2, and 50.4% of patients were younger than 40 years of age at the time of asthma onset. The majority of patients had experienced no hospitalization (80.6%), no emergency department admission (63.6%), and had no systemic steroid (SS) use (71.1%) during their disease course. According to GINA Asthma Control Test scores, most patients had "well-controlled" asthma (59.9%); the rest had either "partially controlled" (20.2%) or "uncontrolled" asthma (20.2%).

When considering aeroallergen sensitization patterns, 32.2% of our adult asthmatic patients were non-sensitized, 33.1% had sensitivity to

Table 2. Comparison of patients' characteristics according to asthma course

Variables	Total (242)	SPT negative (n = 78, 32.2%)	SPT positivity without mold sensitization (n = 80, 33.1%)	SPT positivity with mold sensitization (n = 84, 34.7%)
Asthma diagnosis age (years)	39.3±15.2	44.9 ± 14.5[#¶]	36.1 ± 15.6	37.3 ± 14.1
Asthma onset age				
< 40 [n%]	122 (50.4)	28 (23.0)[#¶]	47 (38.5)	47 (38.5)
≥ 40 [n%]	120 (49.6)	50 (41.7)	33 (27.5)	37 (30.8)
Asthma duration (years)	9.36 ± 8.9	11.2 ± 10.9[#]	9.7 ± 8.5	7.3 ± 6.7
Lifetime hospitalization due to asthma				
Present	47 (19.4)	23 (48.9)[#]	14 (29.8)	10 (21.3)
Absent	195 (80.6)	55 (28.2)	66 (33.8)	74 (37.9)
Mean (± SD)	0.77 ± 2.7	1.53 ± 4.26[#¶]	0.34 ± 1.06	0.49 ± 1.76
Emergency department visits				
Present [n%]	88 (36.4)	38 (43.2)[#¶]	25 (28.4)	25 (28.4)
Absent [n%]	154 (63.6)	40 (26.0)	55 (35.7)	59 (38.3)
Mean (± SD)	2.68 ± 6.52	4.33 ± 9.7[¶]	1.8 ± 3.73	1.99 ± 4.33
Systemic steroid use				
Present [n%]	70 (28.9)	29 (41.4)[#]	25 (35.7)	16 (22.9)
Absent [n%]	172 (71.1)	49 (28.5)	55 (32.0)	68 (39.5)
Mean (± SD)	1.76 ± 5.28	2.55 ± 7.53	1.71 ± 4.45	1.07 ± 2.87
Asthma control status				
Well controlled	144 (59.5)	43 (29.9)	50 (34.7)	51 (35.4)
Partially controlled	49 (20.2)	18 (36.7)	17 (34.7)	14 (28.6)
Uncontrolled	49 (20.2)	17 (34.7)	13 (26.5)	19 (38.8)

Note: R2 = 0.31 (Cox–Snell), 0.35 (Nagelkerke). Model $\chi 2 = 91.122$, $p < 0.0001$; goodness of fit; deviance-p = 0.402; pearson-p = 0.183. [#]Compared to the group that had have prick test positivity with mold sensitization; [¶]Compared to the group that had prick test positivity without mold sensitization. SPT — skin prick test

aeroallergens without molds, and 34.7% demonstrated mold sensitivity with or without other common aeroallergens. While those with mold sensitivity were mostly polysensitized (60.9%), those without mold sensitivity were mostly mono-sensitized (83.3%; $p < 0.05$). Identified sensitized mold species included *Cladosporium* (48.2%), *Aspergillus* (43.4%), *Penicillium* (38.6%), and *Alternaria* (21.7%).

In univariate analysis, the mean age and mean BMI of non-sensitized patients were higher than those of the sensitized groups ($p < 0.05$). In addition, the number of patients with a high number of medications used (5 or more medications) was lower in the non-sensitized group. The presences of comorbidities, SNP, and drug allergies were also higher among non-sensitized patients. The mean FEV_1% was lower in the non-sensitized group, compared with sensitized patients without mold sensitization ($p < 0.05$) (Table 1).

Furthermore, univariate analysis revealed that the mean age of asthma diagnosis, prevalence of late-onset asthma (≥ 40 years), mean number of hospitalizations, and number of patients who experienced emergency department (ED) admission were higher in the non-sensitized group ($p < 0.05$). Finally, asthma duration and presence of SS bursts were higher in the non-sensitized group when compared with the mold-sensitive group ($p < 0.05$) (Table 2).

Table 3 shows the multinomial logistic regression analysis findings of other groups in comparison with the non-sensitized group. The absence of drug allergies [odds ratio (OR): 8.794, 95% confidence interval (CI): 1.499–51.603], absence of ED admission (OR: 3.351, 95% CI: 1.116–10.065), and presence of occupational exposure (OR: 7.943 CI: 1.383–45.608) were more associated with sensitization without molds as compared to patients with non-sensitization. Separately, shorter asthma duration (OR: 1.795, 95% CI: 0.829–3.890), absence of SNP (OR: 3.791, CI: 1.207–11.903), presence of AR (OR: 4.132, 95% CI: 1.436–11.886), and well-controlled asthma (OR: 2.647, CI: 1.096–6.392) were more associated with mold sensitivity than with non-sensitization.

Table 4 shows the differences identified between mold-sensitive patients and sensitized patients without following logistic regression analysis. Shorter asthma duration (OR:2.170,

Table 3. Associations between groups compared to nonsensitive group in multinominal analysis

	SPT positivity without mold sensitization	SPT positivity with mold sensitization
Gender (male gender compared to female)	3.534 (0.914–13.672)	2.997 (0.782–11.488)
Age group (< 65 vs ≥ 65)	1.294 (0.444–0.637)	2.511 (0.756–8.348)
Asthma duration (shorter than 10 years vs longer than 10 years)	0.977 (0.461–2.068)	1.795 (0.829–3.890)*
Asthma onset age (< 40 age vs ≥ 40 age)	1.633 (0.717–3.720)	1.135 (0.497–2.595)
BMI (< 30 vs ≥ 30)	1.228 (0.564–2.675)	1.581 (0.706–3.541)
Comorbidity (presence vs absence)	1.004 (0.436–2.315)	0.523 (0.224–1.221)
SNP (absence vs presence)	1.972 (0.707–5.501)	3.791 (1.207–11.903)*
AR (presence vs absence)	2.036 (0.818–5.068)	4.132 (1.436–11.886)*
Drug allergy (absence vs presence)	8.794 (1.499–51.603)*	1.293 (0.347–4.825)
Hospitalization (absence vs presence)	2.071 (0.705–6.080)	2.746 (0.900–8.372)
Emergency admission (absence vs presence)	3.351 (1.116–10.065)*	1.758 (0.636–4.858)
Systemic steroid burst (absence vs presence)	2.747 (0.858–8.793)	0.686 (0.222–2.114)
Omalizumab use (presence vs absence)	3.348 (0.766–14.631)	3.610 (0.808–16.129)
Asthma control (well controlled vs other)	1.219 (0.511–2.912)	2.647 (1.096–6.392)*
Number of medications using for asthma and/or rhinitis symptoms (≥ 5 vs ≤ 4)	1.944 (0.734–5.153)	1.048 (0.366–2.999)
Job		
Housewife	1	1
No occupational risk group	2.722 (0.866–8.555)	1.918 (0.607–6.066)
Occupational risk group	7.943 (1.383–45.608)*	3.899 (0.667–22.787)
Smoking status		
Never smoker	1	1
Former smoker	0.673 (0.184–2.459)	0.299 (0.065–1.369)
Current smoker	0.863 (0.186–4.008)	1.461 (0.0355–6.018)
FEV_1% predicted (< 80 vs ≥ 80)	0.260 (0.651–1.628)	1.019 (0.419–2.478)

Note: R2 = 0.31 (Cox–Snell); 0.35 (Nagelkerke). Model χ^2 = 91.122, p < 0.0001; goodness of fit; deviance-p = 0.402; pearson-p = 0.183. *p < 0.05, shows the statistically significant difference. BMI — body mass index; FEV_1 — forced expiratory volume in first second; SPT — skin prick test

95% CI: 1.028–4.583), presence of drug allergy (OR:7.462, 95% CI:1.053–52.887), and absence of SS (OR:3.647, 95% CI:1.108–12.006) were more associated with the mold-sensitive group in comparison to the sensitized group without mold-sensitization.

Discussion

This study revealed that nearly one-third of adult asthmatic patients treated at this clinic are mold-sensitive; most of that group have polysensitization but well-controlled asthma. When compared with the non-sensitized group, patients in the mold-sensitive group were positively associated with shorter asthma duration, the presence of AR, an absence of SNP, and the presence of well-controlled asthma. Additionally, mold sensitivity was positively associated with the presence of drug allergies and an absence of SS bursts in comparison with sensitized patients without mold-sensitivity. In this study, we evaluated our population using two models. Firstly, univariate analysis was used to compare the three groups (non-sensitized, sensitized without molds, and sensitized with molds). Secondly, a multivariate analysis and logistic regression analysis of factors associated with mold sensitivity, in comparison with other groups, were performed. Due to the existence of confounding variables, we will discuss our findings according to the results of the logistic regression analysis, but also consider univariate analysis results in our comments.

The relationship between mold sensitivity status and asthma has been previously studied. Many prior studies showed that mold had a negative impact on asthma symptoms and asthma control [2]. A recent study that evaluated the relationship between mold burden in house dust and asthma control found that the concentrations of some molds detected in dust samples from the homes of asthma patients were negatively associ-

Table 4. Factors associated with mold sensitivity compared to non-mold sensitive group

	Mold sensitive group
Gender (female vs male)	1.026 (0.304–3.466)
Age groups (< 65 vs ≥ 65 age)	1.728 (0.413–7.221)
Asthma duration (< 10 vs ≥ 10 years)	2.170 (1.028–4.583)*
Asthma onset age (≥ 40 vs < 40 years)	1.421 (0.634–3.182)
BMI (< 30 vs > 30 kg/m^2)	1.327 (0.597–2.950)
Comorbidity (absence vs presence)	1.992 (0.868–4.576)
SNP (absence vs presence)	1.716 (0.469–6.275)
AR (presence vs absence)	2.096 (0.623–7.058)
Drug allergy (presence vs absence)	7.462(1.053–52.887)*
Hospitalization (absence vs presence)	1.489 (0.455–4.876)
Emergency admission (presence vs absence)	1.587 (0.533–4.717)
Systemic steroid use (absence vs presence)	3.647(1.108–12.006)*
Omalizumab (absence vs presence)	1.086(0.292–4.041)
Asthma control (others vs well controlled)	2.513 (1.086–5.816)
Number of medications used for asthma and/or rhinitis symptoms (≤ 5 vs ≥ 5)	2.126 (0.878–5.164)
Job	
Occupational risk group	1
No occupational risk group	1.588 (0.419–6.028)
Housewife	1.878 (0.460–7.665)
Smoking status	
Never smoker	1
Current smoker	1.720 (0.484–6.119)
Former smoker	0.592 (0.147–2.379)
FEV$_1$% predicted (< 80 vs ≥ 80)	1.380 (0.551–3.459)

Note: R2(Cox–Snell: 0.174, Nagelkerke:0.233) model $p < 0.05$. *$p < 0.05$, shows the statistically significant difference.
AR — allergic rhinitis; BMI — body mass index; FEV$_1$ — forced expiratory volume in first second; SPT — skin prick test

ated with parameters of asthma control in male subjects, but not in female ones. The researchers attributed their finding to males demonstrating a stronger immunoglobulin (Ig) E response following exposure to some molds. This speculation was also supported by the higher IgE concentrations of males in population studies and the higher capability of males to produce stronger allergic responses to fungal infections. Also, potentially due to the protective effects of sex hormones, women were expected to have a stronger immune response than that of men [7]. In our study, only 18.6% of our study population was male. The fact that the majority of our study population was female may be one of the explanations for finding a reverse relationship between mold sensitivity and asthma control. In another study from China, mold-sensitive asthmatics appeared to have higher asthma severity scores than those of the sensitized group without mold-sensitization, but they had lower FEV$_1$ values than those of the non-sensitized group [16]. These authors, however, excluded asthmatics with smoking histories; all of their participants were nonsmokers. In this study, our findings showed a positive impact on patients' asthma control status as well as SS bursts. We included asthmatics who smoke, but neither the univariate nor multivariate results differed with respect to smoking status.

In a cohort study, the effects of mold or dampness exposure during infancy on the risk of asthma, rhinitis, or IgE sensitization was evaluated in children followed from birth to 16 years of age. During this investigation, sensitization was assessed using blood samples in 3,293 children. Exposure to any mold or dampness was associated with asthma in patients up to 16 years of age, while exposure to mold odor and visible mold were associated with rhinitis. Increased risks were observed for nonallergic asthma and rhinitis [17]. Considering this study, it is possible that mold exposure also adversely affects nonallergic/ /nonatopic asthmatics rather than only mold-sensitive cases. In our study, data on environmental mold exposure could not be measured due to the experimental design; therefore, this confounding factor should be considered in future research.

In a recent review article [2], the authors considered studies that measured mold exposure both by qualitative and quantitative methods in order to evaluate the association between asthma, asthma development, asthma exacerbation, and rhinitis. Exposure to molds by using a qualitative metrics approach (i.e. observation of visible mold or smell) was found to have an association with asthma development. In the same review, it is also mentioned that there is currently insufficient evidence to determine whether an association exists between quantitatively measured mold species/ /components and the occurrence of asthma. In our study, we did not evaluate the indoor or outdoor mold exposure status of our study population. This is because it was previously reported that, regardless of sensitization patterns, asthma control was worse in a high-mold exposure group [18].

There are other dissenting studies in the literature to consider when analyzing the findings of this study. Al-Ahmad et al. did not find molds to have a significant triggering role, despite the

high rate of sensitization leading to asthma exacerbation in the desert environment. These authors evaluated asthma exacerbations and mold concentrations across the four seasons and found a higher average concentration of *Alternaria* and *Cladosporium* during September and November. Among the asthmatic participants, the mold-sensitive patients had higher rates of asthma exacerbations in that season. They additionally suggested that the climate and season can affect the presence of molds and asthma exacerbation [19]. Our region, the Eastern Black Sea of Turkey, has an oceanic climate with a narrow annual temperature range. This makes indoor mold growth and the outdoor mold rate similar during all four seasons.

Based on our findings, it is obvious that the mold-sensitive patients had shorter asthma durations. Younger mean age and younger mean asthma diagnosis age, and thus a higher rate of early-onset asthma, were also determined in univariate analysis in the mold-sensitive group. Previous studies have suggested a link between new-onset asthma and mold exposure; prolonged exposure to molds was also found to have an effect on new-onset asthma [20]. Further, it was concluded elsewhere that exposure to damp and moldy work places can induce new-onset adult asthma [21]. Mold exposure in early childhood was found to have an effect on asthma-related symptoms and the development of asthma at earlier ages [22]. Severe asthma with fungal sensitization was also found to be characterized by an early onset of the disease [23]. The mold-sensitive patients' shorter asthma durations and younger ages in this study could be related to the new-onset and early-onset effects of mold exposure in light of these prior investigations. However, Thacher *et al.* evaluated the effects of mold exposure on the development of asthma and rhinitis from birth to the age of 16 years in a cohort study and concluded that exposure to mold and dampness during infancy increased the odds of asthma and rhinitis. Further, exposure was associated with persistent asthma but not with early-transient or late-onset asthma [17].

The mold-sensitive group in our study had higher rates of AR. The effect of mold sensitivity on allergic rhinitis has been presented in previous studies [24]. Exposure to mold is associated with the development of asthma in occupants of damp buildings, and rhinitis is known to be a risk factor for asthma. However, there is little information about the degree of risk for the progression of rhinosinusitis to asthma owing to mold exposures in damp buildings [25]. Another study, from Poland, suggested AR patients' clinical distinctness. They found elevated nasal nitric oxide levels during seasons when the air concentrations of grass pollen and *Alternaria* spores were very high but there was no correlation during or after the pollen season [26]. The presence of a lower rate of SNP in our mold-sensitive group compared to the non-sensitized group is thus in line with the literature. It was previously reported that the SNP is more prevalent among nonatopic asthmatics than atopic asthmatics. Also, late-onset asthma was thought to have an association with the development of nasal polyposis [27].

Strengths and limitations

This study allows us to compare asthma patients' characteristics according to their mold sensitivity status, tested via SPT during their regular follow-ups. Data was gleaned from files from an expert ear-nose-throat outpatient clinic. Beside its ability to address a gap in the literature, there are some limitations that should be mentioned. The retrospective design of the study, as well as the heterogenous distribution of patients regarding gender and occupation, is a limiting factor. Another confounding factor is the sensitivity level of SPT, which limits the ability to determine the exact sensitization status of patients. Sensitivity of SPT in determining aeroallergen sensitization could be an issue. Therefore, future studies should consider using more specific tests. Of our patients, 5 who were treated with Omalizumab had negative SPT results. This may be due to steroid use or use of other medications that can produce false negative SPT results. Also, 26.4% of our asthmatics with an AR diagnosis had negative SPTs. In a previous national study, that rate was found to be 43.7% [28]. Evaluating all of the asthmatics that meet our broad inclusion criteria helped to make the groups more diverse. However, it also led to more confounding factors that could affect the evaluation of the direct effects of mold sensitivity. The lack of evaluation of indoor and outdoor mold exposure in the study design is another limiting factor.

In conclusion, asthmatic patients determined to be mold-sensitive by SPTs were found to have better asthma symptom control. The measurement of the mold exposure that patients encounter in their unique environments can lead to better accuracy regarding the effects of mold on asthma control and comorbidities. Based on the findings of this study, it should be kept in mind that mold sensitization in adult asthmatics is not always a poor prognostic factor.

Conflict of interest

None declared.

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Lung density in the trajectory path — a strong indicator of patients sustaining a pneumothorax during CT-guided lung biopsy

Abstract

Introduction: The purpose is to evaluate the prognostic significance of lung parenchymal density during percutaneous coaxial cutting needle lung biopsy (PNLB).

Materials and methods: Retrospective analysis of 179 consecutive patients (106 males, 73 females; mean age 59.16 ± 16.34 years) undergoing PNLB was included. Mean lobar parenchymal lung density, mean densities anterior to the lesion and posterior to the chest wall in the needle trajectory path were measured in HU. Lesion location and needle trajectory were also measured. Fisher's exact test and Chi-square test were conducted to analyze the categorical variables. ANOVA test was done to examine continuous and normally distributed variables. Statistical significance was considered when $p < 0.05$.

Results: Mean lobar parenchymal lung density ($p < 0.05$) and mean parenchymal lung density relative to the needle trajectory path were below -800 HU in patients who sustained a pneumothorax. Increase in the number of pleural passes was significantly associated with the risk of patients having pneumothorax ($p < 0.05$). The mean distance from the skin to the lesion and needle trajectory angle were not statistically different among patients with and without pneumothorax ($p > 0.05$).

Conclusion: Lobar parenchymal density and lung parenchymal density anterior to the lesion and posterior to the chest wall in the needle trajectory path could be used as predicting parameters in patients undergoing PNLB who sustained a pneumothorax. These findings can help interventional radiologist further assess risk of pneumothorax when preforming such procedure.

Key words: lung biopsy, iatrogenic pneumothorax, lung parenchymal density, parenchymal mass, needle trajectory

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Introduction

Percutaneous computed tomography (CT) guided needle pulmonary lesion biopsy is an effective and safe method for the diagnosis of lung nodules. In order to facilitate repeat sampling and decrease procedural duration, the coaxial technique for percutaneous lung lesion biopsy can be used. This method achieves all the aforementioned objectives without increasing the number of passes through the pleura [1]. Thus, percutaneous coaxial cutting needle lung biopsy (PNLB) has replaced open surgical biopsies as the gold standard for obtaining tissue samples from intraparenchymal lung masses to establish an accurate pathological diagnosis. The American College of Radiology and Society of Interventional Radiology have reported that there are no absolute contraindications that are addressed prior to PNLB [1]. However, there are relative contraindications for PNLB, including, but not limited to significant coagulopathy, compromised cardiopulmonary function, lack of a safe pathway to the lesion, an inability of the patient to cooperate with positioning and instruction, and pregnancy. Nevertheless, success rates are affected by many variables. The success rates in many quality improvement task forces are reliant on a combination of factors such as interventionist skill set, years of experience and rate of complications post PNLB.

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One important complication of PNLB is pneumothorax. Pneumothorax is reported to occur in 17 to 26.6% of patients, with 1 to 14.2% requiring chest tube insertion [2]. Risk factors for pneumothorax during PNLB include as follows: patient's age [3], greater lesion depth [4], lower lobe lesion [5], needle trajectory angle less than 45 degrees [3, 5, 6], lesion size [7], increased number of pleural passes [8] and radiographic emphysema [9].

This study aims to evaluate the possibility that lobar lung parenchymal density and lung parenchymal density relative to the trajectory path are linked to an increased risk of pneumothorax during PNLB. The primary end point of the study is to evaluate the ability of interventional radiologists to assess parenchymal lung density accurately as a quantitative specific threshold in predicting pneumothorax in patients undergoing PNLB. A secondary end point is to evaluate which factors related to the patient, the lesion, or the techniques of PNLB act as a strong indicator for pneumothorax. Such findings may be relevant to formulating a model that aids in assessing risks quantitatively, prior to intervention.

Materials and methods

Inclusion and exclusion criteria

The retrospective study included 179 patients (106 males and 73 females) who underwent CT-guided percutaneous lung biopsies and were classified according to a lung biopsy technique. Exclusion criteria were the following: lesion < 5 mm diameter, uncorrectable coagulopathy, positive pressure ventilation, severe respiratory compromise, pulmonary arterial hypertension, and incapacity to follow instructions or refusal of the procedure. The study had full IRB approval.

Region of interest measurements

One expert in medical imaging (C.S.) measured the attenuation of lung parenchyma and lesion in all 179 patients. Lung parenchymal attenuation that was in the trajectory path of the needle (Figure 1) was determined by placing the routinely used region of interest (ROI) within the lung segments of greatest dimension in the transaxial plane and was calculated by using the average measurement over three ROI. Attenuation was measured at the level of each angle (I–V), then the average measurement was taken to calculate lung parenchymal attenuation anterior to the lesion and posterior to the chest wall as per Figure 1. Total lobular parenchymal density was calculated employing an automated dedicated lung density software (Intellispace, 5.0, Philips Healthcare, Best, The Netherlands) to determine the mean attenuation of the lung parenchyma in each lobe of the lung.

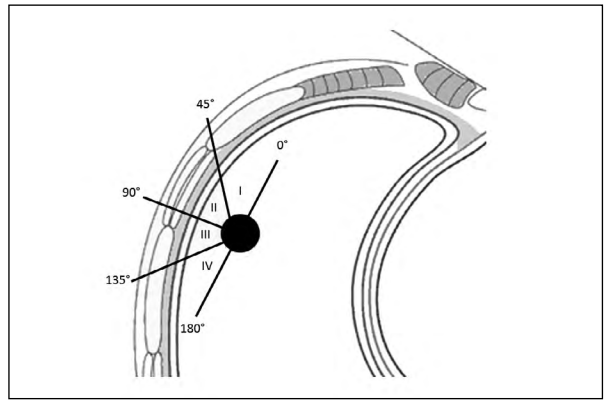

Figure 1. Lung parenchyma density within the trajectory angle from the chest wall

Biopsy protocol

Informed consents were obtained from all patients prior to the lung biopsy. Coagulation parameters of all subjects were checked to ensure platelet counts > 50,000/mL and an international normalized ratio < 1.5 as recommended in consensus guidelines [10]. All patients had chest CT scans that were checked and cross-referenced with the referring physician and the patient history, prior to the procedure. The scan parameters prior to the biopsy were the following: detector width 256 × 0.625 mm; pitch 1.1; rotation time 0.4 sec; exposure factors 100 kVp, 200 mA, with z-axis modulation; and a scanning time of 2.1 sec. The patients were then positioned in the prone, supine, or lateral decubitus position on the basis of the location of the target lesion to minimize the number of pleural reflections, avoid major fissures, predetermine the needle trajectory, the shortest distance to the lesion, and the amount of crossed lung parenchyma . The study subjects were instructed to take a reproducible breath inspiration and abstain from talking during scans, needle positioning, and sampling. The localizing CT scan was used to determine the position of the target lesion.

By using sterile technique, local anesthesia was employed with 1% lidocaine. A small subcutaneous incision was made for needle entry, and a 16-gauge guiding needle was placed to the thoracic wall just proximal to the pleura. Subsequently, the pleura was passed with a single puncture (Quick Core, Cook, USA), and the needle was placed into the lesion. Then, the inner

part (stylet) of the biopsy needle was removed and the orifice of the needle was water sealed using normal saline while asking the patient to hold his breath. Then, the 18-gauge cutting needle was inserted into the lesion over the introducer. All PNLB was performed by 4 interventional radiologists with a mean of 17 years of experience.

Limited CT scan was obtained of each patient that underwent lung biopsy to identify complications such as pneumothorax and bleeding. If the pneumothorax was confined and not symptomatic, a repeat limited CT scan was taken 2 hours post biopsy. If on repeat scan, the pneumothorax was stable, the patients with normal vital signs were sent home with safety instructions. If the pneumothorax increased and/or the patients became unstable, an 8 French pleural drain was placed, and the patient was admitted to hospital for 24 hours.

Statistical analysis

Statistical analysis was performed using SPSS (statistical package for social sciences, version 21, SPSS Inc., Chicago, IL, USA). Fisher's exact test, and Chi-square test were conducted to analyze the categorical variables. Fisher's exact test was done when the sample size within the categories was very minimal (i.e. when comparing lesion lobes and needle angles). Using the central tendency theorem stating that when the sample size is greater than 30 patients, the non-normally distributed variables are considered normal , we were able to conduct ANOVA test. The tested variables were gender, age, smoking status, the number of pleural passes, the distance from the skin and the chest wall, lung density posterior to the chest wall and anterior to the lesion, lung density relative to the needle trajectory (Figure 1) and total lobar parenchymal lung density. Lung lesion density ratio was compared between each group and the ratio of lobar lung density to lesion density was calculated. Logistic regression approach was implemented to conduct bivariate and multivariate analysis to assess the crude and adjusted odds ratios of the risk of having a pneumothorax. Only variables that showed statistical significance of having pneumothorax on the bivariate level with a p-value less than 0.2 were included in the multivariate analysis. Results with a p-value less than 0.05 were considered statistically significant.

Study protocol

A single investigator, who was an expert in medical imaging and was not included in the proper study, reviewed clinical records of each patient. All technical parameters were measured and the lung attenuation values employed an ROI of 2 mm. Lung density, lesion characteristics (location, size measured in greatest transverse diameter, and depth from the pleura along the biopsy track, if the lesion abutted the pleural surface), and the presence of a lung fissure or blood vessel intersecting the biopsy path were assessed and recorded (Figure 1).

Results

Patients' demographics

During the study period, 179 consecutive patients fulfilled the inclusion criteria and underwent image-guided lung biopsy at our institution. The mean age of males (61.58 ± 15.53 years) demonstrated a statistical difference compared to that of females (55.55 ±16.97) ($p < 0.014$). Whereas, the mean age of smokers (60.77 ± 15.03) was not statistically different from that of non-smokers (57.89 ± 17.24; $p < 0.247$).

Of the 179 lung lesions, 48 (27 %) were located in the right upper lobe, 17 (9%) in the right middle lobe, 27 (15%) in the right lower lobe, 47 (26%) in the left upper lobe, 29 (16%) in the left lower lobe, and 11 (7%) within the mediastinum. Malignant and benign lesions were diagnosed in 138 and 15 patients, respectively, with 26 non-diagnostic sample. The final diagnosis was adenocarcinoma (n = 55), small-cell carcinoma (n = 16), squamous cell carcinoma (n = 23), large-cell carcinoma (n = 12), lymphoma (n = 7) and metastatic tumors (n = 45).

Lung parenchymal characteristics and lung lesion density ratio

The lobar parenchymal lung density in all patients with pneumothorax demonstrated a threshold lower than -800 HU (increased aeration and reduced lung density), which included the segmental range (I – IV; 0–180°; Figure 1) of the needle trajectory (Table 1). Average lung density anterior to the mass and posterior to the chest wall showed an overall decrease in the subjects with pneumothorax compared to the patients without pneumothorax with varying significance. Significant decrease was noted at the right upper lobe ($p < 0.02$) and left upper lobe ($p < 0.02$).

Additionally, mean lesion density did not differ between both groups (Table 1). Finally, the lung (anterior to the lesion and posterior to the

Table 1. Mean lobar lung parenchymal density among the patients with and without pneumothorax

Pneumothorax	Yes N = 79	No N =100	P-value
Right lung			
Right upper lobe	-831.95 ± 52.03	-791.41 ± 51.88	< 0.001
Right middle lobe	-830.15 ± 54.05	-792.94 ± 59.69	< 0.001
Right lower lobe	-823.52 ± 67.48	-757.08 ± 58.97	< 0.011
Left lung			
Left upper lobe	-830.21 ± 53.36	-786.82 ± 58.97	< 0.001
Left lower lobe	-817.31 ± 75.88	-756.45 ± 172.10	< 0.030

Note: (±) is standard deviation

Table 2. Mean lung density anterior to the lesion and posterior to the chest wall, mean lesion density, and lung to lesion tissue ratio among the patients with and without pneumothorax

Pneumothorax	Lung density anterior to the lesion			Lesion density			Ratio	
	Yes	No	P-value	Yes	No	P-value	Yes	No
Right lung								
Right upper lobe	-819.4 ± 45.90	-768.9 ± 80.13	0.02	37.41 ± 15.93	36.26 ± 14.15	0.80	1.31	1.29
Right middle lobe	-841.0±42.90	-800.93 ± 47.90	0.10	38.59 ± 14.76	31.27 ± 23.17	0.44	1.13	4.17
Right lower lobe	747.87± 156.12	-782.10 ± 65.90	0.47	31.26 ± 12.40	30.16 ± 15.94	0.84	1.22	1.59
Left lung								
Left upper lobe	-830.05±61.70	-777.18 ± 48.67	0.02	32.28 ± 18.71	32.45 ± 20.29	0.98	1.14	1.24
Left lower lobe	-799.26±97.84	-755.55 ± 110.22	0.27	37.08 ± 14.02	35.73 ± 16.99	0.82	1.16	1.41
Mediastinal	-811.05±75.65	-725.15 ± 147.55	0.15	39.85 ± 12.33	42.71 ± 7.42	0.58	1.10	1.048

Note: (±) is standard deviation

chest wall) to lesion density ratio in the patients with pneumothorax ranged from 1.24 to 4.14, compared to a narrower range from 1.1 to 1.3 in individuals who sustained a pneumothorax (Table 2). This study suggests a possible risk of pneumothorax in patients with a lung to lesion ratio less than 1.3.

Lung parenchymal density relative to the needle trajectory

The mean lung density relative to needle trajectory in the patients with pneumothorax was lower than -800 in all five angles (0–180°; Table 3). Mean lung densities of 135° and 180° relative to trajectories showed statistically significant difference between the subjects with and without pneumothorax ($p < 0.001$ and $p < 0.012$, respectively). Although there was no statistically significant difference for the other angles ($p > 0.05$), all had a mean lung parenchymal threshold below -800 HU, which is compatible with the results shown in Table 1. This finding was irrespective of the trajectory needle angle.

Needle entry, trajectory, depth and distance characteristics

The mean of the number of pleural passes in the patients with and without pneumothorax was 2.61 ±1.85 and 1.95 ± 1.234, respectively, which was not statistically significant ($p < 0.061$). Needle trajectory angle showed no statistical significance ($p = 0.79$). The majority of needle angles fell within 0 to 45 degrees (Table 3).

The total mean distance from the chest was statistically significant comparing the patients with versus those without pneumothorax ($p < 0.032$). This was not the case when we were looking at the total mean distance from the skin ($p < 0.823$; Table 3). There was no statistical significance between lesion lobes and lesion depth for both populations nor between depth and having pneumothorax in each lesion lobe ($p > 0.05$).

Bivariate and multivariate analyses of risk of having pneumothorax

At the bivariate logistic regression level, smoking status, the mean distance from the chest

Table 3. Needle trajectory angle, lesion lobes and distance characteristics among the patients with and without pneumothorax

Pneumothorax	Yes N = 79	No N =100	P-value
Needle angle			
0–45 °	72	80	0.791
46–90 °	6	17	
91–135°	1	3	
Mean lung density relative to needle trajectory			
0° left	-806.89 ± 194.71	-780.46 ± 78.87	0.211
45° middle left	-801.86 ± 200.35	-771.17 ± 94.22	0.172
90° perpendicular to pleura	-804.38 ± 112.19	-791.96 ± 81.22	0.232
135° middle right	-810.48 ± 79.58	-764.75 ± 111.47	0.001
180° right	-811.39 ± 117.70	-763.76 ± 119.44	0.012
Mean distance from skin			0.281
Right lung Right upper lobe	60.07 ± 17.902	55.34 ± 20.683	
Right middle lobe	56.32 ± 17.521	51.98 ± 21.161	
Right lower lobe	56.25 ± 20.634	55.57 ± 18.892	
Left lung Left upper lobe	48.93 ± 13.134	59.64 ± 20.131	
Left lower lobe	55.42 ± 23.472	60.28 ± 17.397	
Mediastinal	57.26 ± 36.421	41.11 ± 20.702	
Total	55.3 ± 20.842	55.97 ± 20.023	0.823
Mean distance from chest wall			0.836
Right lung			
Right upper lobe	20.36 ± 13.382	11.63 ± 12.784	
Right middle lobe	15.47 ± 13.025	10.07 ± 14.418	
Right lower lobe	17.14 ± 18.866	10.85 ± 13.816	
Left lung Left upper lobe	13.04 ± 11.53	13.72 ± 17.409	
Left lower lobe	18.83 ± 19.02	16.31 ± 19.174	
Mediastinal	18.33 ± 24.521	4.19 ± 9.921	
Total	17.02 ± 16.199	12.06 ± 15.202	0.032
Number of passes	2.61 ± 1.85	1.95 ± 1.234	0.061

Note: (±) is standard deviation

wall, and the number of pleural passes were significantly associated with the risk of having pneumothorax ($p < 0.05$). For instance, smokers were 2.75 times more liable to have pneumothorax compared to non-smokers (OR = 2.75; 95%CI: 1.51–5.03). Whereas, age, gender, lesion depth and lesion lobes were not statistically significant with the risk of having pneumothorax ($p > 0.05$; Table 4).

Whereas at the multivariate logistic regression level, adjusting for age and gender, smoking status and the number of pleural passes remained in statistically significant association with the risk of having pneumothorax: smokers were 2.67 times more prone to having pneumothorax compared to non-smokers (OR = 2.67; 95%CI: 11.39–5.15). With every unit growth in the number of pleural passes, the odds of having pneumothorax increased multiplicatively by 1.33 folds (OR = 1.33; 95%CI: 1.69). However, the mean distance from the chest wall was not associated with the risk of having a pneumothorax ($p = 0.39$). Moreover,

Table 4. Bivariate and multivariate analyses of risk of having pneumothorax and other covariates showing adjusted ORs

	Bivariate analysis			Multivariate analysis		
Variables	**OR**	**95%CI OR**	**P-value**	**OR**	**95%CI OR**	**P-value**
Age	1.01	0.99–1.02	0.62	1	0.98–1.02	0.92
Gender						
Male	Reference		0.45	Reference		0.78
Femalez	0.79	0.44–1.44		0.91	0.46–1.79	
Smoking status						
No	Reference		0.001	Reference		0.003
Yes	2.75	1.51–5.03		2.67	1.39–5.15	
Mean distance from chest wall	1.05	1.00–1.1	0.04	1.01	0.99–1.04	0.39
Mean number of passes	1.34	1.08–1.66	0.007	1.33	1.05–1.69	0.02
Mean lesion depth						
Peripheral	Reference		0.12	Reference		0.23
Central	2.33	1.04–5.21	0.04	2.16	0.83–5.65	0.12
Deep	1.14	0.55–2.36	0.71	0.98	0.38–2.54	0.96
Lesion lobes						
Left lung						
Left upper lobe	Reference		0.33			
Left lower lobe	2.05	0.80–5.233	0.13			
Right Lung						
Right upper lobe	1.09	0.48–2.49	0.83			
Right middle lobe	3.06	0.96–9.69	0.06			
Right lower lobe	1.44	0.56–3.72	0.45			
Mediastinal	1.88	0.61–5.73	0.27			
Types of lung lesions						
Benign	Reference		0.23			
Malignant	0.56	0.19–1.66				

CI — confidence interval; OR — is odds ratio

lesion depth remained unassociated with the risk of having pneumothorax ($p > 0.05$; Table 4).

Discussion

The decision to perform PLNB depends on the site of the abnormality, the performance status, the co-morbidities, and how much of an impact the procedure will carry in terms of management. In the literature, there is insufficient data on the ability of radiologists and other clinicians to predict the occurrence of iatrogenic pneumothorax [9]. Previous studies have reported an increased risk of pneumothorax with lesion depth, angle of trajectory, and visually assessed emphysema on chest CT [9, 11–14]. Our findings suggest that although radiologists can predict the proportion of pneumothorax occurring in a cohort of patients, they are unable to do this in an individual case. To the best of our knowledge, this is the first study aiming to evaluate the quantitative lung parenchymal density and the risk of pneumothorax when performing PLNB.

Previously reported studies demonstrated a significant increase in pneumothorax rates from 13% for lesions abutting the pleural surface to 29% for lesions where the needle traverses the aerated lung, which is likely due to the decreased stability of the needle in a short intrapulmonary course leading to pleural tears [15]. Another study demonstrated that higher rates of pneumothorax were associated with needle paths greater than 4 cm [16]. Additionally, the interventional radiologist experience is the third major risk

factor for pneumothorax. The incidence rate of pneumothorax among experienced radiologists was 17% compared to 30% among unexperienced ones [15]. Our study demonstrated that overall lobar parenchymal density below -800 HU was associated with having pneumothorax. Also, we showed that lower lung densitities (less then -800 HU) relative to needle trajectory (0° to 180° anterior to the lesion and posterior to the chest wall) are associated with having pneumothorax. Adding to the current literature and data, quantification of the lung parenchyma in the trajectory path of the biopsy could further reduce the rate of pneumothorax during PNLB.

Other suggested risk factors for pneumothorax during PNLB include smoking, older age, needle thickness, biopsy needle angle, lesion position, lesion volume and distance from the pleura to the tumor [17]. In our study, initial bivariate analysis showed that pneumothorax was associated with a positive smoking history, increased mean distance from the chest wall, and the higher number of pleural passes. However, when we adjusted for age and gender, the mean distance from the chest wall was not associated with having a pneumothorax. Moreover, age was not linked to an increased risk of pneumothorax, which is not compatible with the current literature [17]. Finally, gender, lesion depth (peripheral, central or deep) and lesion lobes were not associated with having pneumothorax.

There were shortcomings in our study. First, the retrospective design of the research made it difficult to determine the clinical indication for ordering a lung biopsy in each patient accurately. Also, for reasons such as lack of follow-up, final results from surgical pathology or autopsy were not available to confirm a benign or malignant etiology for all of the non-diagnostic core biopsies. Second, the relationship between the distance of a target from the diaphragm and the likelihood of a diagnostic success on the basis of the etiology of the lesion was not addressed. Third, our study did not measure the associated post-biopsy complication rates such as bleeding, infection and clinical outcome. Finally, there was no comparison between operators.

Conclusions

Pneumothorax is still a major issue in PNLB with a myriad of risk factors. This is the first study to investigate and prove that the density of lobar lung parenchyma and parenchyma anterior to the lesion and relative to the needle trajectory path could be used as parameters in predicting pneumothorax in patients undergoing percutaneous coaxial cutting needle biopsy. This finding can help interventional radiologist further assess risk of pneumothorax when preforming such procedure.

Conflict of interest

Authors declare no grants, financial support or conflict of interest.

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The -463G/A and -129G/A myeloperoxidase-encoding gene polymorphism in chronic obstructive pulmonary disease

Abstract

Introduction: Neutrophils are involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). Myeloperoxidase is an important bactericidal granulocytic enzyme. It is of interest to question whether or not the polymorphic variants of the myeloperoxidase-encoding gene are associated with the risk of developing COPD.

Material and methods: The study determined the risk of COPD development in 186 COPD patients and 220 healthy subjects in the context of two selected polymorphic sites of the promoter region of the myeloperoxidase-encoding gene.

Results: It has been demonstrated that the AA genotype of locus -463 in the myeloperoxidase-encoding gene increases the risk of developing COPD (OR: 2.87; CI: 1.651–4.997). This genotype also correlates with a higher gene expression in patients (0.56 ± 0.12 *vs* 0.31 ± 0.18 in patients with AG genotype and 0.29 ± 0.17, $p < 0.01$ in those with GG genotype). In healthy individuals, the AA genotype was also characterized by increased expression of the myeloperoxidase-encoding gene (0.41 ± 0.16 *vs* 0.29 ± 0.15 for AG genotype, $p < 0.01$ and 0.25 ± 0.16 for GG genotype $p < 0.01$). Patients with the AA genotype had a significantly higher gene expression than healthy subjects with this genotype.

Conclusions: The polymorphic site -129 of the myeloperoxidase-encoding gene was unrelated to the development of COPD. The gene expression did not differ for the individual genotypes. Our studies indicate that the polymorphism of the myeloperoxidase--encoding gene may be related to chronic obstructive pulmonary disease.

Key words: genotype, gene expression, polymorphic sites

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Introduction

Chronic obstructive pulmonary disease (COPD) inflammatory disease of the respiratory system which leads to irreversible airway obstruction and the formation of *emphysematous bullae* [1]. The neutrophil is one of the important cells involved in airway inflammation in COPD [2]. Neutrophils are phagocytes which secrete numerous inflammatory mediators, including reactive oxygen species [3]. An enzyme important for the antimicrobial defense of neutrophils is myeloperoxidase (EC 1.11.1.7) [4, 5]. This enzyme belongs to the group of hemoproteins catalyzing the formation of hydrogen peroxide, chloride anions of toxic hypochlorites and, in lesser amounts, of tyrosine and nitrotyrosine radicals, singlet oxygen and ozone [4–6]. The above products kill bacteria and pathogenic fungi and cause damage to the genetic apparatus of some viruses such as HIV-1 [5]. The products of catalytic activity of myeloperoxidase also damage the host's tissue [5]. An unfavorable contribution of this enzyme's activity to the pathogenesis of many inflammatory diseases has been noted. These diseases include chronic obstructive pulmonary disease, ARDS, Alzheimer's disease, multiple sclerosis, myocardial infarction, stroke and many others [5].

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Myeloperoxidase (MPO) is encoded by the gene localized in the 17q23.1. fragment of chromosome 17 in the cluster coding for numerous growth hormones [7]. Myeloperoxidase is one member of a gene family of mammalian peroxidases that also includes eosinophil peroxidase, lactoperoxidase, thyroid peroxidase, and prostaglandin H synthase [7]. The mature enzyme is a 140-kDa dimer of identical halves, each containing two polypeptide chains of 108 and 466 amino acids resulting from post-translational excision of 6 amino acids from a single polypeptide precursor. Each half-molecule contains a covalently bound heme that exhibits unusual spectral properties [7]. The significance of polymorphism of the myeloperoxidase-encoding gene in numerous human diseases has been demonstrated. Among others, the myeloperoxidase gene polymorphism plays a role in the risk of gastric cancer [8], preeclampsia [9], lung and prostate cancer [10], coronary heart disease [11], periodontitis [12], chronic lymphocytic leukemia and multiple myeloma [13], as well as in many other pathologies. In our own studies, we have not demonstrated the correlation of the myeloperoxidase gene polymorphism with macular degeneration [14].

Research and discussion of whether polymorphism of the gene coding for myeloperoxidase plays a role in the pathogenesis of COPD is warranted. Neutrophils and mediators produced by them are one of the main pathogenic pathways of this disease [15]. On the one hand, myeloperoxidase is an enzyme of the antimicrobial defense system but on the other, its harmful effect on the tissue has been demonstrated [16–18].

The purpose of this study was to evaluate the frequency of alleles and two polymorphic sites of the myeloperoxidase-encoding gene.

Material and methods

In our study, we examined 186 patients with COPD and 220 healthy controls. There were no statistical differences in sex and age between the groups of patients and the healthy controls. The study exclusion criteria for the patients were as follows: a history of malignant tumors, collagenosis and systemic vasculitis, type 2 diabetes mellitus, bronchial asthma, and asthma and COPD overlap. All of the subjects forming the study group and the control group were smokers or ex-smokers with a minimum number of 10 smoking pack-years. The age of the participants ranged from 45 to 70 years. The group of patients with COPD included 50 at risk level A, 56 at level B, 34 at risk C, and 46 at risk level D. The allele frequency distribution was not analyzed with respect to the level of COPD risk because of an insufficient size of the groups.

In the group of 186 patients, the mean age was 62 years of age with a standard deviation of 7 years, and in the control group the mean age was 60 with a standard deviation of 9. The differences were not statistically significant. The youngest patient was 48 years old while the youngest healthy subject was 45 years old. Among 186 patients, 56 were women and 130 were men. The control group consisted of 66 women and 154 men. Gender distribution did not differ between the two groups.

Reagents

All reagents used were of analytical grade. The restriction enzymes as well as Taq DNA polymerase were procured from Promega (Madison, WI, USA). Oligonucleotides were synthesized at IDT (Coralville, IA, USA).

DNA extraction

Blood DNA was purified on a QIAamp spin column (Qiagen, Hilden, Germany) using the protocol for DNA isolation from body fluids provided by the manufacturer and modified as follows: 5 μg of RNA poly(A) (Pharmacia Biotech, Uppsala, Sweden) was added to 1 mL of serum to serve as a carrier to improve the recovery of small amounts of DNA. Lysis was ensured by adding 20 μL of Qiagen Proteinase K solution and 1 mL of AL buffer (QIAamp® DNA mini kit). After 10-min of incubation at 56°C, 1 mL of ethanol was added. The mixture was loaded on the QIAamp spin column and centrifuged at 20000 g for 1 min. The column was washed twice by adding 500 μL of AW buffer (QIAamp® DNA mini kit) and centrifuged at 20000 g for 1 min. Finally, DNA was incubated for 5 min at room temperature with 50 μl of AE buffer and eluted by centrifugation.

Polymorphism of MPO gene (-463, -129)

The isolated DNA was used for amplification of -463 and -129 SNP sequences of MPO human gene. The PCR reactions and conditions were identical for both polymorphisms by applying starters: 5'CGGTATAGGCACACAATGGTGA 3', 5'GCAATGGTTCAAGCGATTCTTC 3' specific for -463 polymorphism and 5'TGGGCAACAGAGCAAGATAA3', 5'CTCTTT CTCCTCCCCCACTG3' specific for -129. Amplification was performed in a total volume of 25 μL containing 50ng of

DNA, 50 pM PCR primers, dNTP, each at a concentration of 80 μM and 1U of GoTaq polymerase (Promega). PCR conditions were as follows: 94°C for 30 sec, 59°C for 30 sec and 72°C for 30 sec (35 cycles). The final elongation step was 10 min at 72°C. The amplification products of 350 bp length (-463 polymorphism) were digested with AciI restriction enzyme at 37°C and separated on 3% agarose gel. The digestion of homozygote GG yields three fragments of 169 bp, 120 bp and 61 bp, heterozygote GA 289 bp, 169 bp, 120 bp and 61 bp fragments and homozygote AA 289 bp, 61 bp fragment. The genotyping of the -129 SNP sequences of the MPO human gene was analyzed by directly sequencing the PCR product. Amplification was performed under the same conditions using 0.1 μg genomic DNA, 200 μM each dNTP, 5 × GoTaq buffer solution, 1U GoTaq polymerase (Promega, Madison WI USA), 0.5 μM primers. Amplification of the product of 129 bp was sequenced using a specific primer labelled with biotin molecule 5'ATTTCAGG '3' by DNA sequencing service IBB PAN (Warsaw Poland).

RNA purification and real time RT-PCR

The human myeloperoxidase and GADPH gene expression was quantified by real-time PCR using ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA USA) according to the manufacturer's protocol. Total cellular RNAs were extracted from the patients' whole blood cells using the Trizol reagent (Invitrogen, Groningen, Nederlands) method, a single-step purification protocol [19]. Polyadenylated RNA was isolated using an Oligotex kit (Qiagen, Chatsworth, CA USA). 50 ng of poly(A) RNA was then used for cDNA synthesis using the TaqMan Reverse Transcription Reagents kit (Applied Biosystem) according to the manufacturer's protocol. Briefly 2.5, 2.0; 1.5, 1.0; 0.5 and 0.25 μL of synthesized cDNA were amplified in triplicate for both GADPH and each of the target genes to create a standard curve. Likewise, 2 μl of cDNA was amplified in triplicate in all isolated samples for each primer/probe combination and GADPH. Each sample was supplemented with both respective 0.3 μM forward and reverse primers, fluorescent probe, and made up to 50 μL using qPCR™Mastermix for SYBIR Green I (Eurogentec Seraing Belgium). All following PCR primers were designed using software PrimerExpress (Applied Biosystem) forward 5'CCACCAAAACCGATCACCAT 3', reverse 5'CACTCCTCGCCTG CATCAT 3'forward, 5'AGCCACATCGCTCAGACAC 3', reverse 5' GCCCAATACGAC CAAATCC 3' specific for mRNA of human myeloperoxidase and GADPH respectively. GADPH was used as an active and endogenous reference to correct for differences in the amount of total RNA added to the reaction and to compensate for different levels of inhibition during reverse transcription of RNA and during PCR. Each target probe was amplified in separate 96-well plate. All samples were incubated at 50°C for 2 min. and at 95°C for 10 min. and then cycled at 95°C for 30 sec, 56°C for 1 min. and 72°C for 1 min. for 40 cycles. SYBR Green I fluorescence emission data were captured and m-RNA levels were quantified using the critical threshold (C_t) value. Analysis were performed with ABI Prism 7000 (SDS Software). Controls without RT and with no template cDNA were performed with each assay. To compensate for variations in input RNA amounts, and to maximize efficiency of reverse transcription, GADPH mRNA was quantified and results were normalized to these values. Relative gene expression levels were obtained using the $\Delta\Delta C_t$ method [20]. Amplification-specific transcripts were further confirmed by obtaining melting curve profiles.

Results

The frequencies of the investigated genotypes of locus -463 in the myeloperoxidase-encoding gene in the study population are summarized in Table 1 and Table 2. The AA genotype at locus -463 of the myeloperoxidase-encoding gene has a 2.87-fold increased risk of developing COPD

Table 1. Frequency of genotypes and alleles at -463 polymorphic site of the MPO-encoding gene in COPD and in subjects without COPD. The distribution of genotypes in the group of patients with COPD differs from the distribution in healthy subjects ($Chi^2 = 114.19$, $p < 0.001$)

	Allele frequency		Genotype distribution		
	A [%]	G [%]	AA [n%]	AG [n%]	GG [n%]
COPD patients	46	54	45 (25)	77 (41)	64 (34)
Healthy subjects	24	76	22 (10)	40 (18)	158 (72)

COPD — chronic obstructive pulmonary disease

Table 2. Frequency of genotypes and alleles at -129G/A genomic site of the MPO-encoding gene in COPD patients and in the healthy group. The distribution of genotypes in the group of patients with COPD does not differ from the distribution in healthy subjects ($Chi^2 = 5.48$, $p = 0.064$)

	Allele frequency		Genotype distribution		
	A [%]	G [%]	AA [n%]	AG [n%]	GG [n%]
COPD patients	25	75	4 (2)	60 (32)	122 (66)
Healthy subjects	26	74	6 (3)	69 (31)	145 (66)

COPD — *chronic obstructive pulmonary disease*

(95% CI: 1.651–4.997) as compared to AG and GG genotypes. Carriage of the AA and AG genotypes *vs* the GG genotype did not significantly affect the risk of developing COPD (OR: 0.813, 95% CI: 0.521–1.270). Carriage of the AA genotype relative to the GG genotype increases the risk of COPD in smokers (OR: 5050, 95% CI: 2.808–9.080). The AG genotype as compared to GG was also found to be a risk factor for COPD (OR: 4.752, 95% CI: 2.941–7.679). Carriage of the AA and AG genotype vs. the GG genotype is also a risk factor for the development of COPD (OR: 4.857, 95% CI: 3.187–7.406). In contrast, the AA genotype did not increase the risk of developing COPD relative to carriage of the AG genotype (OR: 1.0634, 95% CI: 0.562–2.001).

Genotypic distribution of the polymorphic locus -129 of the gene encoding MPO was not different in healthy subjects and patients and was not associated with the risk of developing COPD.

The myeloperoxidase-encoding gene expression for the -463AA genotype was significantly higher in both healthy subjects and COPD patients (Table 3). Patients with the AA genotype demonstrated a higher gene expression than healthy individuals with the AA genotype. The gene expression did not differ for the remaining genotypes.

No differences in gene expression were reported for the polymorphic variants of locus -129 of the myeloperoxidase-encoding gene (Table 4).

Table 3. Expression of the myeloperoxidase-encoding gene at site -463 according to the genotype in patients with COPD and in healthy subjects. The gene expression in the serum expressed as $2^{-\Delta\Delta Ct}$

Genotype	Gene expression
COPD patients	
AA	$0.56 \pm 0.12^{*,\#}$
AG	0.31 ± 0.18
GG	0.29 ± 0.17
Healthy subjects	
AA	$0.41 \pm 0.16^{**}$
AG	0.29 ± 0.15
GG	0.25 ± 0.16

*Significantly higher than gene expression for phenotypes AG and GG in patients, $p < 0.01$; **significantly higher than gene expression for AG and GG phenotypes in healthy subjects, $p < 0.01$; #Significantly higher in patients with AA phenotype than in healthy subjects with AA phenotype.
COPD — *chronic obstructive pulmonary disease*

Table 4. Expression of the myeloperoxidase-encoding gene at site -129 according to the genotype in patients with COPD and in healthy subjects. The gene expression in the serum expressed as $2^{-\Delta\Delta Ct}$

Genotype	Gene expression
COPD patients	
AA	0.42 ± 0.12
AG	0.31 ± 0.18
GG	0.30 ± 0.17
Healthy subjects	
AA	0.41 ± 0.16
AG	0.29 ± 0.15
GG	0.31 ± 0.16

No inter-group differences in expression were observed. COPD — *chronic obstructive pulmonary disease*

Discussion

The obtained results show the polymorphic locus -129 of the gene encoding human myeloperoxidase is not associated with the risk of developing COPD. The AA genotype at the polymorphic site -463 of the myeloperoxidase-encoding gene has a clear association with the occurrence of chronic obstructive pulmonary disease. It is also associated with an increased expression of mRNA of the enzyme-encoding gene and probably with the enzyme activity that was not studied in this research. As reported in the literature, neutrophil granulocytes are the most important cells responsible for the development of COPD [21, 22]. The mediators released by neutrophils damage the

respiratory epithelium, cause free radical and proteolytic tissue damage, and initiate respiratory tract remodeling typical of COPD [21, 22]. The increased expression of the AA genotype of locus -463 in the myeloperoxidase-encoding gene is associated in our studies both with an increased risk of developing COPD and with an increased expression of the enzyme mRNA. It is well known that neutrophils are the cells responsible for the development of COPD, however, the significance of the mediators released by myeloperoxidase is controversial. On the one hand, myeloperoxidase kills bacteria that cause exacerbations of infection in the course of COPD but, on the other hand, it damages the tissues of the respiratory tract [1, 2, 21, 22]. The lack of "tuning" the magnitude of the reaction involving the secretion of mediators by neutrophils against the infection may be responsible for the remodeling of the respiratory tract and the formation of emphysematous bullae [23]. Such a situation resembles an excessive immune response in autoimmune diseases, but this is only our hypothesis. Similarly, peroxidase deficiency is associated with a disease similar to chronic granulomatous disease and has a predisposition to severe Candida albicans infections [24]. It has recently been demonstrated that myeloperoxidase itself is a mediator of post-ischemic arrhythmogenic cardiomyopathy [25]. Conceivably, it is either through its own actions, or through the peptides resulting from its proteolytic degradation, a mediator of bronchial remodeling. The effects of myeloperoxidase on cardiac vessels and on the heart itself suggest that it contributes to the development of pulmonary hypertension and pulmonary heart disease in COPD, which requires further investigation. The role of HClO, the main product of myeloperoxidase enzyme activity in inducing bronchial remodeling and emphysema, cannot be ruled out, although there has been no research work on this subject to date. Undoubtedly, reactive oxygen species, including hypochloride produced by myeloperoxidase, have a well-established role as COPD development factors [26]. It is interesting what transcription factors affect the expression of the myeloperoxidase gene and what their association is with the polymorphic sites of the promoter region of the gene in question. The polymorphism of locus -463 in the myeloperoxidase-encoding gene is associated with a risk of developing Alzheimer's disease [27]. On the other hand, studies of Asian populations have shown that the AA genotype is associated with a reduced risk of lung cancer [28]. Also, the GG genotype reduces the risk of developing type 2 diabetes [29]. Subjects predisposed to developing lung cancer in the course of COPD may be those who carry the GG genotype. The AA/GA genotype at locus -463 promote the development of hypertension in people with a low BMI and without diabetes [30]. It would be interesting to check the blood pressure distribution in our study group, but this was not the subject of the research. The AA/GA variant reduced the risk of colorectal cancer in the Chinese population [31].

The disadvantage of our research was the fact that we did not determine myeloperoxidase activity and expression in sputum. This would give a more realistic and detailed picture of the correlations between the polymorphic variants of the myeloperoxidase gene and the enzyme activity, as well as its expression in the airways. On the other hand, COPD is a systemic disease and changes in its expression in the blood may have some significance in its pathogenesis. The studies need to be repeated in larger groups and in other populations coming from different regions. In addition, it needs to include the gene expression in induced sputum, or in bronchoalveolar lavage. In our work, we did not analyze the relationships between the polymorphic variants and the severity/risk of COPD. An analysis with division into subgroups would have resulted in there being too few patients to obtain reliable results. It would be interesting to see whether the genotype distributions in the non-smoking group would be similar to the distributions in smokers without COPD. Such studies, however, have not been conducted. That being said, in a study by Kowalski *et al.* concerning the polymorphic variants of the myeloperoxidase-encoding gene (the same as in our study), distributions of genotypes obtained in healthy subjects were similar to those obtained in our work for smokers (the control group in our study) [14]. The aforementioned study was also conducted in the Polish population. It indicates that the studied gene variants are unlikely to be associated with the risk of nicotine dependence syndrome. The distribution, which is similar to ours, supports the probable repeatability of the results we obtained. The contribution of genetic factors to the pathogenesis of nicotine addiction is likely to be low and involves only the genes coding for cytochrome C450 variants and receptor proteins in the central nervous system [32].

Conclusions

1. The polymorphism of locus -463 in the myeloperoxidase-encoding gene is associated

with the risk of developing COPD. Carriage of the AA genotype is associated with a 2.87-fold higher risk of COPD. Also, the myeloperoxidase gene expression in the blood is higher in subjects with the AA genotype, both healthy and sick; however, it is significantly higher in the patient group than in healthy subjects

2. The polymorphism of locus -129 in the myeloperoxidase-encoding gene has no association with the risk of COPD.

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Conflicts of interest

The authors have no conflict of interest to report.

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Transbronchial lung cryobiopsy guided by radial *mini-probe* endobronchial ultrasound in interstitial lung diseases — a multicenter prospective study

Abstract

Introduction: Transbronchial lung cryobiopsy (TBLC) is commonly used in diagnosing interstitial lung diseases (ILDs). A general anesthesia with endotracheal intubation, balloon blockers and fluoroscopy control is the most common modality. Simplifying the procedure without decreasing it's safety could result in wider use.

Prospective, observational study was conducted in three Polish pulmonology centers to evaluate safety and diagnostic yield of TBLC under conscious sedation, without intubation and bronchial blockers and with radial-EBUS guidance instead of fluoroscopy.

Material and methods: In patients suspected of ILD, in accordance with high resolution computer tomography (HRCT) selected lung segments were examined with radial-EBUS mini probe without a guide sheath. If the lung infiltrations were visible this locations were preferred. If not, specimens were taken from two different segments of the same lobe. Two to five biopsies with freezing time 5–8 seconds were performed. Moreover ultrasound examination was used to avoid injury of lung vessels.

Results: From March 2017 to September 2019 — 114 patients (M: 59, F: 55) of mean (SD) age 54 (14) years were included to the study on the basis of medical history and HRCT. Histopathology was conclusive in 90 (79%) patients and included 16 different diagnoses (sarcoidosis, EAA, COP predominantly). 24 inconclusive biopsies of unclassifiable pulmonary fibrosis were followed up. Complications included five cases (4.4%) of pneumothorax requiring a chest tube drainage and a minor and moderate bleeding in few cases. There was no need for use of balloon bronchial blockers.

Conclusions: TBLC under conscious sedation guided by radial EBUS mini-probe is novel, reasonable and safe technique for histological diagnosis of ILDs.

Key words: cryobiopsy, interstital lung diseases, endoscopy, endobronchial ultrasound, bronchoscopy

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Introduction

Interstitial lung disease (ILD), also known as diffuse parenchymal lung disease is a heterogeneous group of conditions. The common pathological mechanism of these illnesses is the presence of inflammation and/or fibrosis in the lung parenchyma. When clinical outcome and radiology findings (especially in high resolution computed tomography, HRCT) are not sufficient to establish proper diagnosis, a pathological assessment of the involved lung tissue may be necessary in selected

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patients. A transbronchial lung biopsy (TBLB) with endoscopic forceps is the most common and the least invasive approach. Whereas its high value in diagnosing only few ILDs (as sarcoidosis) is well established, TBLB is not recommended for identifying pulmonary fibrosis. Regarding idiopathic pulmonary fibrosis (IPF), either ATS/ /ERS/JRS/ALAT (American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association) or Fleischner Society guidelines recognize a surgical lung biopsy (SLB, usually video-assisted thoracic surgery lung biopsy — VATS-LB) as a gold standard and recommend it if HRCT is not conclusive [1, 2]. The other important indication for lung biopsy (LB) in patients with diffuse lung infiltrations is an exclusion of malignancy. Considering that the most common primary lung cancer — adenocarcinoma is quite often diagnosed among patients with no smoking history and with radiological appearance of diffuse lung infiltrates imitating ILD, histological assessment is crucial in many cases [3, 4]. Patients after VATS-LB often require a few days of hospitalization and face a risk of some possible complications, as prolonged air leak and bronchopleural fistula, involving in some cases reoperation and low but significant mortality risk [5, 6]. Considering all these complications, the development and widespread introduction of minimally invasive methods of LB into the practice in ILD with pulmonary fibrosis is necessary.

A biopsy with the use of a flexible cryoprobe was introduced in clinical practice a few years ago, and initially proved its diagnostic value both in endobronchial and transbronchial tissue sampling [7, 8]. Studies published to date have suggested that diagnostic yield of transbronchial lung cryobiopsy (TBLC) in establishing proper diagnosis of ILD reaches 70–80% [9–12].

The review of cryobiopsy literature has revealed a lack of TBLC procedure standardization [1, 10, 11]. While the basic technique of TBLC is nearly the same, the approach how to avoid and manage the most common complications: pneumothorax and severe bleeding differs. The risk of pneumothorax increases when a cryoprobe is inserted too far into the bronchi and freezing hurts the visceral pleura. On the other hand, when a cryoprobe is too close to the pulmonary vessels, the risk of severe bleeding is higher.

There is no doubt that choosing a correct site for LB and controlling the cryoprobe position during the procedure is crucial both for its efficacy and safety. The most common method of supervising a position of a cryoprobe's tip is fluoroscopy [10–12].

In our center, a radial endobronchial ultrasound miniature probe (r-EBUS) is routinely used for diagnosing peripheral solid and semisolid lung lesions. A few years ago we introduced r-EBUS before performing conventional forceps TBLB in diagnosing patients suspected of ILD. In our experience in some cases, if lung opacities are visible in ultrasound imaging, r-EBUS allows the bronchoscopist to choose the best biopsy site. It is very useful in localizing vessels of the hilum and sometimes even shows the visceral pleura. Based on these observations, we designed a study for the diagnosis of ILDs with the use of TBLC without fluoroscopy but controlled with r-EBUS.

Material and methods

This study was designed as an open-label observational prospective trial. It was a multicenter study conducted in Pulmonary Hospital, Zakopane, in John Paul II Hospital, Cracow, and in Clinical Hospital of the Silesian Medical University, Katowice, Poland. A study protocol was approved by Bioethical Committee of District Medical Association, Cracow, Poland (129/KBL/ /OIL/2017).

Consecutive patients in whom the decision of lung biopsy was made, in the process of ILD diagnosing were included in the study. Qualification for LB was made on the basis of clinical outcome and radiology assessed by experts in pulmonology and radiology according to the British Thoracic Society and previous edition of ATS/ERS/JRS/ALAT guidelines [13, 14]. The main exclusion criteria were as follows: lack of patient's agreement, age below 18 years and standard contraindications for invasive endoscopy such as inappropriate hemostasis parameters, instable cardiovascular disease and/or heart failure and pulmonary hypertension. After receiving the informed consent, the TBLC procedure was performed. During the endoscopy, the patient was in the supine position under conscious sedation: fentanyl (0.05–0.1 mg) and midazolam (2.5–7.5 mg) were administered intravenously. A pulse oximetry monitoring was mandatory and oxygen supply was provided when it was necessary. A whole procedure was performed by a trained endoscopist with the assistance of two endoscopy nurses. A flexible videobronchoscope (BFT180 or BFT190 Olympus, Tokyo, Japan) with a 2.8–3.0 mm working channel was introduced orally, without endotracheal intubation with a tube or rigid bronchoscope. After topical administration of a 2% lignocaine and bronchial

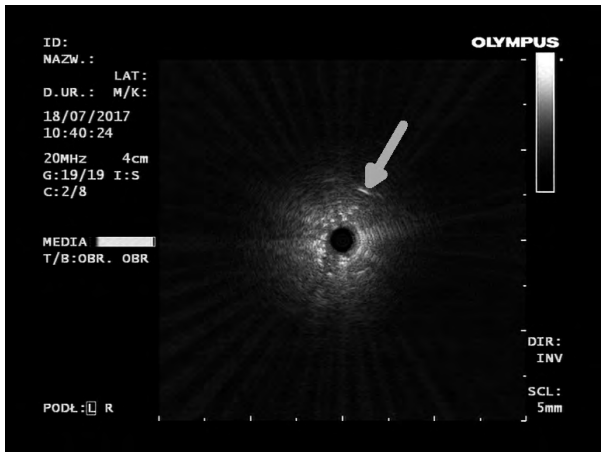

Figure 1. Ultrasound imaging of the visceral pleura (white arrow) obtained with radial endobronchial ultrasound mini-probe — rEBUS. With the consent of the Archives of Endoscopy Unit of Pulmonary Hospital, Zakopane

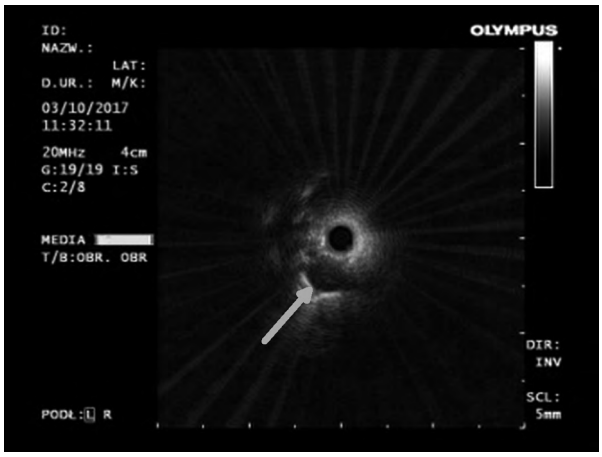

Figure 2. Ultrasound imaging of the subsegmental pulmonary artery (white arrow) obtained with radial endobronchial ultrasound mini-probe (rEBUS). Biopsy in this site would cause significant bleeding. With the consent of the Archives of Endoscopy Unit of Pulmonary Hospital, Zakopane

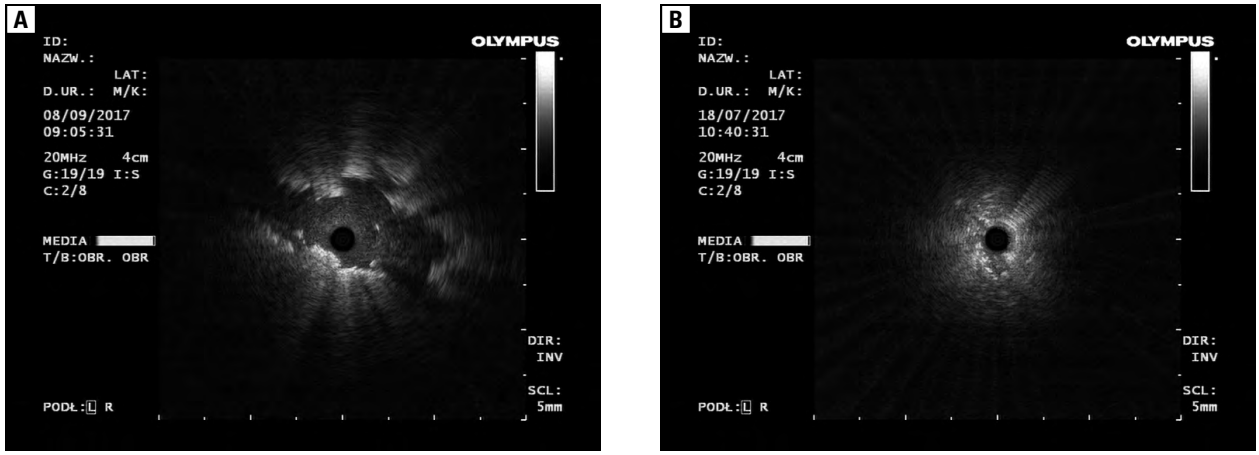

Figure 3. Ultrasound imaging of interstitial lung infiltration obtained with radial endobronchial ultrasound mini-probe (rEBUS). **A.** Cryptogenic organizing pneumonia; **B.** Usual interstitial pneumonia. With the consent of the Archives of Endoscopy Unit of Pulmonary Hospital, Zakopane

tree inspection, the ultrasound radial miniature probe UM-S20-20R (Olympus, Tokyo, Japan) was used. Selected, according to HRCT, lung regions were examined with r-EBUS in order to choose the optimal site for TBLC. The ultrasound probe without a guide sheath was inserted as far as possible (sometimes a specific ultrasound sign of the visceral pleura was visible — Figure 1). A slow withdrawing of the ultrasound probe with controlling the distance between the pleura (if visible) and hilar vessels (Figure 2) allowed to identify the safest area for the biopsy. The additional advantage of this method was that in some cases the lung infiltrates were visible in the ultrasound image (Figure 3), and if there were no vessels close to the lesions, the exact location of the biopsy could be established. Following an ultrasonographic examination, the flexible cryoprobe (diameter 1.9 mm, length 900 mm; ERBE, Tubingen, Germany) was introduced to the working channel of the bronchoscope. After placing its tip in previously selected site (according to r-EBUS examination), TBLC was performed with freezing time of 5–8 sec (mean 7 sec). Two to five biopsies were taken from two different segments of the same lobe, whereas in diffused lung infiltrates, lower lobes were chosen. If ultrasound examination was negative for the presence of lung infiltrations, distance from the visceral pleura was approximately 1–2 cm. The bronchoscope with the inserted cryoprobe and lung tissue adhered to the tip of the probe was taken out of the airways *en bloc*. As the specimen was placed in the saline (Figure 4), the cryoprobe was pulled out, and the same scope was inserted into the bronchial tree

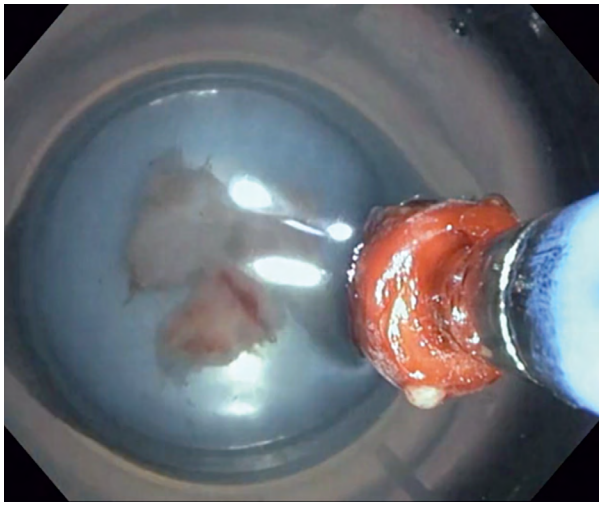

Figure 4. Two lung tissue specimens obtained with flexible cryoprobe and the third one attached to the cryoprobe's tip. With the consent of the Archives of Endoscopy Unit of Pulmonary Hospital, Zakopane

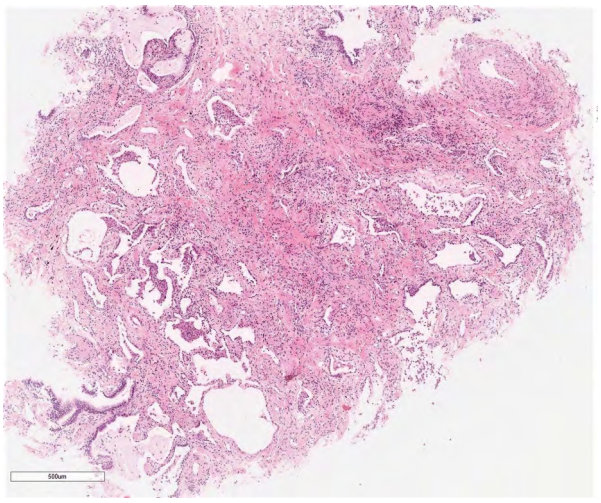

Figure 5. A microscopic imaging of cryobiopsy (hematoxilin and eosin staining). Usual interstitial pneumonia (UIP) associated with histologic pattern (patchy fibrosis and honeycomb). With the consent of the Archives of Pathology Department of Pulmonary Hospital, Zakopane

again to assess and, if necessary, to manage the bleeding. The time without eye control of the bronchial tree was about 15–30 seconds. The balloon catheter for lobar occlusion after TBLC was not used. For safety reasons, the double-lumen endotracheal tube was prepared in case of major bleeding requiring patient's intubation and separated lung ventilation. The bleeding after biopsy was qualified as minor, moderate and major according to its management: minor — no action needed, self-limiting bleeding; moderate — bleeding managed endoscopically (cold saline, adrenaline solution, segmental bronchi blockage with the scope); major when additional actions were needed (intubation, admission to the intensive care, surgery). The chest X-ray due to the pneumothorax control was done within 2–4 hours after TBLC or immediately according to the physician decision. After the procedure, the specimens were transferred from saline to a 10% formaldehyde solution and then sent to the Pathology Department, where were assessed by two independent pathologists after standard Hematoxylin & Eosin (HE) staining (Figure 5). The final diagnosis was established in the discussion by the multidisciplinary team comprising experts in pulmonology, radiology and lung pathology.

Statistical analysis

A statistical analysis was conducted on the basis of descriptive statistic as the arithmetic mean with standard deviation. The results were used to calculate the diagnostic yield.

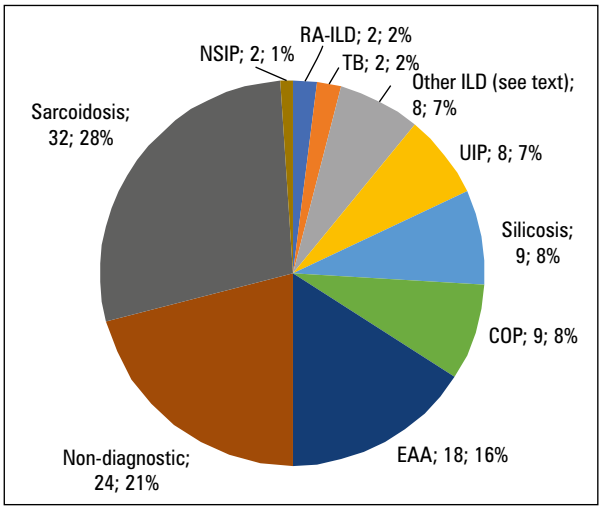

Figure 6. Histological diagnoses in a group of 114 patients with ILD (n; %). EAA — extrinsic allergic alveolitis; COP — cryptogenic organizing pneumonia; ILD — interstitial lung disease; NSIP — nonspecific interstitial pneumonia; RA-ILD — rheumatoid arthritis-related ILD; TB — tuberculosis; UIP — usual interstitial pneumonia.

Results

From March 2017 to September 2019 one hundred and fourteen patients, 59 (52%) males and 55 (48%) females of mean (SD) age 54 (14) years were enrolled in the study. The time of the whole procedure was 10–20 min (mean 15 min). The lung tissue obtained by r-EBUS-TBLC was found sufficient for pathological evaluation in all cases. A diameter of specimen was 5–10 mm (mean 7 mm). Histopathology was conclusive for final diagnosis in 90 (79%) cases. The most com-

mon histological diagnosis was sarcoidosis — 32 (28.1%) patients, then extrinsic allergic alveolitis (EAA) — 18 (15.8%) cases, silicosis and cryptogenic organizing pneumonia (COP) — 9 (7.9%) individuals each. Usual interstitial pneumonia (UIP) was diagnosed in 8 (7%) cases. Tuberculosis, rheumatoid arthritis-related ILD (RA-ILD) and non-specific interstitial pneumonia (NSIP) were diagnosed in two cases each, and hemosiderosis, Langerhans cell histiocytosis (LCH), lymphocytic interstitial pneumonia (LIP), exogenous lipoid pneumonia (ELP), smoking-related interstitial fibrosis (SRIF), vasculitis, scleroderma and mixed connective tissue disease-related ILD (SCL-ILD and MCTD-ILD) in one case each (Figure 6). Among twenty four non-diagnostic biopsies, unclassifiable lung fibrosis and normal lung (in few cases with the presence of macrophages with brown deposits characteristic for smokers) were the most common pathological findings. Patients with non-conclusive TBLC are followed up and further subgroup analyses are planned after establishing final diagnoses for the whole group.

Complications included only five cases (4.4%) of pneumothorax requiring a chest tube drainage and minor and moderate bleeding in few cases.

Discussion

Whereas specimens obtained by conventional TBLB are often found by pathologists not adequate for histological evaluation because they are too small and damaged by forceps, those obtained by TBLC are bigger and free of crushing artifacts. In some studies, the results of TBLC in ILD were even comparable to VATS-LB, and the procedure was safer [9]. A diagnostic yield of TBLC in different reports varies from 51% to 98%. Among the procedure-related complications, the most common is pneumothorax (0–33%). Other reported complications include serious bleeding up to 20%, acute exacerbation (AE) of ILD up to 4% and death in most cases due to AE-IPF (up to 4%) [10–12]. The results of our study have shown the diagnostic yield on the level of 79% and the complication rate of 4.4% (exclusively pneumothorax). No serious bleeding was noted in our study. It can be connected with guidance method we used to visualize the pulmonary vessels. Almost all data about the safety of TBLC come from studies with fluoroscopic guidance. In two studies, in which authors didn't use any guidance for TBLC, complication ratio was 0% and 12% for serious bleeding and 4.7% and 8% for pneumothorax [15, 16]. Taking into consideration the mentioned data and our results in safety area, it is disputable whether the use of fluoroscopy has significant impact on the procedure safety. More data, especially from comparative randomized trials is needed.

The high percentage of sarcoidosis diagnosed in our study (28.1%) must be noted and commented due to its possible influence on the diagnostic yield. It must be underlined that only patients without lymphadenopathy (stage III and IV) and with negative conventional bronchoscopy (endobronchial mucous biopsy, TBLB) were included in the study.

Although the usefulness of TBLC for collecting adequate samples seems to be out of the question, there are no standards when it comes to the exact procedure technique. The fluoroscopic guidance, endotracheal tube or rigid bronchoscope intubation with deep sedation or general anesthesia is the most common way of performing TBLC. For safety reasons, it is important to know if a freezing tip of the flexible cryoprobe is distant enough both from the chest wall and hilar vessels. In twenty six of TBLC reports from 2009 to 2017 analyzed by Lentz RJ *et al.*, only in 2 studies fluoroscopic guidance was not used (in one only in 40% cases) [10].

Experts preparing recommendations for IPF management very carefully analyzed data regarding safety and efficacy of the TBLC, and they found it promising but not standardized enough to be widely recommended. They have pointed out that "a standardized procedure for lung cryobiopsy that optimizes the balance between diagnostic yield and complications needs to be developed among experts currently engaged with the procedure" [1]. They have suggested the experts already performing this procedure should work on its standardization and do not recommend introducing this procedure by new centers before its value and safety will be determined. However it seems to be a very cautious statement, but it might be justified in the light of recently published data showing very poor concordance between TBLC and surgical lung biopsy (SLB). Romagnoli *et al.* showed that TBLC would have led to a different treatment if SLB was not performed in 11 of 21 (52%) cases [17]. This small but well-designed study cooled down the enthusiasm about TBLC in ILDs and showed a lack of strong data comparing SLB with TBLC.

Our idea of using r-EBUS control simplifies the whole procedure and avoids X-ray exposure without decreasing diagnostic yield, and the safety of the procedure is high. Whereas the

fluoroscopy provides guidance mainly for safety reasons, the r-EBUS assessment of the lung before TBLC may also sometimes help to choose the most proper biopsy site (because some types of infiltrations are visible in ultrasound image). Although cost/effectiveness analysis was not the purpose of the study, in our opinion, it is worth noticing that simplifying the method (no use of endotracheal intubation, balloon blockers or fluoroscopic control) should lead to the cost reduction.

At the time of preparing our study protocol, there were no data regarding its efficacy and safety profile. During last years a few papers were published and summarized by Gupta *et al.* [18]. Most of the analyzed studies presented the role of r-EBUS in peripheral nodule cryobiopsy. The authors found and examined only three studies (including our preliminary report in 20 patients) describing the role of r-EBUS cryobiopsies in diffuse parenchymal lung disease. In their conclusions they have found it to be a feasible and safe procedure with high diagnostic yield. The studies on the role of r-EBUS-guided TBLC are currently under way (i.e. in Vanderbilt University Medical Center). In our opinion, this technique should be further investigated and compared in randomized trials, both with TBLC under fluoroscopic control and balloon bronchial blockers in intubated patients (how most centers perform it) and with SLB. Without comparative studies, strong recommendation on the use and method of performing TBLC in ILDs will be impossible.

Conclusions

The results of our study have shown that combination of r-EBUS and TBLC is a novel, reasonable and safe technique to diagnose ILDs. It could be an alternative way of performing TBLC in ILD patients but further comparative trials are required.

Conflict of interest

None declared.

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Prevalence of inducible laryngeal obstruction among patients diagnosed as bronchial asthma

Abstract

Introduction: Inducible laryngeal obstruction (ILO) is an important cause of a variety of respiratory symptoms and can mimic bronchial asthma (BA). This study was planned to measure the prevalence of ILO among patients diagnosed with BA and to detect its effect on BA control and severity.

Material and methods: Patients aged 18 years or older who were previously diagnosed with BA were enrolled. Laryngeal obstruction was induced using the patient's specific trigger (e.g. exercise). Visualization of vocal folds was accomplished using a 70-degree rigid laryngoscope (Karl Storz). A visual grade score was utilized to determine the severity of laryngeal obstruction.

Results: Results showed that 38.3% (n = 46) of the patients had ILO with the majority being classified as grade 2 (80.4%) (n = 37). The most common subtype was glottic ILO (63%). Bronchial asthma duration, level of control, and severity were not associated with ILO (P values: 0.2, 0.3 and 0.8 respectively).

Conclusion: Asthma and ILO commonly co-exist. An accurate classification of patients is very important and must be considered in order to determine whether the symptoms are directly related to ILO or whether they are caused by BA. Ceasing inappropriate treatment may be necessary. Objective diagnostic modalities of ILO are essential.

Key words: inducible laryngeal obstruction; bronchial asthma; bronchial asthma control and severity

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Introduction

Inducible laryngeal obstruction (ILO), otherwise referred to by many other terms including vocal cord dysfunction and paradoxical vocal fold motion, describes an inappropriate, transient, reversible narrowing of the larynx in response to external triggers. ILO is an important cause of a variety of respiratory symptoms and can mimic bronchial asthma (BA) [1, 2]. Typical clinical features of ILO include wheezing, dyspnea, and cough, but these symptoms are highly variable. In most cases, individuals with ILO will exhibit inspiratory breathing difficulties, although a pure expiratory form of ILO has also been described [1].

Exercise-induced ILO can impair patients ability to exercise and can be confused with BA. This can lead to unnecessary treatment with BA medications and can result in increased healthcare resource utilization. It is characterized by attacks of shortness of breath and noisy breathing that generally occur during high work rates [2].

A definitive diagnosis of ILO is dependent on laryngoscopic visualization of abnormal glottic or supraglottic collapse resulting in airway narrowing during a spontaneous event or provocation challenge [2, 3].

Treatment modalities of ILO include removal of the irritant, voice therapy, physiotherapy and psychological support [1].

Although ILO has long been recognized as mimicking BA, it is increasingly becoming recognized as coexisting with BA as well [1]. As a result, this study was conducted in order to measure the prevalence of ILO among patients diagnosed with BA and to detect its effect on BA control and severity.

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Table 1. The 12-item vocal cord dysfunction questionnaire (VCDQ)

Question	Disagree Strongly	Disagree	Neither Agree nor Disagree	Agree	Agree Strongly	Score
	1	2	3	4	5	
1. My symptoms are confined to my throat/upper chest						
2. I feel like I can't get breath past a certain point in mythroat/ /upper chest because of restriction						
3. My breathlessness is usually worse when breathing in						
4. My attacks typically come on very suddenly						
5. I feel that there is something in my throat that I can't clear						
6. My attacks are associated with changes in my voice						
7. My breathing can be noisy during attacks						
8. I'm aware of other specific triggers that cause attacks						
9. My symptoms are associated with an ache or itch inmy throat						
10. I am frustrated that my symptoms have not beenunderstood correctly						
11. I am unable to tolerate any light pressure around the neck, e.g. tight clothes or bending the neck						
12. The attacks impact on my social life						
Total						(12–60)

Patients will replay on a 12-item questionnaire and their final score will be recorded. Total score ranges from 12 to 60; higher scores suggest VCD.

Material and methods

Study design: cross-sectional study. Patients enrolled in the study attended the outpatient clinic of the chest medicine department at Mansoura University Hospitals between May 2018 and December 2019. These patients were previously diagnosed with BA and were 18 years or older. Patients who refused to participate in the study, patients who were pregnant, patients with a known history of vocal fold immobility, patients with acute exacerbations of BA, and patients who were current smokers were excluded.

Enrolled patients were submitted to:

— thorough history taking and clinical examination;
— assessment of the level of BA control and severity according to GINA 2018;
— vocal cord dysfunction questionnaire (VCDQ, Table 1) [4]. Patients responded to a 12-item questionnaire and their final score was recorded. Total scores ranged from 12 to 60; higher scores suggest VCD [1];
— induction of laryngeal obstruction by the patient's specific trigger (e.g. exercise) with visualization of vocal folds using 70-degree rigid laryngoscope (Karl Storz) interfaced with a camera (LEMKE MC 204). A visual grade score was utilized to determine the severity of the laryngeal obstruction [5]. This scoring system grades laryngeal closure at both the glottic and supraglottic levels; scores ranged between 0 (complete patency) and 3 (almost complete closure) [6].

Fibroptic-nasoendoscopy using Henke-Sass-Wolf type 10 was used in patients with high gag reflex.

Statistical analysis

Data was analyzed using SPSS V. 16. Categorical data were presented in the form of numbers (percent), while continuous data were presented either as mean (SD) or median (min-max) depending on the results of Shapiro-Wilk test which was used to test the assumption of normal distribution of data. The associations of different parameters with ILO were tested using the Chi^2 test or Fisher's exact test in case of categorical data (with respect to the minimal expected values in the contingencies tables), Welch's t-test in case of continuous data with normal distribution due to unequal variance of the groups, and the Mann-Whitney U test for continuous data with non-normal distribution. Paired data (pre/post treatment data of ILO cases) was compared using the paired t-test for continuous variables with normal distribution and the Wilcoxon Signed

Table 2. Characteristics of the participants (n = 120)

Age mean (SD)	36 (11)
Sex [n%]	
Male	26 (21.7%)
Female	94 (78.3%)
Marital status [n%]	
Currently married	94 (78.3%)
Not currently married	26 (21.7%)
Occupation [n%]	
Non-working	77 (64.2%)
Manual work	17 (14.2%)
Professional/administrative work	26 (21.7%)
BMI mean (SD)	30.2 (7.2%)
Comorbidities* [n%]	
No	72 (60)
Allergic rhinitis	31 (25.8)
Others	17 (14.2)
Asthma duration median (min–max)	4 (0.2–32) years
Asthma control [n%]	
Uncontrolled	20 (16.7%)
Partly controlled	69 (57.5%)
Well controlled	30 (25%)

*Classes are not mutually exclusive; Others: DM (4), HTN (7), Adenoid (2), peptic ulcer (2), GERD (6), OSA (1). BMI — body mass index

Ranks Test for ordinal variables (ILO grade: none, grade 1, grade 2, grade 3; severity of asthma: mild, moderate, severe; control of asthma: uncontrolled, partially controlled, well controlled). The correlation of asthma control with ILO control was tested using Spearman's correlation. Significance level was set at 0.05.

Results

The study included 120 patients previously diagnosed with BA. 78.3% of them were females. Their mean age was 36 years (± 11). About a quarter of the patients had allergic rhinitis, and 60% of them had no comorbidities. More than half of the patients were partly controlled while 16.7% were uncontrolled as per BA (Table 2).

Prevalence of ILO among studied patients

Results showed that 38.3% (n = 46) of the patients had ILO, mostly grade 2 (80.4%) (n = 37) with the most common manifestation being glottic ILO (63%) (Table 3, Figure 1).

Many provocation techniques have been used to induce ILO for diagnosis. Most of our patients (about 100 patients) reported exercise as a trigger for their symptoms. However, GERD, uncontrolled allergic rhinitis with posterior nasal discharge and emotional stress were reported in other cases.

Table 3. Prevalence of ILO among studied patients

ILO [n%]	46 (38.3%)
Sites of ILO (n = 46) [n%]	
Supraglottic	8 (17.4%)
Glottic	29 (63%)
Supraglottic and glottic	9 (19.6%)
Severity of ILO (n = 46) [n%]	
Grade 1	5 (10.9%)
Grade2	37 (80.4%)
Grade 3	4 (8.7%)

ILO — inducible laryngeal obstruction

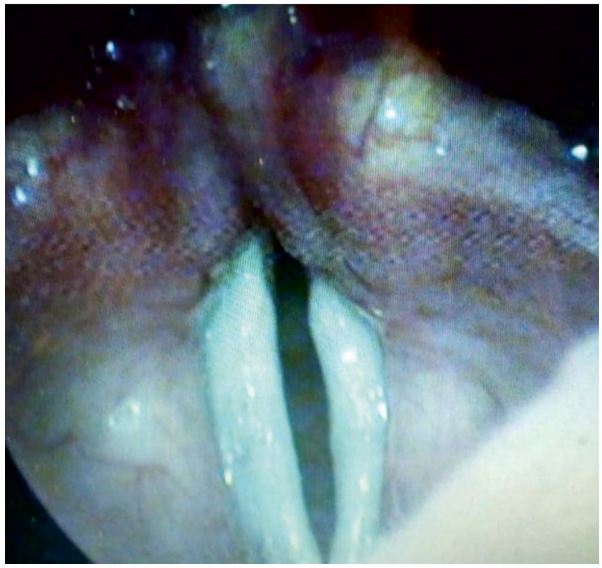

Figure 1. Grade 2 glottic inducible laryngeal obstruction

The association of ILO with different epidemiological characteristics of the patients

Table 4 shows that ILO increased with age with significant differences between age groups. For example, patients aged between 31 and 50 had significantly different results from those below 30 years of age (p = 0.023 and 0.038, respectively). However, there was no significant difference in people aged over 50 years of age. Also, being married was associated with a higher risk of ILO. Other parameters such as sex and occupation were not associated with ILO. Similarly, ILO was not associated with BMI of patients or with other comorbidities.

Table 4. The association of ILO with different epidemiological and clinical parameters of the patients (n = 120)

Parameter	ILO absent n = 74	ILO present n = 46	Significance
Age			
≤ 30	29 (74.4)	10 (25.6)	R
31–40	22 (50)	22 (50)	$\chi^2 = 5.18$, p = 0.023*
41–50	10 (47.6)	11 (52.4)	$\chi^2 = 4.2$, p = 0.038*
≥ 50	13 (81.2)	3 (18.8)	P = 0.734**
Sex [n%]			
Male	15 (57.7)	11 (42.3)	
Female	59 (62.8)	35 (37.2)	
Marital status [n%]			
Currently not married	21(80.8)	5(19.2)	$\chi^2 = 5.12$, p = 0.02*
Currently married	53 (56.4)	41(43.6)	
Occupation [n%]			
Non-working	49(63.6)	28(36.4)	$\chi^2 = 0.86$, p = 0.649*
Manual work	11(64.7)	6 (35.3)	
Professional/administrative work	14(53.8)	12 (46.2)	
BMI mean (SD)	29.7 (5)	31 (5.9)	T = -1.23, p = 0.219***
Comorbidities [n%]			
No	43 (59.7)	29 (40.3)	$\chi^2 = 1.79$, p = 0.407*
Allergic rhinitis	22 (71)	9 (29)	
Others	9 (52.9)	8 (47.1)	

*Chi² test; **Fisher's exact test; ***Welch's t-test.
BMI — body mass index; ILO — inducible laryngeal obstruction; r — reference

The association of ILO with the vocal cord dysfunction questionnaire

Mean of VDCQ was 44 in ILO patients and 35.5 in non-ILO patients. These values had no clinical significance despite its statistical significance (P value < 0.001).

The association of ILO with BA duration, severity and control

Bronchial asthma duration, level of control and severity were not associated with ILO (P values = 0.2, 0.3 and 0.8 respectively; Table 5).

Discussion

Inducible laryngeal obstruction (ILO), an induced, inappropriate adduction of the vocal cords, can coexist with bronchial asthma. Accurate differentiation has been challenging because of overlapping symptoms and the absence of sensitive diagnostic criteria for either condition. Although challenging, an accurate diagnosis of patients is very important due to the differing treatment modalities for asthma and ILO [7].

This study included 120 patients previously diagnosed with bronchial asthma and receiving asthma medications for many years. About 38.3% of them were diagnosed with ILO for the first time [80.4% were grade 2 with the most common presentation being glottic ILO (63%)]. The diagnostic difficulty in this study is demonstrated by a mean delay of about 4 years before reaching an ILO diagnosis. Accurate classification of patients is very important to differentiate if symptoms are directly related to ILO or to BA. Ceasing inappropriate treatment may be needed.

The presence of comorbidities, abnormal vocal cord dysfunction questionnaire results, bronchial asthma duration, level of bronchial asthma control, and level of bronchial asthma severity could not aid in the diagnosis of ILO in studied patients. Therefore, objective diagnostic modalities are essential. Lee *et al.* also concluded that clinical assessment, questionnaire scores, and presence of comorbidities were not sufficient enough to diagnose ILO [7].

Table 5. The association of ILO with BA duration, severity and level of control

Parameter	ILO absent n = 74	ILO present n = 46	Significance
Duration of asthma (median, min-max)*	4 (0.1–32)	5 (0.2–30)	Z = -1.2, p = 0.226
Severity of asthma**			
Mild	42 (62.7)	25 (37.3)	X^2 = 0.02, p = 0.898
Moderate/sever	32 (61.5)	20 (38.5)	
Control of asthma**			
Uncontrolled	15 (75)	5 (25)	X^2 = 1.84, p = 0.399
Partly controlled	42 (60.9)	27 (39.1)	
Well controlled	17 (56.7)	13 (43.3)	

*Mann-Whitney U test; **Chi^2 test.

ILO — inducible laryngeal obstruction

Conclusion

Asthma and ILO commonly co-exist. Accurate classification of patients is very important in order to determine whether symptoms are directly related to ILO or to BA. Ceasing inappropriate treatment may be necessary.

The presence of comorbidities, abnormal vocal cord dysfunction questionnaire results, BA duration, and its BA level of control/severity could not aid in diagnosing ILO in studied patients. Therefore, objective diagnostic modalities are essential.

Conflict of interest

None declared.

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Posterior Mediastinal Paravertebral Müllerian cyst (cyst of Hattori): literature review

Abstract

Mediastinal cysts are typically of bronchogenic, thymic or neurenteric origin, but may also represent oesophageal duplication. Posterior paravertebral mediastinal Müllerian cysts of undetermined pathogenesis are very rare occurrences. The first case of a ciliated cyst arising in the mediastinum, of probable Müllerian origin, was reported by Hattori in 2005, which gave rise to the name cyst of Hatorri (COH) The number of reported cases in literature of a similar nature have since then increased significantly. One of the main concerns about this pathology is the possibility of malignant transformation of the Müllerian tissue. Over the course of this paper we will discuss the pathogenesis, immunohistochemistry and its role in differential diagnosis as well as optimal treatment of such cysts.

Key words: Mullerian cyst, cyst of Hattori, posterior mediastinum, paired box gene 8

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Introduction

The pathogenesis of Müllerian cysts, which are located in the posterior mediastinum, is not yet fully understood. It was suggested by Ludwig *et al.* in his study of Mayer-Rokitansky-Kuster-Hauser syndrome [1–3], that such cysts are possibly remnants of embryonic Müllerian tissue. The pathomorphology showed that the cysts were lined with ciliated columnar/tubal epithelium surrounded by a thin smooth muscular layer (Figure 1). The immunohistochemical profile demonstrated positivity to oestrogen as well as progesterone receptors. Such cysts, in relation to this condition, were first described by Hattori in 2005 as being female sex hormone receptor sensitive and situated in the posterior mediastinum [4].

Pathogenesis

In 1998, Ludwig reported that "in stage 16 embryos, a thickening of the coelomic epithelium develops on the cranial end of the plica mesonephrica at the level of the 3rd to 5th thoracic vertebral blastema and forms the anlage of the funnel area (of the fallopian tube)" [1]. There is a possibility that some of the rudimentary tissue lined by fallopian tubal epithelium persisted in the paravertebral mediastinal areas at T4–6 levels [5]. Accordingly, a Müllerian cyst could develop anywhere along the path of Müllerian duct regression [3, 6]. However, the origin of these cysts is still not fully understood. Firstly, mediastinal structures are not regarded as part of the secondary Müllerian system. Secondly, embryonic Müllerian structures have not been identified in the mediastinum, pleura or pericardium [7].

In 2010, a paper discussing the pathogenesis of cyst of Hattori by Batt *et al.* suggested that the cyst originated from the primary Müllerian tissue similar to the postulated pathogenesis of Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH). This syndrome is characterized by the failure of the Müllerian duct to develop, resulting

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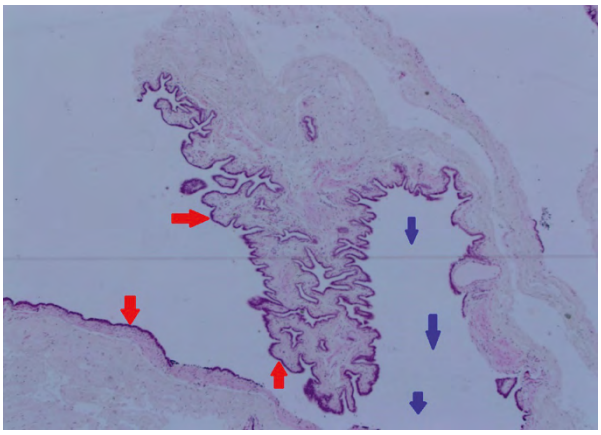

Figure 1. Haemotoxylin and eosin × 4, stained section of cyst of Hattori shows cystic spaces (blue arrows) with slightly fibrotic walls lined by ciliated columnar epithelium (red arrows)

in absence of the uterus and variable degrees of vaginal hypoplasia or agenesis [8]. Other theories include the notion that Müllerian cysts of Hattori may represent metaplastic changes of the mesothelium into ciliated epithelium [7].

Clinical presentation

The most common sites of Müllerian cysts are within the pelvis [9], however, they can also be found, albeit less frequently, on the skin [10], the retroperitoneum or the mediastinum [11, 12]. Mediastinal cysts may present with chest symptoms, whereas abdominal cysts may present as an acute abdomen [13]. Mediastinal manifestations include cough (22%) [2, 9, 14, 15] chest pain (17%) [9, 16–18], shortness of breath [18], dysphagia [9], and arm numbness [18] (Table 1). However, amongst the 40 reported cases of COH, 55% were asymptomatic and detected as an incidental finding on imaging.

It is suggested that mediastinal cysts may account for up to 12–30% of mediastinal masses [19, 20] and Thomas-de-Montpréville at the Marie-Lannelong Surgical Centre in France, found that of a series of 163 cases of operated mediastinal non-malignant cysts, COH represented 5.5% of cases [21].

All reviewed cases of COH in literature are found in the female population, often amongst those with high body mass indexes, and in some instances associated with gynaecological pathology [3, 5, 9, 21, 22]. The presence of aromatase in fatty tissue may result in high levels of oestrogen, which could be a contributory factor in the development of COH [23]. Moreover, a possible association between COH and the use of hormone replacement therapy (HRT) has been reported in two cases [3, 5, 24]. Similarly to that of the female population, Müllerian cysts in men typically develop in the pelvis [25].

Imaging

Cyst of Hatorri are often incidental findings of chest imaging [3, 5, 26–28]. Age of presentation are predominantly amongst woman between 40 to 60 years [22]. They are typically found between T3–T8 vertebrae [15] (Tables 1, 2); commonly occurring as a solitary cyst. However, multiple cysts have also been reported [2]. Imaging may detect an abnormality in a chest X-ray [18, 22], but computed tomography [24, 15, 17, 27] (Figure 2) or magnetic resonance imaging are recommended for detailed evaluation [15, 27] (Figure 3).

Histology

Histologically, Müllerian cysts are lined with ciliated columnar/tubal epithelium, surrounded by a thin smooth muscular layer without atypia or mitosis. Whilst this is identical to the histology of the fallopian tube (Figure 1), differential diagnoses should include mesothelial, gastro-enteric, thoracic duct or pericardial cysts [5, 9, 26] (Table 3).

Immunohistochemistry

The immunohistochemistry of COH shows positive expression for oestrogen (Figure 4) and progesterone receptors (Figure 5) [2, 11, 14, 15, 21, 22, 24]. The hormone receptor expression in the cyst wall, as well as the histological similarity to fallopian tube, supports the theory of its origin being remnants of embryonic Müllerian tissue [26]. This is further reinforced by the fact that the immunohistochemical profile of retroperitoneal Mullerian cysts are indistinguishable from that of the paravertebral mediastinal cysts [11]. Furthermore, all known reported cases in literature have been found within the female population [2, 3, 4, 9, 11, 15, 16, 18, 19, 21, 22, 24, 26, 28].

Cytokeratin 7 (CK7) is type II keratin of simple non-keratinizing epithelia which is present in uterine tubal epithelium as well as COH [11, 22, 27]. It has shown expression in our case, however, CK7 is not a specific marker as it has shown positivity in carcinoid [29] as well. Furthermore, pan-Cytokeratins (CK AE1/AE3) has also been detected in the COH [20, 24].

Table 1. Müllerian cyst of Hattori presentation, size and hormonal status in 40 cases reported in literature

Author/year	Age/sex	Clinical symptoms	Paravertebral level	Diameter	ER/PR	CK7	WT1	Paired Box Gene 8 (PAX8)
Hattori 2005	52/F	Persistent cough	Right T6	2.5 cm	+/+			
	18/F	Incidental	Right T5	2.0 cm	+/+			
	49/F	Cough	Left T4	2.0 cm	+/+			
Thomas-de-Montepreville 2007	40/F	Chest pain	Left T4	1.5 cm	+/+			
	46/F	Cough	Left T4	3.3 cm	+/+			
	47/F	Cough	Right T4/5	5.0 cm	+/+			
	48/F	Incidental	Left T5	3.0 cm	+/+			
	50/F	Chest pain	Right T3/4	3.2 cm	+/+			
	51/F	Incidental	Left T3/4	3.0 cm	+/+			
	56/F	Incidental	Left T8	1.3 cm	-/+			
	58/F	Cough	Pre-vertebral T5	4.5 cm	-/-			
	59/F	Chest pain	Right T2–4	2.5 cm	-/-			
Businger 2007	54/F	Incidental	Left T4–6	4.5 cm	+/+			
Batt 2010	41/F	Chest pain	Left T6	2.1 cm	+/+			
Kobayashi 2012	53/F	Incidental	Right T5	2.0 cm	+/+			
Liao 2012	48/F	Chest tightness	Right T7	5.1 cm	+/+			
Simmons 2013	52/F	Shortness of breath	Pre-vertebral T4	4.1 cm	+/+			
	47/F	Incidental	N/A	5.0 cm	+/			
Lee 2014	42/F	Incidental	Right T6	2.6 cm	+/+			
Miyawaki 2014	51/F	Incidental	Left T5/6	4.0 cm	+/+			
Takahashi 2014	47/f	Incidental	Right T4/5	2.0 cm	+/+	+		
Chon 2015	51/F	Chest pain	Left T6	3.0 cm	+/+			
Dakak 2015	51/F	Dysphagia	Pre-vertebral T5	2.7 cm	+/+			
Skancke 2015	35/F	Cough	Bilateral	8.0 cm 4.0 cm	+/+			
Lochowski 2017	53/F	Incidental	Right T4	3.0 cm	+/+			
Tsai 2017	44/F	Incidental	Left T4	1.2 cm	+/+	+		
Chandra 2017	52/F	Chest pain/SOB	Left T3–5	3.9 cm				
Mowad 2017	49/F	Cough	Left T4	3.6 cm	+/+	+	+	+
Karpathiou 2017	51/F	Incidental	Left T4	3.0 cm	+/+	+	+	
Oshima 2017	48/F	Incidental	Left T4–5	3.1 cm	+/+			
	40/F	Incidental	Right T3–4	3.5 cm	+/?			
Lee 2018	22/F	Incidental	Left T10	2.4 cm	+/+		+	
Chao 2018	49/F	Incidental	Right T5	1.3 cm	+/+	+		+
Miura 2018	50/F	Cough	Left T6–7	1.9 cm	+/+			
	52/F	Incidental	Right T3–4	5.2 cm	+/+			
	46/F	Incidental	Right T4–5	4.1 cm	+/+			
	52/F	Incidental	Left T1–2	3 cm	+/+			
Sekimura 2018	40/F	Incidental	Left	1.2 cm	+/+			+
Idaewor 2018	56/F	Cough	Left T3–4	3 cm	+/+			
Adachi 2018	53/F	Incidental	Right T5	3 cm	+/+			+
Yasukawa 2018	41/F	Incidental	Pre-vertebral T10	3 cm	+/+	+		+

Table 2. Clinical picture in the 40 COH reported cases

Clinical characteristics	N = 40
Age	Average (18–59)
Sex	
F	40
M	0
Size (cm)	Average (1.2–8.0)
Lateralisation	
Right	15
Left	20
Pre-vertebral	3
Bilateral	1
N/A	1
Vertebral level	
T2	1
T3	5
T4	12
T5	8
T6	5
T7	1
T8	1
T9	0
T10	1
Multiple	3
N/A	3
Symptoms	
Asymptomatic	22
Cough	9
Shortness of breath	1
Chest pain	7
Dysphagia	1
Immunohistochemistry	
ER+	36
ER-	3
ER N/A	1
PR+	35
PR-	2
PR N/A	3
PAX8+	4
PAX8 N/A	36

ER — oestrogen receptors; PR — progesterone receptors; PAX8 — paired box gene 8

Wilms' tumor protein 1 (WT1) is transcriptional regulator protein that is thought to inhibit transcription of growth promoting genes. This protein is expressed in tissue of the fallopian tube as well as the Müllerian system [30] and in COH [2, 15].

Paired box gene 8 (PAX-8); a gene found at 2p13, is associated with tumours of thyroid gland, kidney/upper urinary tract and Müllerian system [30, 31] and is positive in COH [2, 15, 22, 25, 27]. Cancer antigen 125 (CA-125), a protein encoded by MUC16 gene, is used as a tumour marker with a 79% sensitive for ovarian cancer. As it is present in amnion, coelomic and Müllerian epithelium [32–34], it has been found to be useful in the diagnosis of COH [2, 11] (Figure 6). Cytokeratin 5/6, specific for mesothelial differentiation and usually positive in squamous epithelia, myoepithelial cells, and mesothelial cells, are negative in COH [21].

Cytokeratin 20(CK20), an epithelial marker often used to distinguish colon carcinoma (80% are CK20+) [35], shows positivity in Merkel cell carcinoma and is positive only in some cases of extra-pulmonary small cell lung carcinoma [29]. Müllerian cysts show immunonegativity for CK 20 [11, 36].

Calretinin, a calcium binding protein, is positive in epithelioid pleural mesothelioma [40] but is negative in COH [7, 11, 22, 27] (Figure 7).

Thyroid transcription factor (TTF-1), also called thyroid specific enhancer binding protein, distinguishes primary lung (TTF1+) from metastatic (usually TTF1-) lung carcinoma [37]; it is negative in Müllerian cysts [15, 17, 19].

BerEP4 marker, also known as epithelial cell adhesion molecule, is an antibody to cell

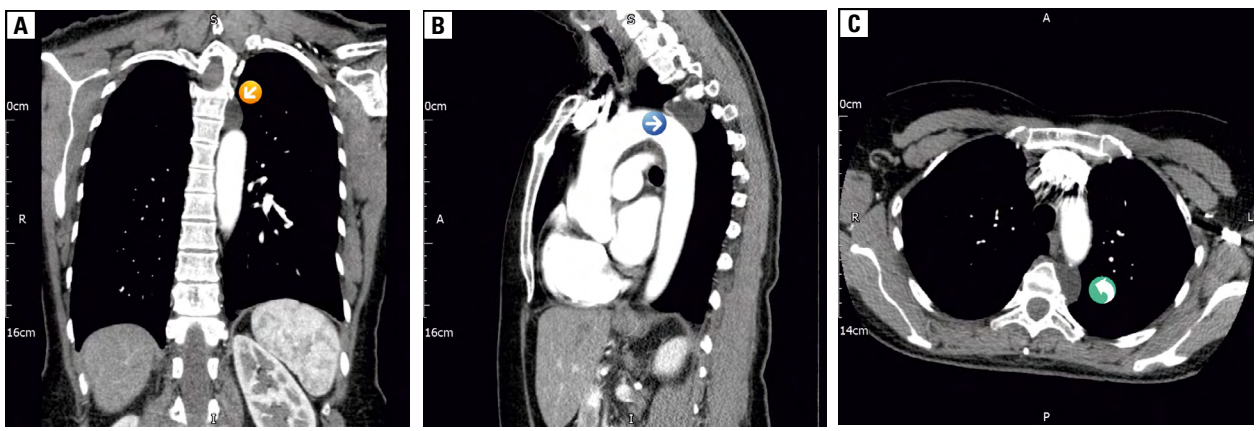

Figure 2. A. Chest computed tomography (CT) coronal view; soft tissue mass at the level of aortic arch (orange arrow); **B.** CT chest-sagittal view; soft tissue mass at the level of aortic arch (blue arrow); **C.** CT chest-axial view; soft tissue mass at the level of aortic arch (green arrow)

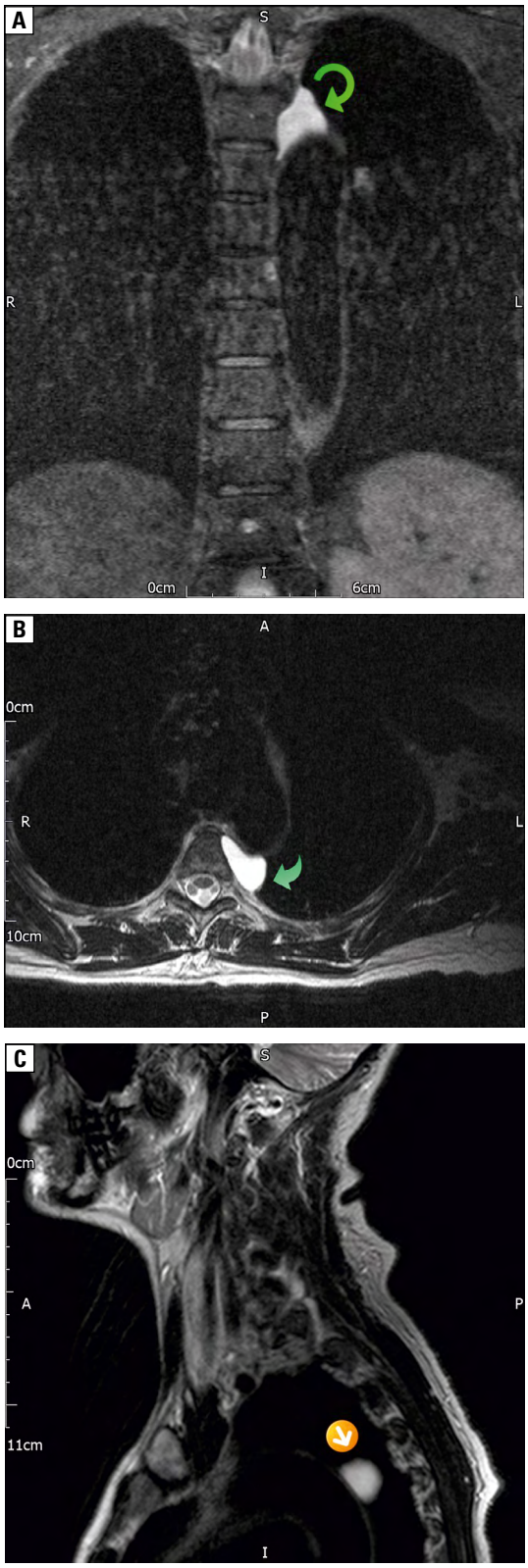

Figure 3. A. Magnetic resonance image (MRI) chest-coronal view; left paravertebral mass of cystic nature seen very close to the aortic arch (green arrow); **B.** MRI chest-axial view; left paravertebral mass of cystic nature seen very close to the aortic arch (green arrow); **C.** MRI chest-sagittal view; left paravertebral mass of cystic nature seen very close to the aortic arch (orange arrow)

Table 3. Histopathology of the mediastinal cysts [5, 9, 14, 39, 41]

Origin	Histology
Mullarian (Hattori)	Ciliated columnar/tubal apocrine-like secretory cells, surrounded by smooth muscles. They are Oestrogen Receptor and progesterone receptor positive (ER+/PR+)
Bronchogenic	Pseudostratified, ciliated columnar epithelium, smooth muscles, cartilage and bronchial gland
Enterogenous	Gastrointestinal mucosa
Mesothelial	Single-cell layer of mesothelial cells surrounded by external fibrous capsule
Neurenteric	Enteric and neural tissue
Pericardial cysts	Single layer of mesothelial cells
Thoracic duct cysts	Lymphatic duct lining
Thymic cyst	Flattened cuboidal epithelium and Hassal's corpuscles
Hydatid cyst	Germinal layer with nucleoli . Protoscolices with double row hooklets round suckers that comprise "hydatid sand". Daughter cysts

ER — oestrogen receptors; PR — progesterone receptors

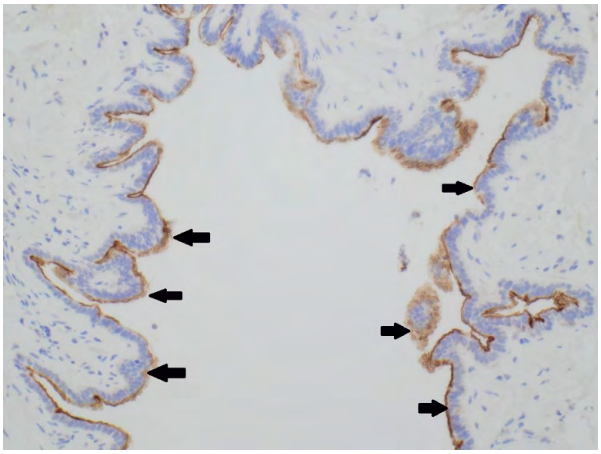

Figure 4. ×10, the lining epithelial cells are strongly positive for oestrogen receptors (arrows)

membrane glycoprotein expressed on healthy epithelia and in various carcinomas. This marker is reported to be negative in mesotheliomas [38]. Conversely, BerEP4 is reported to be positive in COH [7] (Figure 8).

Thrombomodulin, a transmembrane glycoprotein and cofactor for the thrombin-mediated activation of protein C, has a 95% specificity and 65% sensitivity to mesothelioma [39]. This marker is negative in COH [7] (Figure 9).

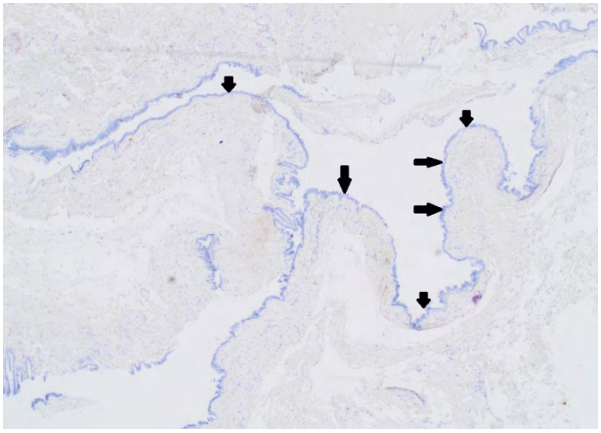

Figure 5. ×10, the cells are also strongly positive for progesterone receptors (arrows)

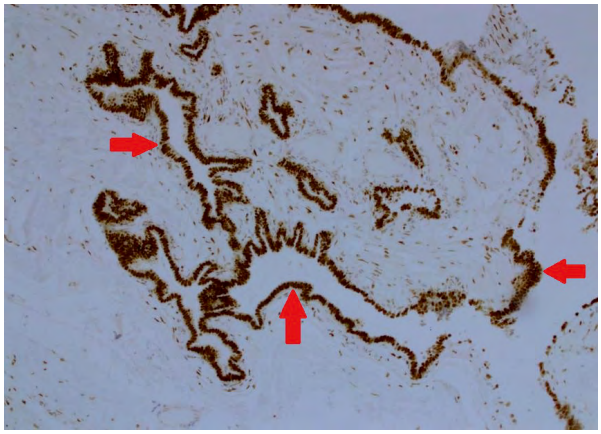

Figure 8. ×10, the epithelial cells are strongly positive for BerEP4 (arrows)

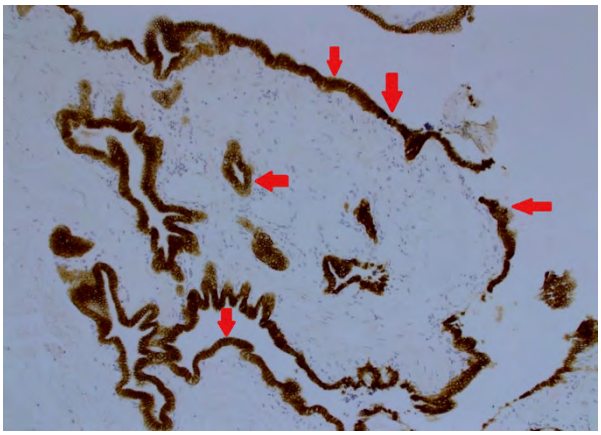

Figure 6. ×10, there is weak luminal staining of the epithelial cell with cancer antigen 125

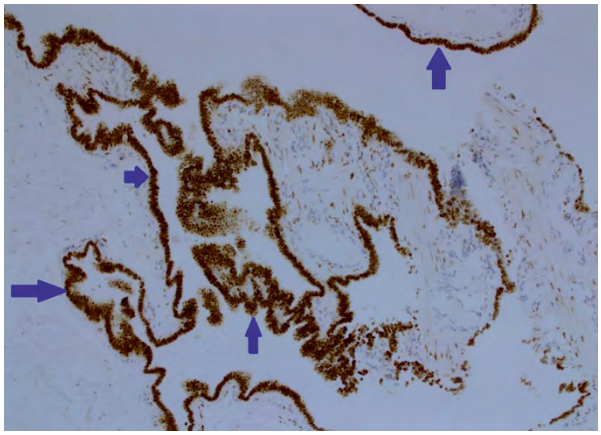

Figure 9. ×10, the epithelia cells are negative for Thrombomodulin (arrows)

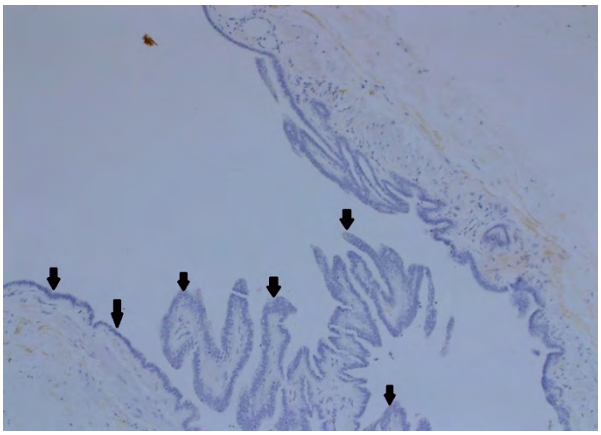

Figure 7. ×10, the epithelial cells are negative for Calretinin (arrows)

Differential diagnosis

Generally, mediastinal cysts are rare; they can be either acquired or congenital and their origins may be of Müllerian, thymic [40], pericardial, bronchogenic or neuroenteric tissue. They may also represent oesophageal duplication [18, 28]. Other differential diagnoses may include mesothelial, gastro-enteric, thoracic duct cysts [26] or due hydatid disease [41] (Table 3). Anterior spinal meningocele containing cerebrospinal fluid, located in the posterior mediastinum, may appear as a cystic lesion, however, a meningocele will communicate with the spinal canal unlike a true cyst [3].

Treatment

The long-term prognosis of Müllerian cysts is unclear. However, the risk of malignant transformation must not be discounted. The evidence suggests that presence of embryonic tissue away from its typical site, may give rise to malignant transformation. For example, cryptorchidism

within the male population significantly increases the risk of developing testicular cancer. Furthermore, patients with mediastinal goitres are more susceptible to thyroid cancer. Surgical excision is advisable to ensure formal analysis and diagnosis, symptomatic relief and elimination of possible malignant transformation of the Müllerian tissue [18, 42]. Surgical approaches taken include open thoracotomy [7, 9] or video-assisted thoracoscopic surgery (VATS) [3, 15, 25, 27, 43–45].

Conclusion

Of all reviewed cases in literature, COH are found with the female population; often amongst those with high BMI's and in conjunction with gynaecological pathology. Histological appearance shows ciliated columnar epithelium surrounded by a smooth muscular layer, identical to the histology of the fallopian tube, supporting the theory of probable Müllerian origin. Surgical excision of such cysts are the treatment of choice as this allows for definitive diagnosis, symptomatic relief and a reduced risk of malignant transformation of Müllerian tissue.

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Conflict of interest

None declared.

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Acute eosinophilic pneumonia associated with non-cigarette smoking products: a systematic review

Abstract

Acute eosinophilic pneumonia (AEP) is characterized by an acute onset respiratory illness with bilateral chest infiltrates and evidence of pulmonary eosinophilia. Cigarette smoking is the main risk factor, but drugs and other inhalational exposures have also been reported. Herein, the association between AEP and smoking devices other than cigarettes is reviewed

The PubMed database was searched using terms such as "smoking", "vaping", "e-cigarette", "waterpipe", and "marijuana", along with other commonly used synonyms for these terms. In addition, eosinophilic lung diseases were also searched for using the same database. All cases of AEP were identified using the modified Philit criteria in association with the use of marijuana, waterpipe, e-cigarettes or heat-not-burn cigarettes. Cases associated with illicit drug use were excluded.

Twelve cases were included with a median age of 20 (15–60). 75% of patients studied were male. Exposures included marijuana smoking (n = 5), waterpipe usage (n = 2), heat-not-burn cigarette use (n = 2), e-cigarette use (n = 2) and synthetic cannabinoid use (n = 1). A recent change in smoking habits was reported in 50% of patients. Presenting symptoms were dyspnea (91.6%), cough (66.6%), fever (66.6%) and chest pain (25%). 90% of patients had leukocytosis on presentation, but only 16.6% had peripheral eosinophilia. The median eosinophil percentage in bronchoalveolar lavage was 67.5% (0 to 78). Two patients had a lung biopsy performed. Bilateral involvement on chest imaging was reported in all patients. Five patients (41.6%) required invasive mechanical ventilation and ten patients (83.3%) were treated in an intensive care unit. All patients responded to corticosteroid therapy with no relapses reported.

Acute eosinophilic pneumonia is reported with smoking that does not include traditional cigarette smoking such as waterpipes, e-cigarettes, heat-not-burn cigarettes, and marijuana and can have a similar presentation and clinical course.

Key words: eosinophilic pneumonia, smoking, e-cigarette, waterpipe, vaping, marijuana, cannabis

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Introduction

Acute eosinophilic pneumonia (AEP) is an eosinophilic lung disease characterized by an acute onset febrile respiratory illness with bronchoalveolar lavage eosinophilia that promptly responds to treatment with steroids. While the disease can be idiopathic, cigarette smoking is a major risk factor [1, 2] and commonly occurs in the setting of new exposure or a recent change in smoking habits [3, 4]. Drug use and other inhalational exposures are also known triggers [5–7]. Here, the reported cases of AEP in association with non-cigarette smoking such as e-cigarette, marijuana, and waterpipe smoking are reviewed.

Material and methods

The PubMed database was searched using the following keywords: smoking, electronic nicotine delivery systems, waterpipe, hookah, shisha, marijuana, cannabis, non-cigarette tobacco, vaping, e-cigarettes, electronic cigarettes and cannabis sativa. These keywords were searched for in combination with the following medical complications: eosinophilic pneumonia, respiratory distress syndrome, ARDS, respiratory insufficiency, and pulmonary eosinophilia. The "Related articles" section in PubMed was used to screen for additional relevant articles. The reference lists of the relevant articles were also screened to look

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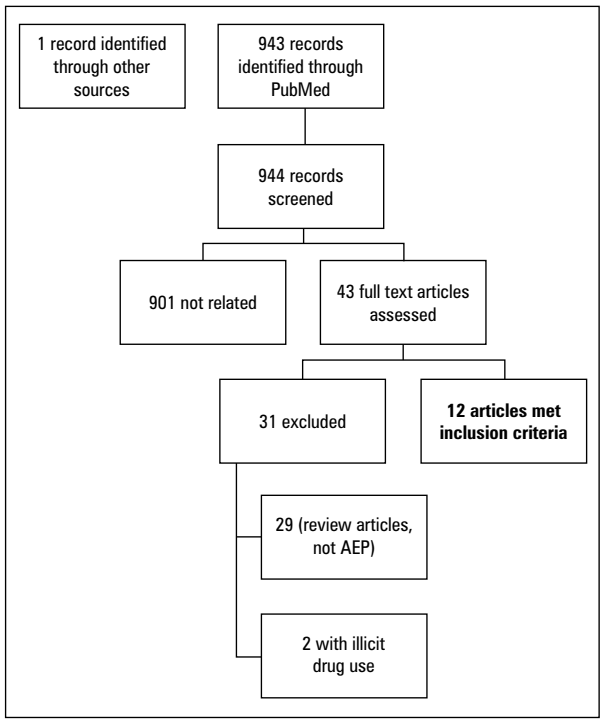

Figure 1. Study flowchart
AEP — acute eosinophilic pneumonia

for other manuscripts eligible for the current review. Cases were included if they involved cases of AEP defined as: 1) Acute respiratory illness of 1-month duration or less; 2) Bilateral pulmonary infiltrates on chest imaging; 3) Eosinophilic pneumonia detected by Bronchoalveolar lavage (BAL) with eosinophils totaling more than 25% or showing eosinophilic pneumonia on lung biopsy. In addition, exposure to smoking items other than traditional cigarettes was required and included cannabis, electronic cigarettes, heat-not-burn cigarettes, waterpipes and hookahs. Cases with exposure to illicit drug use were excluded. The results of this search are summarized in Figure 1. 943 abstracts were reviewed and 42 of them were identified for full text review (in addition to one relevant report identified by screening the references of the reviewed articles). Two were excluded because of illicit drug use exposure and 29 articles were not relevant (review articles or no relevant exposure). Twelve cases met the inclusion criteria (Figure 1).

Results

Patient characteristics and exposures

Table 1 summarizes the reported cases and patient demographics. 12 cases of AEP linked to non-cigarette smoking were reported with the patients having a median age of 20 (range 15–60). 42.6% were younger or equal to 18 years of age and 75% were male. Exposures reported included patients who smoked marijuana (n = 5), used a waterpipe (n = 2), smoked heat-not-burn cigarettes (n = 2), used e-cigarettes (n = 2), and one who used synthetic cannabinoids (n = 1). 25% reported associated cigarette smoking. A recent change in smoking habits was reported in 50% of patients and included the following: new initiation within 1 month in one patient, recent product change in four patients, and restarting less than 1 month in 3 patients. One patient also reported starting cigarette smoking within 1 month of symptom onset. Time from the last inhalational exposure to onset of symptoms was reported in 5 cases; three of them occurred within 1 day, one occurred after 2 days, and one occurred after 2 weeks. Two patients reported comorbid asthma.

Clinical manifestations, laboratory and radiological findings

Patients presented with dyspnea (91.6%), cough (66.6%), fever (66.6%) and chest pain (25%). Lung auscultation findings were reported in 9 out of 12 patients and findings included crackles (55.5%) and wheezing (22.2%). White blood cell count (WBCs) was reported in 11 patients with a median of 19,95 $\times$ 10^9/L (range 5.8 to 28) with 90% having leukocytosis as defined by WBC count greater than 11 $\times$ 10^9 cells/L. Only 2 patients (16.6) had peripheral eosinophilia on presentation. Three additional patients (25%) developed peripheral eosinophilia during their hospitalization.

Bronchoalveolar lavage eosinophil cell count was reported in 11 out of 12 patients. In one of the patients, the procedure had to be aborted due to hypoxemia which resulted in an inadequate sample. Of the remaining ten patients, the median eosinophil percentage was 67.5% (range 0 to 78) with 90% of patients having an eosinophil count totaling more than 25%. The only patient who had less than 25% (0 eosinophils) was treated with corticosteroids prior to bronchoscopy. One patient's sputum was sent for evaluation and their eosinophil count was 14%. IgE levels were reported in 4 patients; they were normal in three patients and elevated in one. Lung biopsies were obtained in two patients and confirmed eosinophilic pneumonia. One of these biopsies was conducted using video assisted thoracoscopic surgery (VATS), while the other sampling method was not specified.

Bilateral involvement on chest imaging was reported in all patients. Computed tomography

Table 1. Articles reviewed and patient characteristics

Publication	Exposure	Study design	Number of patients with AEP	Age	Gender	Current cigarette smoker
Sauvaget *et al.* 2010 [8]	Marijuana	Case report	1	15	M	Yes
Liebling *et al.* 2013 [9]	Marijuana	Case report	1	60	M	No
Natarajan *et al.* 2013 [10]	Marijuana	Case report	1	29	M	Yes
Dyal *et al.* 2014 [11]	Hookah	Case report	1	26	F	No
Thota *et al.* 2014 [12]	E-cigarette	Case report	1	20	M	No
Kang *et al.* 2016 [13]	Hookah	Case report	1	NA	M	No
Kamada *et al.* 2016 [14]	Heat not burn	Case report	1	20	M	No
Schlossma *et al.* 2017 [15]	Synthetic cannabinoids inhaled	Case report/ /poster presentation	1	18	F	No
McElligott *et al.* 2017 [16]	Marijuana	Case report/ /poster presentation	1	20	M	Yes
McGraw *et al.* 2018 [17]	Marijuana	Case series	1	17	M	No
Aokage *et al.* 2019 [18]	Heat not burn	Case report	1	16	M	No
Arter *et al.* 2019 [19]	E-cigarette	Case report	1	18	F	No

AEP — acute eosinophilic pneumonia

Table 2. Computed tomography (CT) of the chest findings

CT chest findings	N (%)
Ground glass opacities	7 (58)
Consolidation/infiltrates	6 (50)
Nodules/nodular infiltrates	2 (16.6)
Interlobular septal thickening	3 (25)
Tree in bud	1 (8.3)
Pleural effusions	4 (33.3)

of the chest (CT chest) findings are summarized in Table 2.

Treatment and outcomes

Two patients (16.6%) did not require respiratory support, two patients (16.6%) required supplemental oxygen therapy, 1 patient (8.3%) was treated with noninvasive positive pressure ventilation, and 5 patients (41.6%) required invasive mechanical ventilation. One patient required extracorporeal membrane oxygenation (ECMO) and another patient was transferred for ECMO to another institution. Ten patients (83.3%) were treated in an intensive care unit. All patients received corticosteroids with varying regimens with five of them (41.6%) receiving oral corticosteroids. All patients responded to steroid therapy with no relapses reported.

Discussion

Acute eosinophilic pneumonia is a disease characterized by an acute respiratory illness with bilateral involvement on chest imaging, pulmonary eosinophilia and a prompt response to treatment with corticosteroids. It commonly progresses to respiratory failure requiring invasive mechanical ventilation and intensive care [1, 4, 20]. Philit *et al.* [21] suggested diagnostic criteria which required subsequent modifications [1, 4] seeing as hypoxemia is not present in all patients and recovery without steroids was reported. Cigarette smoking is a strong risk factor [1, 2] and patients frequently report recent initiation of smoking, a change in smoking habits [3, 4], drug use and/or other inhalational exposures [5, 6, 22, 23]. Non-cigarette forms of smoking are increasingly used. These include e-cigarettes [24, 25], waterpipe/hookah smoking [25–27], and marijuana use [28]. Younger populations are mainly at risk and include adolescents as well as college and university students [26, 29–31]. Recently, an outbreak of vaping induced lung injury was reported [32]. Other pulmonary effects of vaping include: bronchial toxicity [33], lipoid pneumonia [34], diffuse alveolar hemorrhage [35], hypersensitivity pneumonitis [36], and organizing pneumonia [37]. In the current review, literature was researched for reported cases of AEP associated with these exposures. The median age in the

reviewed cases was 20 with 42% being 18 years of age or younger. This reflects the increasing use of such products by the young and adolescent population, and the need for public health policies and awareness to address this problem as they are frequently falsely perceived as safe by the public. In addition to the direct health consequences of these products, they increase the risk of traditional cigarette smoking in users [38]. As reported by Rhee *et al.* [4], the exposure to other types of smoking was frequently recently started or altered (50% of patients) similarly to traditional cigarette smokers. Fever was present in 66.6% of the patients, but higher percentages were reported in other studies (mean 80%) [1]. Peripheral eosinophilia was not common during presentation and this is a related finding to Giacomi *et al.* [5] who reported a lower incidence of peripheral eosinophilia in smoking associated AEP (36%) compared to other causes. Cigarette smoking and other types of smoking likely share common pathophysiological mechanisms which might explain why peripheral eosinophilia was uncommon upon presentation in this review similarly to smoking associated AEP. Like in other studies [1, 20], peripheral eosinophilia can arise during hospitalization (25% in the reviewed cases above). Bronchoalveolar lavage eosinophilia was present in most of the reviewed cases. One patient had no eosinophils on BAL, but the diagnosis of eosinophilic pneumonia was later confirmed by biopsy. In this patient, the BAL was done after corticosteroid therapy. In another patient, the procedure had to be aborted due to hypoxemia. The retrieved sample had an eosinophil count of 6% and biopsy obtained by VATS confirmed eosinophilic pneumonia but the sample was deemed ineligible due to the aforementioned complication during the procedure. One patient had sputum cytology showing eosinophilia without a reported BAL and responded promptly to corticosteroid therapy. Like other studies [1, 4], ground glass opacities (GGO) and bilateral infiltrates were the most common chest imaging findings. Pleural effusions were less common in the reviewed cases. Some radiological details might have been missed from the case reports. Invasive mechanical ventilation was required in 41.6% of patients. One patient required ECMO. Rates of mechanical ventilation are lower in AEP studies (mean 25%) [1]. This review included only case reports where there was a possibility of reporting bias where severe cases are reported more than milder ones. Different corticosteroid regimens were used with an excellent response in all patients. The regimens used varied and no conclusions can be drawn from these cases, but prior data in AEP associated with other causes showed that treatment duration could potentially be shortened to 2 weeks [4]. This review is limited by the quality of data available that comes mainly from case reports. Clinical, radiological and laboratory details might have been omitted. Also, reporting bias cannot be excluded as the exposure is sometimes not disclosed by patients.

Conclusion

AEP is reported with smoking outside of traditional cigarette smoking including vaping, waterpipe smoking, marijuana and HNBCs. The disease has a similar presentation and clinical course to AEP associated with cigarette smoking and other exposures.

Conflict of interest

None declared

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Volume-assured pressure support mode plus pirfenidone as resuscitation therapy in patients with exacerbation of idiopathic pulmonary fibrosis

Abstract

Introduction: Treatment among advanced stage idiopathic pulmonary fibrosis is quite challenging, especially considering that no major evidence has been released about it. This case report demonstrates and discusses the benefit of non-invasive mechanical ventilation in volume-assured pressure support (AVAPS) mode plus pirfenidone based on the relief of a patient's symptoms in combination with high-resolution computed tomography (HRCT) evidence.

Material and methods: An 83-year-old female patient with multiple hospital admissions within a six-month period initially presented with cardiac symptoms which were later attributed to a possible exacerbation of her primary diagnosis, idiopathic pulmonary fibrosis.

Conclusion: The addition of non-invasive mechanical ventilation in AVAPS mode plus pirfenidone can improve the survival rates even in patients with current exacerbations of acute respiratory failure due to idiopathic pulmonary fibrosis.

Key words: pirfenidone, idiopathic pulmonary fibrosis, non-invasive ventilator

Adv Respir Med. 2019; 87: 147–152

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most frequent and devastating form of idiopathic interstitial pneumonias. The average survival time from diagnosis is 3 years. It commonly affects people older than 60. IPF is a chronic fibrotic interstitial lung disease (ILD) characterized by progressive loss of lung function with symptoms including dyspnea and cough [1]. The American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Association guidelines indicate that a diagnosis of IPF should be based on: a) Exclusion of other known causes of interstitial lung disease (e.g., domestic and occupational environmental exposures, connective tissue disease, and drug toxicity); b) Presence of a usual interstitial pneumonia (UIP) pattern on high-resolution CT (HRCT) in patients who have not undergone surgical lung biopsy; and c) Specific combinations of HRCT and surgical lung biopsy pathological patterns in patients who have undergone surgical lung biopsy [2]. The American Thoracic Society defines exacerbation as: a) Previous or concurrent diagnosis of IPF; b) Acute worsening, or development of dyspnea typically < 1-month duration; c) Computed tomography with new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent with a usual interstitial pneumonia pattern; d) Deterioration not fully explained by cardiac failure or fluid overload [3]. Patients with IPF frequently suffer from acute exacerbations, which could

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be due to single or multiple factors [4]. Pirfenidone was recently found to be effective for a patient with an acute exacerbation of IPF (AE-IPF) [5].

Based on high in-hospital mortality rates, current treatment guidelines state that many patients with respiratory failure due to AE-IPF should not receive mechanical ventilation, mainly because of the aforementioned high mortality rates. There are no studies suggesting that mechanical ventilation (including non-invasive ventilation) might be successful [6–8]. Furthermore, the use of non-invasive mechanical ventilation in these patients has been questioned due to the possibility of futility [9].

We present this case to show that the combination of noninvasive mechanical ventilation in AVAPS mode plus pirfenidone might provide a different perspective towards the clinical approach of ventilatory management for these patients.

Material and methods

An 83-year-old female patient who was transferred from another hospital presented to the emergency department with a 4-week history of productive cough, low production of white expectoration, and marked dyspnea (class III MRC (Medical Research Council) dyspnea scale). The patient was diagnosed with AE-IPF based on the American Thoracic Society guidelines: a) Worsening dyspnea; b) New findings on HRCT, and c) Significantly decreased PaO_2. According to the criteria, these findings should occur within 1 month of one another without any other causative factor (e.g. infection); c) During admission, the patient was conscious and dyspnea was clearly observed. There was no evidence of cardiac symptoms or possible cardiac involvement and no manifestation of hemodynamic compromise.

IPF had been diagnosed in a tertiary referral hospital approximately 1-year prior to the admission to our hospital. The diagnosis of IPF included a transbronchial biopsy that exhibited characteristic pathological patterns.

Physical examination showed rhythmic heart sounds, hypoventilated lung fields with the presence of bilateral basal crepitations, and a Glasgow score of 15/15. Laboratory tests indicated normal blood count (Leukocytes — 7.87 mm^3), normal inflammatory markers (Monocytes — 14.7%, Lymphocytes — 15.1%, Neutrophils — 64.6%, Eosinophils — 3.99%), Hematocrit — 32.6%, Hemoglobin — 10.8 g/dL, Red Blood Cells — 373 mm^3, Platelets — 429 mm^3, ALT — 35 U/L, AST — 41 U/L, LDH — 246 U/L) and serum creatinine was 0.7 mg/dL.

The HRCT revealed diffuse bilateral pleuropulmonary infiltrations accompanied by areas of fibrosis in the lung bases (Figure 1).

The patient remained under hospital supervision for four days with clinical suspicion of an AE-IPF exacerbation and was discharged after observed improvement.

One week later, the patient returned to the emergency department complaining of cough without expectoration, as well as use of accessory muscles of breathing (especially sternocleidomastoids).

White blood cell count, prior to admission, was as follows: Leukocytes — 9.93 mm^3; Neutrophils — 66.8%. CRP value was: 2.0 mg/L.

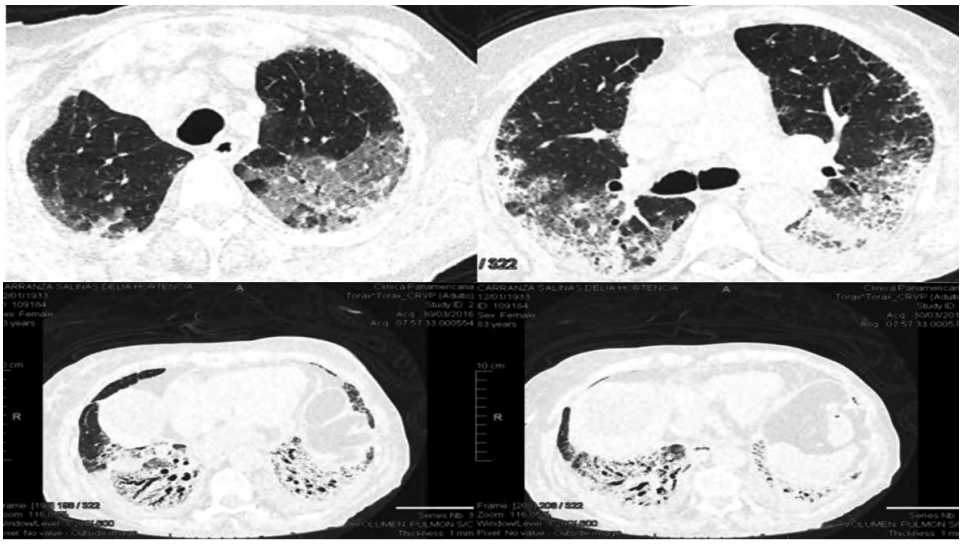

Figure 1. High-resolution computed tomography of the chest on initial presentation. Peripheral ground-glass, traction bronchiectasis. Reticulated opacities were distributed throughout the basal lungs; honeycombing was absent

Blood and sputum cultures, along with molecular investigations for atypical respiratory pathogens remained negative.

After assessment in the emergency department, the patient was admitted to the intensive care unit (ICU). The patient was started on treatment consisting of sodium chloride for hydration, ampicillin-sulbactam, salbutamol, fluticasone, salmeterol, enoxaparin, furosemide, and hydrocortisone 400 mg/day.

The initial arterial blood gas analysis showed: pH — 7.47, $PaCO_2$ — 33.6 (mm Hg), PaO_2 — 45.2 (mm Hg), HCO_3 —24 (mmoL/ L), excess base — 1.9, and SO_2 — 85%.

The Echocardiogram reports showed evidence of preserved global and segmental contractility with an ejection fraction (EF) of 72.8% and an estimated systolic pulmonary artery pressure (PSAP) of 32 mm Hg.

Ventilatory parameters

The ventilatory parameters were initially programmed in the BiPAP S/T-AVAPS (volume-assured pressure support) (AVAPS) Tidal volume programmed AVAPS (ml) 400 mL, levels of IPAP programmed maximum — 14 (cmH_2O).

Levels of IPAP patient — 14 (cmH_2O), levels of EPAP — 8 (cmH_2O), RAMP — 3 (msec), inspiratory time — 1.2 (sec), tidal volume patient — 398 (mL), Vmin — 18 (L/min), leak — 10 (cmH_2O), FiO_2 — 70 (%).

During the fifth day in the ICU, treatment with pirfenidone was administered at 200 mg orally three times daily (600 mg/day). This treatment was based on the IPF diagnosis and was initiated after obtaining informed consent from the patient and her surrogates.

The patient was then transferred at which point she and her relatives decided to establish a "**do not resuscitate order**". Through advanced care planning, cardiopulmonary resuscitation (CPR) and/or invasive mechanical ventilation would not be attempted. However, clinical management was sustained. During the hospitalization, she continued with NIV for 9 days (approximately 20 hours a day) with a high percentage of inspired (FiO_2) 70%, and with the use of a rebreather mask during the rest periods of NIV in AVAPS mode.

The patient presented slight erythema on the nasal bridge due to skin irritation from the facial mask. On day 10, we decided to increase the dose of pirfenidone to 1200 mg/day. On day 12, the patient began to decrease daily use of NIV in AVAPS mode to 18 hours/day, requiring less percentage inspired FiO_2 (65%). On day 16, the dose of pirfenidone was increased to 2400 mg/day and the patient presented with ruddiness of the face along with a sensation of heat as a result of the use of pirfenidone. On day 19, NIV was decreased to 10 hours a day with FiO_2 50%. On day 24, the use of NIV in AVAPS mode was decreased to 3 hours, twice daily. On day 26 of hospitalization, the patient began to wear a facial oxygen mask at 5 L/min. After 27 days of hospitalization, the patient was finally discharged with an oxygen mask, receiving pirfenidone at the dose of 1200 mg/d.

Follow-up

The patient had consultations throughout the year after her initial presentation in the hospital, and the evaluation showed a marked decrease in patchy opacities. Figure 3 shows a marked decrease in ground-glass opacities at 18 months after treatment with pirfenidone. Forced and slow spirometry manouvers are shown in Figure 4.

Minute ventilation (VE), peak oxygen consumption (VO_2), and carbon dioxide released (VCO_2) measured breath by breath (Software PistonXP version 1.61 PRE-201 Ergospirometer) (Piston Ltd.'s respiratory diagnostics), and 12-lead ECG (BTL CardioPoint-Ergo E600). Data was continuously recorded. After 3 min of resting

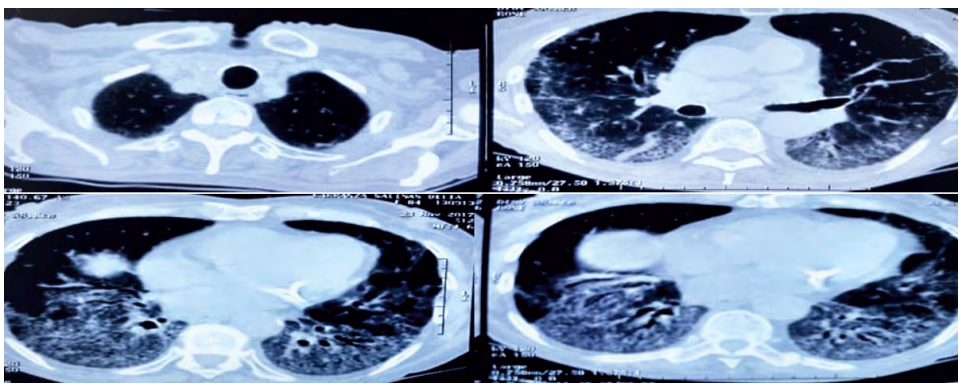

Figure 2. CT notes a notable decrease in opacities in upper lung segments in the axial section

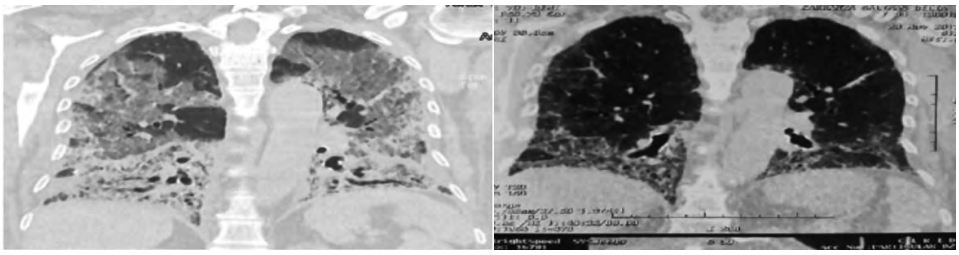

Figure 3. CT note the decrease in opacities in upper lung lobes in the coronal section 18 months after treatment with pirfenidone

Female 84 years 40 kg 149 cm Born: 12/1/1933

Prediction: Crapo-Hsu

Forced Vital Capacity Parameters		Ref	PRE 23/9/2017 13:11:42	%	Ref	POST 23/9/2017 13:31:03	%	DIFFERENCE Abs	DIFFERENCE %
FVC	[l]	2,63	1,35	51	2,63	1,48	56	0,13	10
FEV*1.0	[l]	2,25	1,32	59	2,25	1,46	65	0,14	11
FEV*1.0/FVC	[%]	84,49	98,24	116	84,49	98,71	117	0,47	0
FEF*25-75%	[l/s]	2,89	3,13	108	2,89	3,54	122	0,41	13
FEF*75%	[l/s]	1,68	0,93	55	1,68	1,18	70	0,25	27
FEF*50%	[l/s]	3,27	3,17	97	3,27	3,76	115	0,59	19
FEF*25%	[l/s]	4,62	4,81	104	4,62	3,78	82	-1,03	-21
MEF*25%	[l/s]	1,68	0,93	55	1,68	1,18	70	0,25	27
MEF*50%	[l/s]	3,27	3,17	97	3,27	3,76	115	0,59	19
MEF*75%	[l/s]	4,62	4,81	104	4,62	3,78	82	-1,03	-21

Inspiratory Vital Capacity Parameters		Ref	PRE 23/9/2017 13:12:27	%	Ref	POST 23/9/2017 13:32:04	%	DIFFERENCE Abs	DIFFERENCE %
IVC	[l]	2,70	1,00	37	2,70	0,97	36	-0,03	-3
IRV	[l]	1,28	0,69	54	1,28	0,66	52	-0,03	-4
ERV	[l]	0,88	0,07	8	0,88	0,03	3	-0,04	-57
TV	[l]	0,45	0,21	46	0,45	0,32	71	0,11	52
IC	[l]	1,82	0,91	50	1,82	0,98	54	0,07	8
Temp	[?C]	-	25,50		-	24,60		-0,90	-4
Hum	[%]	-	58,97		-	52,95		-6,02	-10
Press	[mbar]	-	1005,30		-	1005,40		0,10	0

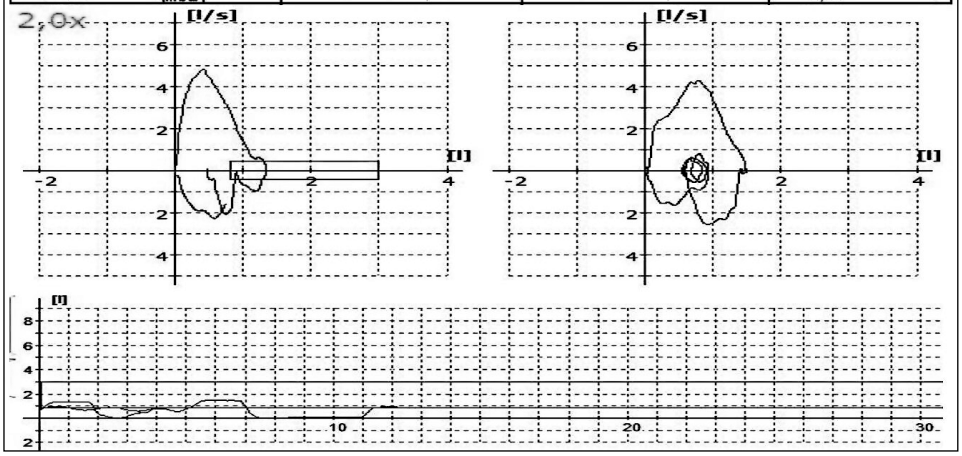

Figure 4. Forced spirometry and slow spirometry maneuvers in which the flow-volume curve shows an ascending branch of rapid rise in expiratory loop with a restrictive pattern and a reduction in inspiratory capacity

and 2 min of warm up, the exercise load was increased every 3 min by 25 watts; as determined for patients with interstitial disease and chronic pulmonary disease. She pedaled at about 50 to 60 revolutions per min. The following data was obtained: VO_2 9.18 ml/kg/min (51% predictor), VCO_2 9.41 ml/kg/min, respiratory exchange ratio (RER) 1.15, Load 50 watts, breaths rate (BR) 47 breat/min, VE 28 (38%), coefficient minute ventilation/VO_2 production (VE/VO_2) 46.7; coefficient minute ventilation/VCO_2 production (VE/VCO_2) 82,5, coefficient VO_2 production/heart rate (VO_2/HR) 8,4 mL/b, coefficient VO_2 production/work rate (WR) 4,70 mL/min/watts, HR maximum 157 heart/min; maximum (Figure 5).

Discussion

IPF is defined as a specific form of chronic, progressive, fibrosing interstitial pneumonia of

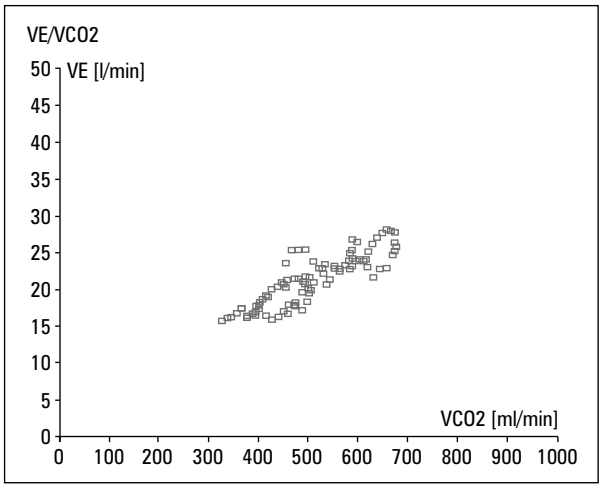

Figure 5. VE/VCO_2 value during incremental cardiopulmonary exercise testing VE/VCO_2 (coefficient minute ventilation/VCO2 production)

unknown cause. IPF shows the usual interstitial pneumonia (UIP) pattern, pathologically and/ /or radiologically. Recently, an official ATS, ERS, JRS, and LATA consensus statement (the current IPF guideline) advocated that the presence of characteristic UIP findings ("UIP pattern") on thin-section CT images is sufficient for diagnosing IPF/UIP without pathologic evaluation by surgical lung biopsy in appropriate clinical settings [1]. Pirfenidone inhibits transforming growth factor-β (TGF-β) and has antifibrotic, anti-inflammatory, and antioxidant effects. Pirfenidone is the first antifibrotic agent that has been approved by the FDA for the treatment of IPF and has recently been found to be effective for a patient with AE-IPF [10].

AE-IPF is defined as any respiratory event characterized by new bilateral ground-glass opacification/consolidation not fully explained by cardiac failure or fluid overload, which parallels the Berlin Criteria for acute respiratory distress syndrome. However, there is difficulty in distinguishing idiopathic from respiratory events triggered by known causes (apart from cases with evident infectious pneumonia); both idiopathic and triggered events (e.g. infection, post-procedural/postoperative, drug toxicity, aspiration) resulting in worsening respiratory symptoms and widespread alveolar damage can be diagnosed as AE-IPF. The difference between AE triggered by infection and pneumonia in terms of therapeutic strategy and prognosis remains unknown.

No treatments have been shown to be effective in the manegement of acute exacerbations. Patients usually require hospital admission, supplemental oxygen, and broad-spectrum antibiotics. International treatment guidelines include a weak recommendation for the use of high-dose corticosteroids in the management of patients with AE-IPF, based on very low-quality evidence.

In the case of our patient, she received noninvasive mechanical ventilation (NIV) plus pirfenidone, the latter therapy beginning on the fifth day of hospitalization. These resulted in clinical improvement with a gradual decrease in oxygen requirement (inspired FiO_2) and time of use of NIV. Hence, current IPF guidelines state that while the majority of patients with respiratory failure due to IPF should not receive mechanical ventilation, it may be a reasonable intervention in a minority of patients (weak recommendation, low-quality evidence) and that NIV may be appropriate in some patients [11].

Management of AE-IPF in the ICU may be justified, particularly in patients in whom the possibility of lung transplantation exists and in those who have not yet undergone clinical evaluation for the cause of their respiratory decline [12].

Patients with IPF often present common comorbidities such as pulmonary hypertension [13], obstructive sleep apnea, lung cancer, chronic obstructive pulmonary disease (COPD) / emphysema, ischemic heart disease, and GERD [14], which demand holistic approach in the disease management.

Conclusion

In our case, we facilitated the recovery of a patient with AE-IPF who had marked hypoxemia and ventilatory work, normal blood chemistry and adequate renal function. The patient remained on NIV in AVAPS mode to avoid intubation. Pirfenidone was added at high doses that were achieved progressively and which were well tolerated.

The patient experienced progressive clinical improvement with declining oxygen requirements and NIV time until her hospital discharge at which time she no longer required NIV. Follow--up after one year demonstrated that the patient maintained a reduced lung function with minimal oxygen requirements at home, and her chest CT showed a notable improvement in opacities in upper lung segments in the axial section.

Conflict of interest:

No conflict of interest.

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Pulmonary oxalosis in pulmonary aspergillosis syndrome

Abstract

The presence of pulmonary oxalosis in bronchoalveolar lavage (BAL) or biopsied tissue samples is considered pathognomonic for *Aspergillus* disease etiology. The finding of calcium oxalate crystals in the tissue samples infected with aspergillosis can serve as a vital diagnostic clue. Detection of calcium oxalate crystals is achievable within 24 hours by most hospital microbiology laboratories. It is much quicker than the time it takes to receive results of other tests like histopathology, sputum cultures, and aspergillus antigen assays. We present this case to emphasize the importance of pulmonary oxalosis as a crucial early diagnostic factor in pulmonary aspergillosis syndromes.

Key words: aspergillosis, *Aspergillus*, oxalosis, calcium oxalate

Adv Respir Med. 2020; 88: 153–156

Introduction

Aspergillus genus comprises hundreds of species that are ubiquitously found in soil, water, or decaying vegetation. A. *fumigatus* and A. *niger* comprise the majority of disease-causing agents in pulmonary aspergillosis syndromes. These include invasive pulmonary aspergillosis (IPA), chronic necrotizing aspergillosis (CNA), allergic bronchopulmonary aspergillosis (ABPA), and aspergilloma.

A frequently overlooked diagnostic clue for tissue infection by aspergillosis is the finding of calcium oxalate crystals. The presence of pulmonary oxalosis in BAL or biopsied tissue samples is pathognomonic for *Aspergillus* disease etiology. Oxalic acid is a byproduct of *Aspergillus* metabolism, which combines with calcium in the blood supply of invaded tissue to form calcium oxalate crystals. The presence of calcium oxalate crystals is detectable under polarized light within 24 hours by most hospital microbiology laboratories, which is earlier than the time to receive results of histopathology, sputum cultures, and sputum and blood aspergillus antigen assays. This article suggests that evaluation for pulmonary oxalosis may be included in the workup of cases suspicious for aspergillosis.

Case report

A 56-year-old African-American male presented with two months' history of weight loss, cough with yellowish expectorate. He also complained of pleuritic right-sided chest pain for one week, and hemoptysis for two days before admission. A review of systems was positive for fever and chills. He smoked one pack/day for 35 years, and had a long-standing history of alcoholism.

At admission, he was tachycardic at 118 bpm, and his respiratory rate was 20 bpm. His blood pressure was 137/88, his temperature was 98.6°F, and SaO_2 was 96% at room air. Other pertinent physical exam findings were cachexia, and inspiratory crackles and diminished breath sounds at right upper lung fields. He was admitted for further testing.

His complete blood count showed leukocytosis at 37.5 K/μL and normocytic anemia with a hemoglobin of 10 mg/dL. A chest X-ray (Figure 1A) revealed right upper lobe infiltrates. He was started on ceftriaxone and azithromycin for the treatment of community-acquired pneumonia.

On day three, his symptoms persisted, which prompted broadening of antibiotics coverage to imipenem/cilastin 500 mg Q6 hours and tri-

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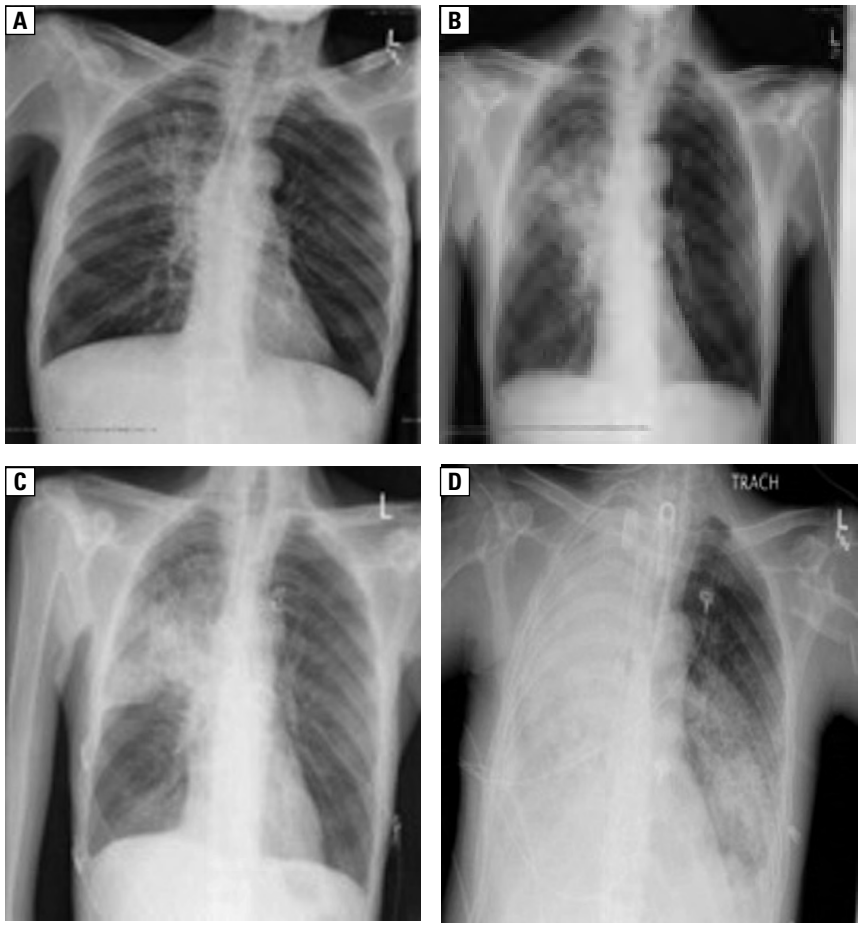

Figure 1. Chest X-ray results showing progression of RUL infiltrates to bilateral lungs from day 1 (**A**), 3 (**B**), 4 (**C**), and 8 (**D**) of admission respectively

methoprim/sulfamethoxazole 800 mg/160 mg 2 tablets BID. A repeat chest X-ray (Figure 1B) revealed worsening infiltrates of the right upper and middle lobes. His leukocytosis persisted at 37 K/μL. AFB of a sputum sample collected at admission returned negative. He continued to be normothermic and was saturating well on room air. On day 4, the patient continued to have leukocytosis at 32.5 K/μL. His chest X-ray (Figure 1C) showed worsening infiltrates. Linezolid 600 mg oral BID was also added to the aforementioned antibiotics. A CT of the chest with contrast (Figure 2) was obtained, which showed the right lung pulmonary consolidation with pleural effusion. His sputum culture results grew *Candida albicans* and *Aspergillus niger*. He was started on voriconazole loading dose of 6 mg/kg IV Q24 hours (which was subsequently followed by 4 mg/kg Q24 h).

On day 8, his leukocytosis worsened to 62 K/μL. Antibiotics were switched to meropenem 1000 mg Q12 hours, azithromycin 250 mg IV

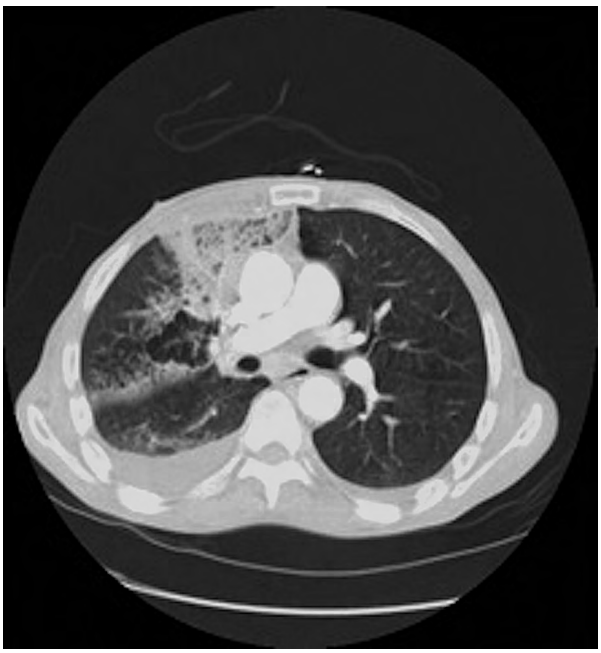

Figure 2. Computed tomography chest with contrast on day 4 of admission, showing right lung infiltrates, and right pleural effusion

Q12 hours, vancomycin 750 mg Q24 hours. He was also started on caspofungin 70 mg IV Q24 hours (followed by 50 mg daily on subsequent days). Based on the clinical course, broadened differential diagnoses were considered. These included pulmonary tuberculosis and nontuberculosis mycobacterial lung disease, pulmonary mycosis (chronic pulmonary aspergillosis or histoplasmosis), cryptogenic organizing pneumonia, pulmonary vasculitis (such as granulomatosis with polyangiitis, microscopic polyangiitis or eosinophilic granulomatosis with polyangiitis), *Pneumocystis carinii* (PCP) pneumonia and lung cancer. He had also developed respiratory failure requiring BiPAP. He was subsequently intubated and underwent bronchoscopy with bronchoalveolar lavage (BAL). Within 24 hours of collection, his BAL sample showed calcium oxalate crystals under polarized light. Histopathology of biopsied tissue sample was negative for malignancy, fungal bodies, but also confirmed calcium oxalate crystals (Figure 3).

His sputum sample was also tested for AFB stain, TB culture, mycobacterium tuberculosis DNA by PCR, cytology, and PCP antigen, which were all negative. Urine histoplasma antigen and pANCA and cANCA blood titers were also negative. One week later, the patient's BAL sputum galactomannan antigen and Aspergillus DNA PCR returned positive. The serum galactomannan antigen was also positive. The patient's health continued to deteriorate; he developed multiorgan system failure and died one week later. Autopsy was not performed and necrotizing pneumonia was identified as the cause of death.

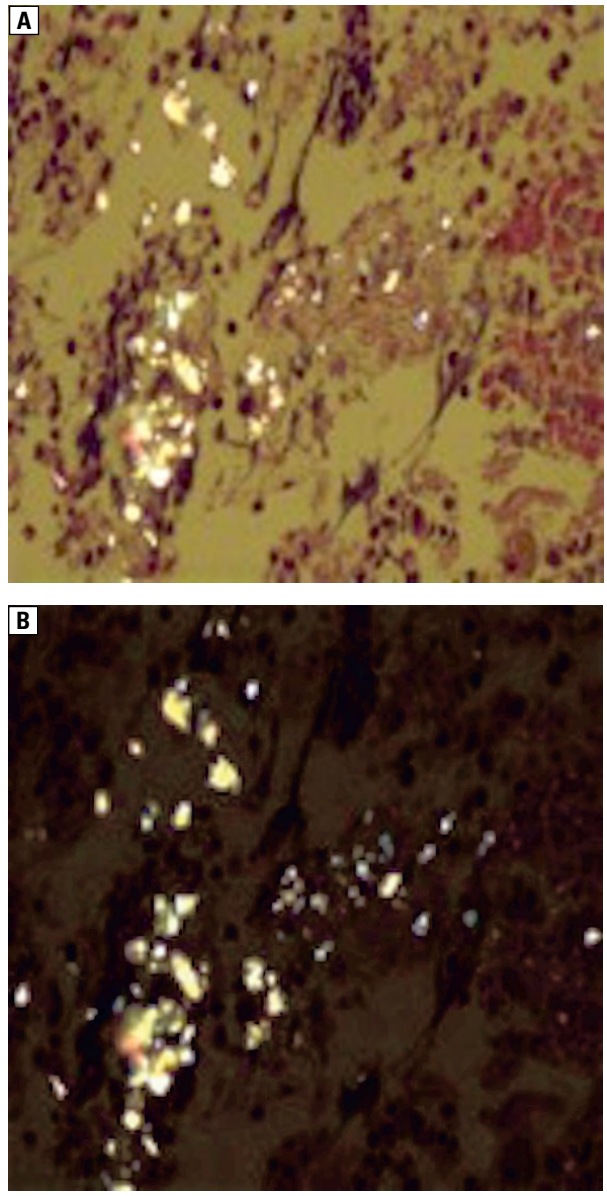

Figure 3. Showing calcium oxalate crystals in pulmonary tissue biopsy

Discussion

Pulmonary oxalosis is pathognomonic for *Aspergillus fumigatus* and *Aspergillus niger* tissue invasion. Oxalic acid is a byproduct of *Aspergillus* spp. metabolism which combines with calcium in the blood supply of invaded tissue to form calcium oxalate crystals. The deposition of calcium oxalate crystals and *Aspergillus* invasion both contribute to further inflammation and tissue destruction. Calcium oxalate crystals can occur in all spectrum of pulmonary aspergillosis such as aspergilloma, allergic bronchopulmonary aspergillosis, chronic necrotizing aspergillosis, and invasive pulmonary aspergillosis (IPA), but is more common in the latter two which are representatives of more invasive disease. Calcium oxalate crystals are readily identifiable under polarized light microscopy as birefringent granules in sputum and tissue samples of the infected host. This result is available at most standard hospital laboratories within 24 hours of sample collection, and in the appropriate clinical situation, it can facilitate early initiation of antifungal treatment, long before the results of other tests for pulmonary aspergillosis return.

Pulmonary aspergillosis is most frequently caused by A. *fumigatus*, and less commonly by A. *niger*. Invasive pulmonary aspergillosis (IPA) is an acute disease that manifests clinically in patients with risk factors which include neutropenia, prolonged use of high dose steroids, critical illness, and immunosuppression associated with organ transplant, chemotherapy or AIDS [1]. Typical symptoms reported are similar

to bronchopneumonia, i.e. cough with expectorating, dyspnea, fever, pleurisy, and hemoptysis. In contrast, CNA is a more indolent, sub-invasive disease in which full symptoms may take weeks to months from onset of *Aspergillus* exposure to become fully evident. CNA experience symptoms similar to IPA, with the addition of weight loss. Risk factors for CNA are centered around mild levels of immunosuppression, which include being elderly, underlying chronic lung diseases such as COPD, history of radiation therapy, diabetes mellitus, alcoholism, chronic liver disease, and low-dose corticosteroid therapy [1–3]. Patients with COPD are known to have pulmonary parenchymal modifications, which increases their risk of infections. Alcoholism is also known to affect innate and adaptive pulmonary immunity resulting in subclinical immunosuppression that becomes apparent with a heavy burden of infection [3, 4]. Our patient's indolent prehospital course was consistent with CNA secondary to chronic alcoholism and COPD, and his disease progressed rapidly to IPA and became critically ill after he was admitted.

The man was diagnosed with IPA based on positive respiratory cultures for *Aspergillus*, bronchial necrosis during bronchoscopy, imaging findings, and positive sputum culture, and galactomannan antigen assay which was positive in both sputum and blood (which returned two weeks later). Histopathology with Grocott's methenamine silver stain of the tissue sample from his right upper lobe biopsy was negative for fungal organisms; however, the diagnostic value of biopsy is highly correlative to the site sampled. The detection of calcium oxalate crystals in BAL within 24 hours of collection, coupled with tracheobronchial necrosis raised the clinical suspicion for IPA, and lead to early initiation of treatment.

The treatment for proven pulmonary aspergillosis is based on intravenous azoles, echinocandins, and amphotericin B [5–9]. Invasive forms of pulmonary aspergillosis require intravenous azoles (voriconazole as first-line), which can be deescalated to oral regimens with clinical improvement, and treatment lasts about 6–12 weeks at which time radiological and clinical symptoms are resolved. Frequently, parenchymal damages are irreversible [10–13].

According to the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG), pulmonary oxalosis is not currently considered a part of the standardized criteria for the diagnosis of aspergillosis. Checking for the presence of pulmonary oxalosis in the BAL sputum during the workup of patients suspected of invasive pulmonary disease is of high yield potential in early diagnosis. Eradication of pulmonary aspergillosis syndromes can be difficult to achieve with a heavy infectious burden and severe immunosuppression, and early initiation of treatment is recommended. We present this case to emphasize the importance of pulmonary oxalosis as an early diagnostic clue for *Aspergillus* tissue invasion prior to results from cultures, lab tests, and tissue biopsy, which can take longer to yield results.

Conflict of interest

None declared.

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An unusual cause of high density radiological opacities

Abstract

Introduction: Metallic mercury poisoning through intravenous injection is rare, especially as part of a suicide attempt. Diagnosis and treatment of the disease are challenging as clinical features are not specific.

Material and metods: A 41-year-old male presented with dyspnea, fatigue, loss of weight, and loss of appetite over two months. Routine radiological examination by chest X-ray and CT showed randomly distributed high density opacities with Hounsfield units (HU) around 500 HU all over the body. The diagnosis was then confirmed with a urinary mercury concentration of > 1000 mcg/24 h.

Results: The patient's clinical condition was getting worse in spite of chelation therapy and hemodialysis. The patient eventually died because of respiratory failure.

Conclusion: Early diagnosis and appropriate treatment are critical for intravenous mercury poisoning especially because there are no specific signs or symptoms. There should be a high level of suspicion in drug abusers. Treatment should involve the combined use of chelating agents and other treatments such as hemodialysis and plasma exchange in advanced clinical settings.

Key words: intravenous injection, mercury poisoning, high density opacities

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Introduction

High density opacities on chest X-ray are an infrequent finding and are highly nonspecific. When such a radiological picture is encountered the differential diagnosis includes pulmonary alveolar microlithiasis, pulmonary hemosiderosis, metal poisoning, barium aspiration, pellet injury, and possible remnants of contrast used for lymphangiography or myelography. However, a thorough history taking, physical examination and other supportive investigations will help in achieving a proper diagnosis. Though uncommon, mercury poisoning can be seen in patients who are frequent drug abusers as well as in individuals working in the manufacturing of industrial chemicals, paints, explosives, batteries, thermometers, electronic instruments, etc.

Mercury exists in elemental, inorganic, and organic forms. Elemental mercury causes pulmonary, neurologic and nephrotoxic injury and its poisoning results most commonly from vapor inhalation as it is absorbed (80%) throughout the lungs [1]. Poisoning due to intravenous injection of mercury usually occurs in connection with attempted suicide, by accident, or in drug addicts exploring new ways to become intoxicated [2].

Case details

A 41-year-old male presented with complaints of breathlessness and cough with watery expectoration for the past 2 months. He also complained of loss of appetite and loss of weight (around 6–7 kg in 2 months). He was a painter by occupation with no known comorbidities. He was a chronic smoker and alcoholic with events of binge drinking for the past 15 years. He also had a history of recurrent IV drug abuse in the last 6 months and it had increased in frequency over the past two weeks before presenting to emergency.

On examination, the patient was conscious, oriented, and afebrile. The patient had a blood pressure of 106/86 mm Hg, a pulse rate of 106/min, a respiratory rate of 26 cycles/min, and saturation was 93% in room air that improved to 96–98% with 2 L oxygen supplementation with nasal prongs. Puncture marks along with thrombosed veins were seen in the right antecubital fossa extending on to

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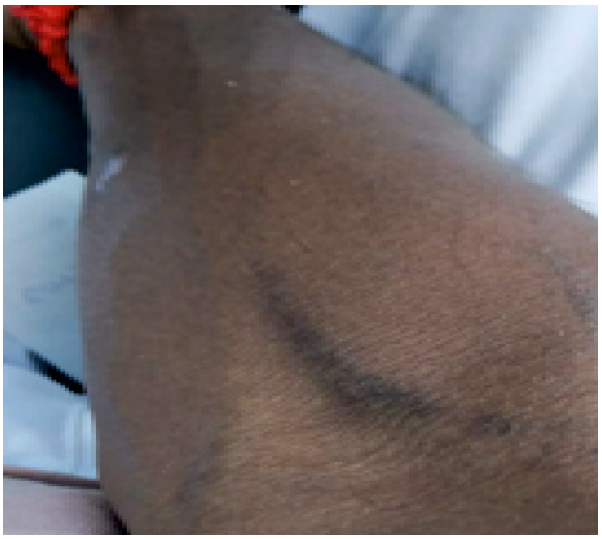

Figure 1. Puncture marks with thrombosed veins over right cubital fossa extending on to forearm

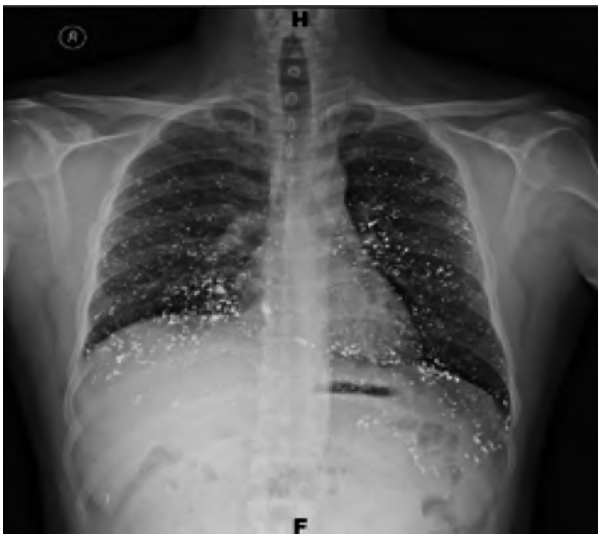

Figure 2. Chest X-ray showed multiple punctate and amorphous opacities of metallic densities noted in bilateral lung fields

the forearm (Figure 1). Chest auscultation revealed bilateral normal vesicular breath sounds. There were no signs and symptoms of other organ involvement. Blood urea, serum creatinine, and urine analysis were normal. Arterial blood gas analysis (ABG) showed a pH of 7.48, a pCO_2 of 24 mm Hg, HCO_3 of 23 mmoL/L, and a PO_2 of 73 mm Hg which were all suggestive of respiratory alkalosis.

Chest X-ray showed multiple punctate and amorphous opacities of metallic densities noted in bilateral lung fields (predominantly in the lower lobes) and overlying the cardiac silhouette (Figure 2). An X-ray of the abdomen showed similar opacities over the liver, kidneys, and dorso-lumbar spine. A lateral radiograph of the skull was found to be normal. CT of the chest showed multiple diffusely distributed metallic densities in bilateral lungs, the pericardium, liver, and kidneys as well as in multiple vertebral bodies and foramina (Figure 3). These features were compared with similar scenarios in literature and thus, IV mercury poisoning was suspected [3–7]. CT Hounsfield unit (HU) values of the high density opacities were 179 to 1065 (mean 527 HU, median 566 HU). Hence, 24 hour urine mercury (ICP-MS) was measured and was found to be > 1000 mcg/24 h. The patient was prescribed the chelating agent penicillamine given at doses of 500 mg orally every 6 hours in combination with pyridoxine (vitamin B6) in doses of 20 mg/day. The patient's condition was worsening in spite of chelation therapy hence hemodialysis and plasma exchange were used. Unfortunately, the patient's condition deteriorated and he ultimately died due to respiratory failure.

Discussion

Elemental mercury, when injected intravenously, can cause widely varying presentations. It can be asymptomatic in some patients while causing respiratory failure, kidney damage, liver damage, neurologic symptoms, and even death in others. Once mercury enters the bloodstream, it's quickly distributed throughout the body, particularly in the lungs as its absorption through the lungs is 80% [8]. Due to its high water–metal interfacial tension and lack of bonding to other materials, mercury either takes the form of tiny spherules or coalesces when it enters the plasma. Because of gas exchange and the extensive capillary network of the lungs through which blood flows back to the heart, the lungs can easily become a depository for the exogenous mercury. Because of this reason, the lungs are often more seriously affected than the other organs when examined on imaging and can give rise to a distinctive radiological appearance. In cases of intravenous self-administration in the forearm veins like in our case, aggregations of mercury at the site of injection may also be radiologically seen [9]. Radiologically small metallic spherules of different sizes scattered throughout the lungs or beaded chains along the pulmonary vasculature and other organs of the body can also be observed [10]. Mercury is mainly excreted from the body by the kidneys, but the rate of excretion is usually very slow and traces of mercury can be seen on radiographs and in urine even two years after the index event [8]. The total body burden of mercury can be determined by assessing twenty-four hour urinary levels of mercury. This total will serve as a useful marker to determine the

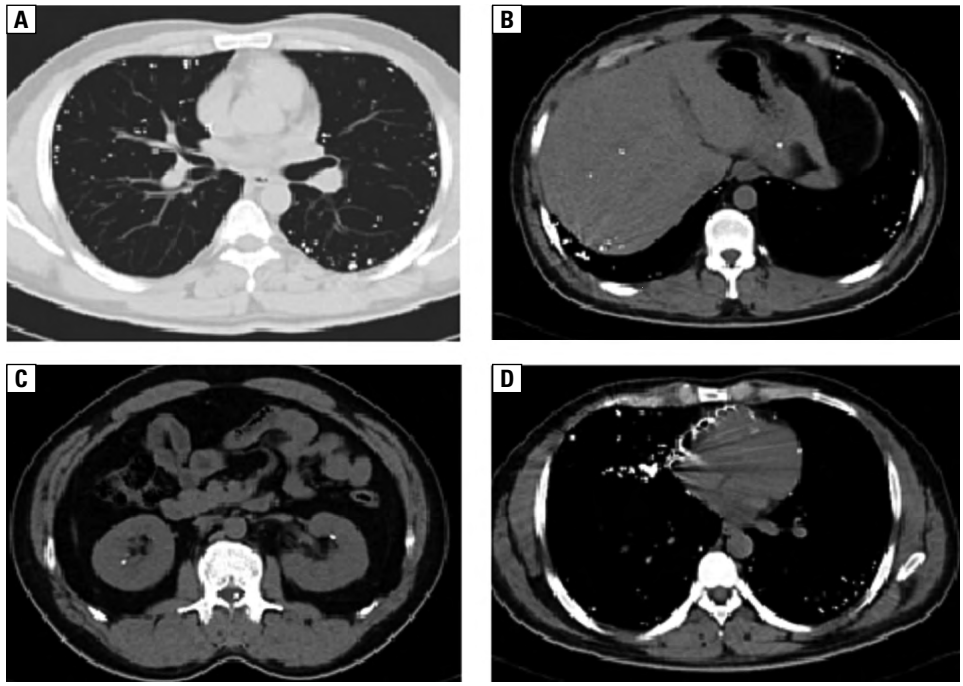

Figure 3. CT chest showed multiple diffusely distributed metallic densities in bilateral lung, pericardium, liver, kidneys and multiple vertebral bodies and foramina

need for chelation therapy and gauge the prognosis in some cases. Dimercaprol (BAL, *British anti-Lewisite*) or succimer, an orally administered analogue of BAL, are effective chelators utilised for treatment. Chelation therapy is the mainstay of treatment because these agents increase the urinary excretion of mercury [11]. Combined use of chelating agents and other treatments like hemodialysis and plasma exchange will enhance the elimination of mercury from the body in severe poisoning.

Conclusion

A clinician must take mercury poisoning into consideration when the imaging results shows radiopaque deposits that are unexplainable as early diagnosis and treatment is critical. Findings in a patient's history such as occupational exposure of mercury and drug abuse are very critical in the diagnosis of mercury poisoning and will affect the patient outcome significantly.

Conflict of interest

None declared

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An unusual case of right upper zone pneumonic patch

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A 32-year-old male heavy smoker presented to outside hospital with history of cough and shortness of breath for 20 days. There was no past history of tuberculosis, cardiac disease or interstitial lung disease. He was diagnosed as right pneumothorax for which chest tube was placed on the right. Immediately after chest tube placement, his dyspnoea increased and he desaturated for which he was referred to our hospital. On admission, his pulse rate was 102/min, BP — 110/70 mm Hg, SPO_2 — 98% with 2 litres of oxygen, respiratory rate — 36/min. On examination, chest crepitations over the right infraclavicular and mammary area were found. Other systemic examinations were normal. His symptom was alleviated after oxygen supplementation. His chest radiograph showed right upper zone opacity (Figure 1A) but preceding chest radiograph left at previous hospital manifested right pneumothorax (Figure 1B). Sputum for AFB samples were sent but report came to be negative, then bronchoscopy was done and bronchoalveolar lavage was taken to rule out tuberculosis. Spirometry confirmed chronic obstructive airway disease which could be the cause of pneumothorax. Then repeated chest radiograph (Figure 1C) was done which showed clearing of opacity, which confirmed the diagnosis of reexpansion pulmonary oedema.

Reexpansion pulmonary oedema (RPE) is a rare and lethal complication with 15 to 20% mortality [1]. RPE was thought to arise from an increased permeability of damaged pulmonary blood vessels, caused by swift reexpansion of lung tissue [2]. Younger age, longer duration of lung collapse (> 4 days), large pneumothorax (> 30% of a single lung), airway obstruction, rate of decompression are the risk factors associated with RPE [3–5].

Large pneumothorax, younger age and history of obstructive airway disease in our case favoured development of RPE. His manifestation was mild and managed symptomatically without a need for mechanical ventilation. However, his age, presentation, post chest tube symptom and chest radiograph, particularly unaccompanied by previous radiograph made us suspect strongly of tuberculosis rather than RPE.

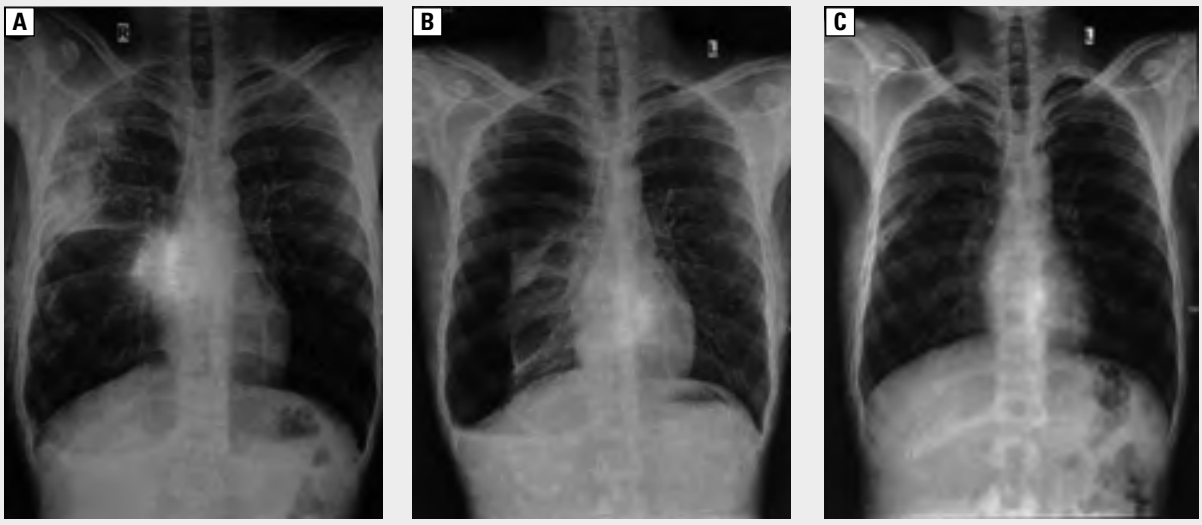

Figure 1. A. On presentation, chest radiograph showing dense, nonhomogenous air-spaced opacity over the right upper zone, horizontal fissure prominence, emphysematous changes with a chest tube *in situ*; **B.** Previous hospital chest radiograph showing large right pneumothorax with minimal fluid, collapsed lung noted medially in the right mid and lower zone; **C.** Repeat chest radiograph showing complete resolution of the radiographic abnormality.

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In India where tuberculosis is endemic, it remains the initial suspicion in case of the patient with three weeks history of cough and right upper zone opacity on chest radiograph. However, reexpansion pulmonary oedema should be kept in first differential diagnosis, particularly in the patient with immediate history of chest tube drainage and thoracocentesis.

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Gynecomastia in multi-drug resistant tuberculosis — ethionamide the villain

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A 59-year-old married male, a known case of line probe assay-confirmed multidrug-resistant pulmonary tuberculosis was treated with kanamycin, levofloxacin, ethionamide, ethambutol, pyrazinamide and cycloserine for seven months. He was responding to treatment, had gained five kg weight and underwent culture conversion. He presented with painful swelling of both the breasts for 15 days. It was not associated with nipple bleeding or discharge. There was no history of any other drug intake. 3.5 cm and 3 cm single, tender, firm nodule concentric with nipple-areolar complex could be palpated in the right and left breasts, respectively (Figure 1). It was not associated with skin dimpling or nipple retraction. On examination, thyroid, external genitalia and secondary sexual characters were normal. Bilateral breast ultrasound revealed glandular tissue hyperplasia. Ultrasound of the abdomen and scrotum was normal. Liver and kidney function tests revealed no abnormality. His serum was non-reactive for HIV-1 and HIV-2 by ELISA. Serum TSH was 1.86 μIU/mL (0.3–5.5 μIU/mL), serum testosterone was 302.6 ng/dL (241–827 ng/dL), serum estradiol was 38.4 pg/mL (0–39.8 pg/mL). Computed tomography of the adrenals showed no abnormality. Ethionamide was suspected to be the cause of gynecomastia and was stopped and replaced by para-aminosalicylic acid. His breast swelling resolved slowly within 1 month after stopping ethionamide. Thus, a clinical diagnosis of ethionamide-induced gynecomastia was made. As per the World Health Organization (WHO) Uppsala monitoring center causality assessment scale [1], the present adverse reaction is probable/likely associated with ethionamide.

Gynecomastia is enlargement of male breast glandular tissue. Drugs are responsible for 10–25% of cases of gynecomastia. Drugs which are known to cause gynecomastia include spironolactone, ketoconazole, cimetidine, estrogen preparations, 5-α reductase inhibitors, human chorionic gonadotropin, human growth hormone, gonadotropin releasing hormone (GnRH) analogs, risperidone, omeprazole, nifedipine, verapamil, efavirenz. Anti-tubercular drugs implicated to cause gynecomastia include isoniazid, ethionamide and thioacetazone [2–3]. Ethionamide is used for treatment of drug-resistant tuberculosis. The mechanism by which it causes gynecomastia is not known. PubMed search using MeSH terms "Ethionamide", "Gynecomastia" revealed only two case reports (Table 1).

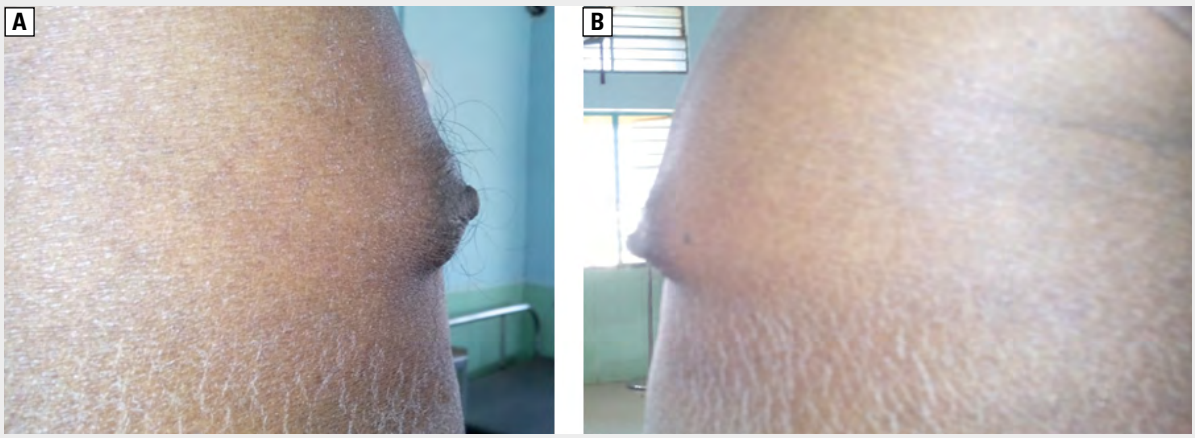

Figure 1. Lateral view of enlarged right breast and enlarged left breast

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Table 1. Case reports of ethionamide- induced gynecomastia

Author	Year	Age of patient in years	Range of onset after starting suspected drug	De-challenge	Re-challenge	Sex hormones
Dixit *et al.* [3]	2012	38	2 months	Yes	Yes	Normal
Sharma *et al.* [4]	2012	43	4 months	Yes	No	Not done
Present case	2019	59	7 months	Yes	No	Normal

Points which favor ethionamide-induced gynecomastia in our patient include: 1. Temporal association — recent onset of gynecomastia while the patient was taking anti-tuberculosis drugs; 2. Ethionamide is known to cause gynecomastia; 3. Exclusion of other causes: there was no history of any other drug intake. Normal biochemical and endocrinological investigations; 4. Dechallenge — gynecomastia disappeared after stopping ethionamide. Gynecomastia in our patient on the WHO Uppsala monitoring center causality assessment scale [3] is probable/likely associated with ethionamide.

Thus, our case report emphasizes the fact that ethionamide can cause painful gynecomastia and clinicians must be aware of this adverse event. In view of the frequent adverse events of second-line anti-tuberculosis drugs, implementation of pharmacovigilance and monitoring of adverse events (aDSM) have been recommended by WHO. Furthermore, a team approach to the management of adverse events known as Tuberculosis Consilium has shown to be useful [5].

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Curative lobectomy in a patient with pulmonary arteriovenous fistula

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A 26-year-old Caucasian male was referred to the Sleep and Respiratory Disorders Centre with a past medical history of polycythemia and hypoxemia with chronic respiratory alkalosis. The patient complained of feeling dyspneic on exertion. On admission, the patient had a normal pulse (82/min) and was normotensive (115/75 mm Hg) with signs of peripheral cyanosis and digital clubbing. Normal vesicular sounds were heard on auscultation.

Prior to referral, the patient was admitted to a cardiac surgery clinic due to low exercise tolerance. Laboratory tests revealed hypoxemia with chronic respiratory alkalosis (pH = 7.45, pO_2 = 45 mm Hg, pCO_2 = 26 mm Hg, HCO_3 = 17.9 mM, SpO_2 = 84%) and polycythemia (Hb 19.2 g/dL; Hct 60.8%). An ECG showed no significant abnormalities. In echocardiography, a type 2 minor atrial septal defect (ASD) with a left-to-right shunt was found, as well as mitral valve prolapse with a preserved ejection fraction of 60%. Right-heart catheterization was performed and revealed a cardiac output of 4.74 L/min and a cardiac index of 2.67 L/min/m^2 with a pulmonary-to-systemic flow ratio of 1.16:1.00. Aortography showed no changes in the aortic arch and descending aorta, and no vascular malformations. Chest radiography did not show any anomalies. The patient was discharged from the hospital with a diagnosis of minor ASD left-to-right shunt that, apparently, did not explain his complaints or abnormal test results. Fortunately, lung scintigraphy was suggested on discharge. Curiously, upon a subsequent visit to his family doctor, the patient was ordered to exercise regularly to improve his fitness because deconditioning was suggested to be the culprit of his symptoms. However, the patient's repeated efforts to exercise resulted in a loss of consciousness.

Following these events, pulmonary function tests were performed at the Sleep and Respiratory Disorders Centre. In body plethysmography, lung static volumes were within normal limits, but carbon monoxide diffusion capacity ($D_{L,CO}$) was decreased (Table 1).

Later, the patient was readmitted to the hospital in order to undergo lung scintigraphy. It revealed a lack of perfusion in the 10th segment of the right lung. Chest radiography in a PA projection revealed a semi-circular shade at the Th12-L1 level in the right paravertebral line with dimensions of 4.5 × 3.0 cm which were not visible in the lateral projection. Based on the gathered evidence, a pulmonary arteriovenous fistula (PAVF) was suspected and the patient was referred to a thoracic surgery clinic where a decision was made to perform right anterolateral thoracotomy and right lower lobectomy. Prior to surgery, laboratory tests were ordered and confirmed polycythemia and hypoxemia with chronic respiratory alkalosis (Table 1). The surgery was uneventful.

One month after the surgery, a normalization of arterial blood gas (ABG) parameters was noted. Three months later, a follow-up D_{LCO} was performed and the results showed a significant improvement. Body plethysmography revealed decreased static lung volumes, which was expected (Table 1).

PAVF is a rare disorder characterized by the presence of an abnormal, non-capillary vessel connecting pulmonary arteries and veins. Over 80% of PAVF cases are reported to be congenital [1], and 70% of all cases are associated with hereditary hemorrhagic telangiectasia [2]. Secondary PAVF can be brought on by various factors such as trauma, infection, systemic amyloidosis, or mitral stenosis. Reported incidence of PAVF is 2–3 per 100 000 [1]. More than half of all patients with PAVF are asymptomatic and the condition is discovered during routine chest radiography. If signs and symptoms are present, they are usually nonspecific and vary from subtle to severe. They include dyspnea, hemoptysis, polycythemia, cyanosis, and digital clubbing, among others [3]. However, the most serious complication of PAVF is paradoxical embolization and as such, even the treatment of small fistulas is warranted [4]. Pulmonary angiography is considered to be the gold standard for the diagnosis of PAVF [5]. Treatment includes embolization (less invasive) or surgery (lower recurrence rate) [2].

Isolated PAVF poses a diagnostic challenge because, as was the case with our patient, their family history of arteriovenous malformations and/or telangiectasias can be negative. A right-to-left cardiac shunt should be

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addressed first as it is more prevalent. If this pathology is ruled out, PAVF should be considered. The diagnosis in our patient was based on indirect evidence including chest X-rays, pulmonary scintigraphy, and a lack of perfusion in one of the lung segments. All of these can be attributed to the phenomenon of perfusion stealing by the fistula. Paradoxically, lobectomy resulting in lower lung volumes markedly improved gas exchange and reversed chronic hypoxemia and polycythemia (Table 1).

Table 1. Comparison of ABG, $D_{L,CO}$ and bodyplethysmography parameters before and after the surgery (lobectomy)

Parameter	Before surgery	After surgery
ABG		
pH	7.42	7.43
pO_2 [mm Hg]	40	69
pCO_2 [mm Hg]	26	36
HCO_3 [mM]	16.9	23.8
SpO_2 [%]	77	94
$D_{L,CO}$-SB		
$D_{L,CO}c$ [mmol/min/kPa] (% of predicted)	7.03 (60)	7.92 (68)
VA [L] (% of predicted)	5.88 (87)	5.37 (80)
$D_{L,CO}c$/VA [mmol/min/kPa/L] (% of predicted)	1.20 (71)	1.48 (87)
Hb [g/100ml]	19.60	15.15
RV-He [L] (% of predicted)	1.09 (67)	1.51 (92)
TLC-He [L] (% of predicted)	6.01 (87)	5.50 (80)
Bodyplethysmography		
RV [L] (% of predicted)	1.63 (100)	1.55 (95)
TLC [L] (% of predicted)	6.90 (100)	5.84 (85)

ABG was determined in arterialized blood sample. ABG — arterial blood gas; $D_{L,CO}$ — transfer factor for carbon monoxide; $D_{L,CO}$-SB — single breath, carbon monoxide diffusing capacity of the lung; $D_{L,CO}c$ — $D_{L,CO}$ corrected for Hb; VA — alveolar volume; $D_{L,CO}c$/VA — $D_{L,CO}$ corrected for Hb and VA (Krogh Factor); RV-He — residual volume assessed by helium dilution method; TLC-He — total lung capacity assessed by helium dilution method; SpO_2 — peripheral blood oxygen hemoglobin saturation

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Coronavirus — preventing an occupational hazard among doctors

To the Editor

It all started in the last week of December 2019 when the World Health Organization (WHO) was alerted regarding several respiratory infections localized to a city in China. In January 2020, China confirmed a new virus, the Novel Coronavirus (nCoV), and the resulting disease was later termed COVID-19 (Coronavirus disease 2019). Since then, the spread, incidence, and death toll has taken a draconian leap with cases reported from over 4 continents with countries including China, Thailand, Hong Kong, Australia, Taiwan, Singapore, Malaysia, Japan, Canada, and Germany just to name a few. India has also reported over 100 confirmed cases as of March 16th, 2020 and the numbers are rising.

The reasons for COVID-19 quickly becoming a pandemic are numerous and include but are not limited to the following: a late reaction from public health agencies, an unrestricted flow of people across various borders, human to human spread even during incubation periods, and non-specific symptoms including fever, running nose, cough, breathlessness and headache.

Health care personnel including doctors are directly exposed during detection, isolation, and treatment of patients and contacts. In a resource constrained setting, this has the potential to turn into an occupational hazard [1]. The situation is further compounded by the fact that a vaccine may be at least one to two years away, even though trials for 35 vaccine candidates are planned for commencement. However, there are a few simple yet effective steps which can easily reduce this risk [2, 3].

Hand hygiene — it's in your hands now

In a time when the general public is mass buying masks and other preventive gear, it is the duty and right of every health care worker (HCW) to use them during the entire time spent in contact with patients or contacts. The advantages are many and include a decreased risk of infection, decreased risk of spread to one's family, and a decreased risk of spread to other patients. Hand sanitizers and hand washing practices followed in operation theatres and intensive care units have to be ensured and used in all areas of the hospital. Hand hygiene (HH) should be performed before and after any contact with patients. If alcohol-based hand rubs are used, the concentration should be ensured to include > 60% alcohol. If hands are visibly soiled, soap and water should be used [3–5].

Personal protective equipment — your very own security shield

Ensure that hand hygiene has been performed before donning (putting on) personal protective equipment (PPE). If a respirator (N95 respirator) is not available, a facemask should be used. For eye protection, goggles or face shields can be employed. It should be remembered that refractive-error correcting personal glasses do not qualify as eye protective gear. Further, care should be given towards not touching the eye/face protection or masks. Other important points to note regarding PPE include: not wearing a facemask inside an N95 respirator (as the former loosens it and renders it useless), doffing (removing PPE) in the order of glove, goggle, gown and mask, and performing

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immediate hand hygiene after doffing. In case of a shortage of facemasks or eye protection gear, centres for disease control and prevention (CDC) advises changing gloves and gown (in addition to HH) while keeping the same facemask and eye protection gear between patients which have the same diagnosis [4, 5]. Diagnostic specimens should preferably be collected by dedicated collection teams, and team members should wear a respirator in addition to PPEs.

Segregation of hospital staff and doctors — divide to prevent I

It may be difficult in view of limited staff, however, every attempt should be made to designate health-care teams dedicated to COVID-19 patient care during a single shift. Adequate training for PPE, sample collection, and waste disposal of personnel caring for COVID-19 patients should be mandatory for all HCWs. Further, it is important to have control checks on the shift hours and number of shifts to ensure adequate rest and avoid physician fatigue. As well, providing emotional support cannot be overstated in these testing times. Changing to hospital scrubs at the time of starting a shift may also contribute to prevention. HCWs should be advised to immediately report if they develop COVID-19 symptoms. Additionally, they should be actively monitored for these symptoms. The CDC has recently published interim guidance on the identification of the risk-level of HCWs [6].

Patient segregation — divide to prevent II

COVID-19 patients requiring admission should be provided with a single room or a common room with at least 1m distance between patients. Hospitals must ensure that the block handling COVID-19 patients has separate entry/exit points, ventilation, and waste management from the rest of the hospital to avoid spreading to health care workers not working in other areas of the hospital. All individuals entering the block should adhere to strict HH/PPE guidance. Suspected/confirmed COVID-19 patients should be advised to wear a facemask as a source control measure to decrease droplet and secretion emission. In case of a shortage of facemasks, patients should be advised to use tissues, handkerchiefs or cloth masks to cover their mouth and nose, in addition to maintaining strict cough etiquette and respiratory hygiene. Engineering controls like proper ventilation of patients rooms with at least 12 air circulation per hour can decrease droplet concentration and thus, decrease contamination of surfaces when these droplets settle. Either mechanical or natural ventilation can be employed but never both together. Further details can be accessed in WHO documents [2, 5].

Patient transport outside of the COVID-19 block should be minimized to reduce contact with non-COVID-19 medical staff. Lastly, early recognition and transfer of patients from non-Covid-19 wards or emergency wards should also be employed.

Additional precautions during aerosol generating procedures

Procedures like induction of sputum, bronchoscopy, nebulization, non-invasive ventilation (NIV), endotracheal intubation/extubation, open endotracheal suction, open tracheostomy etc. generate aerosols and droplets. In addition to the usual precautions, HCWs should use particulate respirators (e.g. N95 respirator). All patient procedures should be performed preferably in the patient's room. In case this is not possible, a dedicated room in the COVID-19 block should be used to avoid exposure of non-COVID-19 staff. Additionally, in the case of the latter, room surfaces should be disinfected between patients. Cheung *et al.* recommend using rapid sequence induction for COVID-19 patients wherever Invasive Mechanical Ventilation is required [7]. They do not recommend using NIV or high flow nasal oxygen (HFNO) until the patient is cleared of COVID-19. However, the strength of these recommendations needs to be assessed in planned clinical trials. A few more points to remember regarding invasive mechanical ventilation include using a viral filter during bag and mask ventilation, using a video laryngoscope in place of a direct laryngoscope, and using closed endotracheal suctioning.

Visitor check

A check on the number of visitors to patients admitted in the non-Covid-19 wards should not be forgotten during the community transmission part of a respiratory epidemic. An infected visitor can easily infect patients, other visitors, and HCWs. Ideally, all visitors should be actively assessed for Covid-19 symptoms.

Avoid public gathering — practice what you preach

All HCWs should practice universal preventive measures like avoiding crowded public places/

/transport, non-essential travel, etc. Practicing 'social-distancing' as much as possible could be an effective method in the prevention of airborne infection transmission [8].

Public education — preach what you practice

Hospitals should display posters and videos with instructions on HH and cough etiquette for visitors and patients alike throughout the whole hospital. In terms of limiting the community transmission phase of the epidemic, patients should be enquired about symptoms of COVID-19 at the time of appointment scheduling in order to ensure minimizing the exposure of non-COVID-19 HCWs. Additionally, since it may be difficult to screen patients at the time of first contact during emergency department (ED) visits, ED-HCWs should use preventive measures universally.

Looking to the future

We should take advantage of technology as much as possible. Smartphones and tablet computers can easily be employed for telemedicine and are already being put into practice at certain institutions around the world [5, 9].Tablet computers may be used to interview patients during their hospital visits or even before they arrive at the hospital. This can lead to easy and early segregation of patients.

The quest for a vaccine is already underway with some experts even calling for simultaneous clinical and pre-clinical trials. A Phase-I clinical trial is already underway [10]. Pharmacologists and molecular experts around the world are working hard to find a cure. Even so, we must not forget that such solutions will take time. When these do become available, national health administrators must ensure early access for HCWs.

Conclusion

The cocktail of high infectivity, a massive susceptible population, and the lack of a preventive vaccine or therapeutic drug has brought an unfortunate realization to long term fears of many health care experts. It has reminded us that the medical profession needs to push its limits further and faster than ever before to overcome this obstacle. As is the case with any pandemic, the healthcare infrastructure will be put through a cauldron and will be stretched to its thinnest limit. While the world measures us with their own yardstick, we must not forget that our own well-being is important to ensure the well-being of the world around us.

Conflict of interest

None declared.

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Amiodarone and diffuse alveolar hemorrhage

To the Editor,

Amiodarone, a common anti-arrhythmic drug, is well known for its pulmonary toxicity. Pulmonary toxicity is reported to occur at a rate of 1–5%, with this rate rising with an increased daily dose >200mg and drug accumulation over several years. Pulmonary toxicity by amiodarone may present just few days up to several years after the initiation of the drug [1]. Most common complications are chronic interstitial pneumonitis, organizing pneumonia, eosinophilic pneumonia, pleural effusion, acute respiratory distress syndrome (ARDS) and pulmonary nodules/masses [2]. Diffuse alveolar hemorrhage (DAH) is described as a rare amiodarone-related toxicity, so far reported in the form of scarce, very few case reports. We report 2 cases of amiodarone-associated DAH in order to highlight the significance of this complication.

Our first case was a 70-year-old female who was receiving amiodarone 200 mg per day due to supra-ventricular arrhythmias for the past 5 months. She was asymptomatic, but resting hypoxemia was noticed by her cardiologist on a regular follow-up visit, along with a new finding of pulmonary hypertension of 65 mm Hg. She had no evidence of heart failure; a high-resolution computed tomography scan (HRCT) then performed revealed ground-glass opacities distributed bilaterally in the upper and lower lung fields, along with mosaic attenuation (Figures 1A, 1B). Blood tests showed a decrease in hemoglobin levels by 1 g/dL. In bronchoalveolar lavage (BAL), 58% of alveolar macrophages were hemosiderin-laden, positive on Perls' stain. Autoimmunity testing and urine sediment were all negative. The patient responded well to 32 mg prednisone as a starting dose. Opacities cleared after 3 months of treatment and pulmonary hypertension completely subsided.

The second case was a 54-year-old male. He received amiodarone for the past 3 weeks at a dose of 600 mg per day to control atrial fibrillation. He complained of shortness of breath for the past 2 days and mild hemoptysis. Cardiac function was normal. HRCT scan showed hazy opacities bilaterally, ground-glass attenuation and increased interstitial markings extending at the lung periphery (Figures 1C, 1D). Hemoglobin was stable and oxygenation normal. BAL was macroscopically hemorrhagic and showed 100% hemosiderin-laden macrophages positive on Perls' stain. He had no evidence of vasculitis or hematouria. Apart from amiodarone, he was receiving apixaban for the past 6 years; however, the latest implemented drug (amiodarone) was considered responsible for DAH in this patient. He was started on methyl-prednisolone 600 mg/day for 5 days and then switched to prednisone 48 mg/day with good response.

Only seven cases of DAH due to amiodarone are reported in the literature [3–5]. In one case, diagnosis was established post-mortem, while the others responded to treatment with corticosteroids and removal of amiodarone. Reinstitution of amiodarone resulted in repeated DAH incidents. Pathogenetically, amiodarone is shown *in vitro* and *in vivo* studies responsible for interstitial pneumonitis by direct cellular cytotoxicity, accumulation of phospholipid complexes and activation of angiotensin converting enzyme leading to alveolar epithelial and macrophage cells injury and apoptosis; indirectly via an immunological

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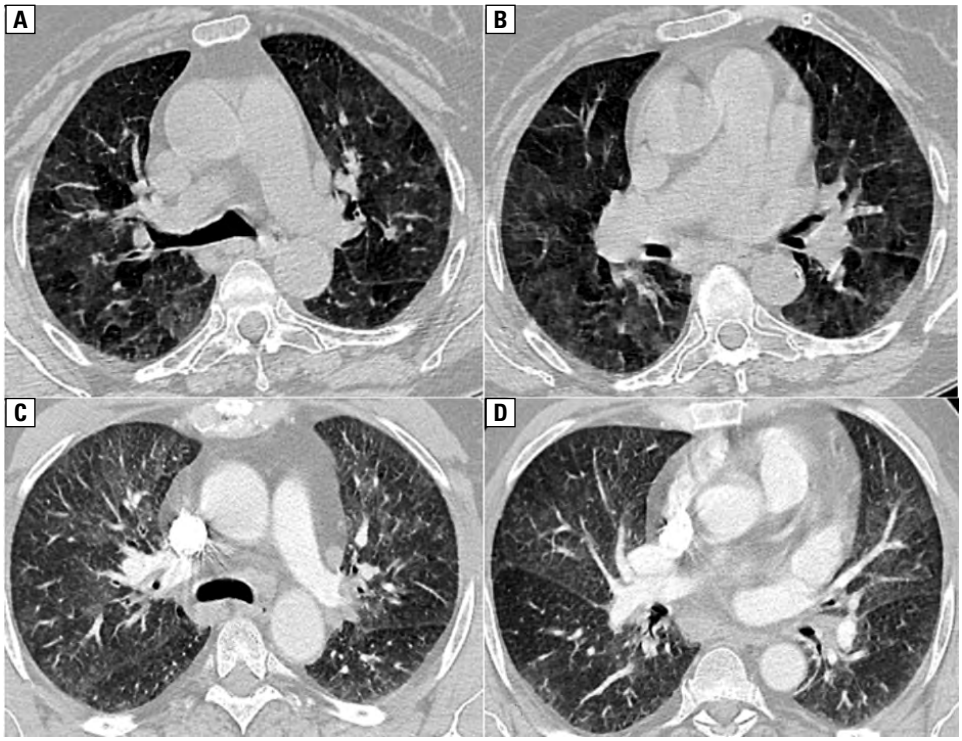

Figure 1. A, B. First case of female patient, ground glass and mosaic attenuation bilaterally; **C, D.** Second case of male patient, hazy ground-glass opacities located centrally bilaterally along with interstitial markings at the periphery

reaction characterized by CD8-T lymphocytosis that resembles hypersensitivity pneumonitis [6, 7]. In the case of amiodarone causing DAH, the underlying mechanism responsible is diffuse alveolar damage (DAD) [8]. DAD pathology associates either with ARDS or with DAH and is characterized by alveolar septa edema and hyaline membranes formation.

DAH in a patient receiving amiodarone who presents with symptoms or new radiology findings has to be differentiated from cardiogenic diseases and from the rest of amiodarone pulmonary toxicities. This is based on radiology and pulmonary function tests. Pulmonary function tests in DAH will not exhibit significant restriction, while diffusing capacity instead of decreased is found normal or increased. HRCT is characterized by ground-glass attenuation and mosaic pattern in adjacent normal lung tissue [9]. Diagnosis is established by BAL, which typically demonstrates hemosiderin-laden macrophages > 20% of total macrophages on Perls' Prussian Blue stain [10]. Lung biopsy is not required for the diagnosis of DAH. Both cases cases we present in this study had typical HRCT and BAL features.

Treatment strategies for amiodarone interstitial pneumonitis or DAH are based solely on case series. While for the former, drug removal and/or prednisone 0.5 mg/kg are proposed, it is possible that for the latter, pulse corticosteroids are required, as in other DAH etiologies.

In conclusion, as illustrated in our study, DAH due to amiodarone may not be as rare as it is believed. Since HRCT is not diagnostic, BAL should be performed to differentiate from other amiodarone-related or other disorders. Awareness is mandatory to quickly remove amiodarone and initiate corticosteroids.

Conflict of interest

None declared.

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