Pulmonology Journal

volume 26 / number 3 / May/June 2020

PULMONOLOGY JOURNAL

Previously **Revista Portuguesa de Pneumologia**

Original articles

volume 26 / number 3 / May/June 2020

ISSN 2531-0437

www.journalpulmonology.org

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Published every 2 months (6 issues per year).
www.journalpulmonology.org

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Anual	120.00 € (VAT not included)

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Indexed in: Science Citation Index Expanded (SCIE), Journal of Citation Reports (JCR), Index Medicus/MEDLINE, Scopus, EMBASE/Excerpt Medica
ISSN 2531-0437 (online)

Register 122.190 of Gabinete para os Meios de Comunicação Social

Printed in Spain
Printed in acid free paper
Legal deposit: B-20.760-2019

PULMONOLOGY

PULMONOLOGY

www.journalpulmonology.org

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EDITORIAL

The COVID-19 outbreak: From ''black swan'' to global challenges and opportunities

The world is in war against the pandemic of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2, the cause of COVID-19) and casualties (like in war: casualties) are increasing dramatically throughout the world. This disease may cause massive diffuse alveolar damage resulting in hypoxaemic acute respiratory failure (ARF) requiring, in a high percentage of cases, mechanical ventilation.[1–3] Behaviours and lifestyles are changing all over the world due to restrictions and lockdowns imposed by national governments to contain the diffusion.

This editorial is written in the middle of the war and only final and complete data will allow to draw solid conclusions. However, even with partial information the scientific community has the moral duty to raise questions and to try to provide interdisciplinary analyses and a prospective vision based on societal, clinical, economical, organisational, technological and ethical challenges which arise from this worldwide emergency.

With respect to the *organisation of the healthcare services*, in most countries the available ICU beds seem not to be properly sized to face the dramatic requirements. In the years, many governments around the world have reduced the *economic resources* to their healthcare systems and the current organisation of our hospitals may not fit present needs. The huge prevalence of the disease requires the distribution of patients according to different levels of interventions from simple medical supervision to oxygen (supplementation or High Flow), to non-invasive ventilation (NIV), to intubation while preserving safety of doctors and nurses.[4,5] Do we have the infrastructure for these purposes? Furthermore, the shortage of ICU beds and the higher mortality among oldest and weakest people pose dramatic ethical questions regarding the triage of the patients with possible need of ''Sophie's Choice''.[6]

Moreover, it is not only a matter of infrastructure, but also of *competences*. Health authorities launch appeals for more troops (doctors and nurses) and weapons (ventilators, masks and other tools). The physiology of COVID-19 induces ARF which requires high skills (e.g. to appropriately set the ventilator either for NIV or invasive ventilation). Do we have such skilled troops?

Furthermore, how to equip our troops? Other than to provide the healthcare professionals with the proper safety equipment, an appropriate use of available *technologies* is required.[7] Robots, Artificial Intelligence, Big Data Analytics, mobile apps and tele-medicine can be effective resources also in fighting pandemics. In fact, robots have a high potential in different infection-related applications, such as environment disinfection, medications and food delivery, assessment of clinical parameters and security checks.[8] For disease prevention autonomous or remote-controlled robots can be deployed for noncontact ultraviolet surface disinfection.[9] Mobile robots embedded with temperature measurement sensors (i.e., thermal sensors) and vision algorithms (for facial recognition) may lead to cost-effective, fast and effective screening of population in hospitals and public areas (airports, railway stations, ports, underground stations, etc.).[10] Robots may also be deployed to optimise nasopharyngeal and oropharyngeal swabbing in terms of increase of the process speed and protection of healthcare professionals. Another potential area of application is represented by delivery of samples and medicines to infected persons by means of drones or autonomous ground robots.[11]

Data collected by robots and mobile apps may be analysed by means of dedicated machine learning algorithms able to extract meaningful information for infection prevention and prediction, diffusion control, and diagnosis and treatment of infected persons.[12] Data can be transmitted to hospital information and security systems and can be matched with data collected by mobile applications to support contact tracing efforts amid the outbreak to limit the infection diffusion: however, data protection and data privacy regulations and laws must be guaranteed.[13,14]

Which lessons from the middle of the storm we are still fighting against? We highlight at least three precious lessons:

1. **The expense in the healthcare field must be considered an investment and not a cost.** We must build strong healthcare systems, able to work well in normal and to

https://doi.org/10.1016/j.pulmoe.2020.03.002

promptly react in extraordinary times. Let us think of the economic impact produced by the pandemic. How much of the economic losses might have been avoided by previous major investments in healthcare? What is the *opportunity cost* today of not having done so? We must not repeat the same mistake! Let's invest in research, innovation, technology, organisational models.

2. **In healthcare we must adopt a strategic approach.** There are some answers that even in emergency conditions can be provided (mask production through the reconversion of some production activities, etc.), others not. How to make available in a few days, skills that take years to be trained? We need to redesign the academic programmes for specializations we may require most in the next years (such as respiratory medicine). We need to scan the horizon, trying to forecast – analysing trends in the population, in the behaviours, in the lifestyles, and in the society, and taking into account the potential large-scale events (although unlikely) – future scenarios.
3. **We must have a common systematic approach in the response.** The international community must share ways of reacting and common protocols for managing these emergencies. A global effort is required in terms of organisation in collaborative networks, and of implementation of international emergency policies. Our countries seem unprepared: (a) absence of codified procedures and predefined plans to manage events like COVID-19 outbreak; (b) different behaviours between country and country in the way of managing the emergency.

As said, we are still in the middle of the storm, more lessons will be taught from this emergency before it is over. It will only depend on us if we will be able to learn them and to turn the ''black swan'' into a growth opportunity for the entire international community.

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Available online 11 April 2020

Pulmonol. 2020;**26(3)**:119–120

PULMONOLOGY

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EDITORIAL

Defining the prevalence of chronic critical illness

Since the survival rates of patients with life-threatening conditions admitted to intensive care units (ICUs), has significantly increased thanks to the improvements in medical care, interest in outcome measures has expanded to include survivors. ICU survivors frequently face a complex recovery trajectory; it is increasingly recognised that chronic critical illness (CI) is a medical condition in itself and these patients are prone to physical, psychological, and cognitive-related dysfunctions during the hospital stay and after discharge.[1,2] So far, more attention has been given to the physical conditions of critically ill patients, and this is particularly evident in respiratory settings. However, limited data are available to guide therapeutic choices in these subjects, and a univocal definition is not available yet to precisely identify chronic CI.

Marchioni and Colleagues report the results of an interesting observational prospective cohort study, exploring the prevalence and the development of chronic CI in a population of patients with de novo acute respiratory failure (ARF) admitted to a specialised respiratory intensive care unit (RICU).[3]

It is well known that about 20–30 % of patients hospitalised in RICUs for ARF will require a tracheostomy to be weaned off mechanical ventilation (MV). These tracheostomies will usually be removed before hospital discharge, although some patients who require prolonged MV keep the tracheostomy longer, experiencing long-term complications.[4]

In the study by Marchioni and Colleagues, chronic CI was defined as the condition of ICU survivors with both a hospital stay >8 days and with tracheostomy due to the need of MV > 21 consecutive days for at least 6 h/day.[3] In the cohort investigated, about one-third of the patients developed chronic CI during the RICU stay. The majority of them were affected by an acute exacerbation of chronic obstructive pulmonary disease (AECOPD), and septic shock; 45 %, and 19 %, respectively.

We must underline the study has some limitations. First, the use of the term 'de novo' respiratory failure is questionable. Although severe end-stage COPD patients, requiring home oxygen and/or ventilatory support, were excluded by definition, 'de novo' respiratory failure refers specifically to a respiratory failure occurring in subjects without prior history of chronic respiratory disease.[5]

Second, the definition of chronic CI in the study excluded patients with persistent signs of organ dysfunction (i.e., renal failure) or without any need for tracheostomy and prolonged MV. As clinicians interpreting these data, it is crucial to understand the target population. A multicentre research would have doubtlessly returned more robust figures. However, we must recognise that the interesting findings from this study highlight the need to understand ICU survivorship better and providing targeted support to this population.

An exciting aspect that emerged from the study by Marchioni and Colleagues is the possibility of enrolling patients, developing chronic CI resulting from de novo ARF, in a daily mobility programme during the RICU stay.[3] Due to profound deconditioning after critical illness, the importance of focusing on attempting to restore physical function is well established. Limiting the period of immobility and promoting early physiotherapy, are interventions directed at enhancing the recovery and preventing physical impairments and poor outcomes. In this context, The European Respiratory Society and European Society of Intensive Care Medicine recommends a comprehensive treatment for ICU survivors during all phases of the recovery pathway.[6] A particular target of rehabilitative interest is the diaphragm muscle, as pointed out by the results of the study which show that diaphragmatic dysfunction represents a risk factor in the specific population of difficult-to-wean patients.[3] This field deserves additional further investigation, aimed at establishing targets for physiotherapy among patients with chronic CI. These subgroups of subjects should be identified to provide them with different types of care, from acute respiratory interventions to early rehabilitation.

The evaluation of the diaphragm muscle with ultrasounds and the early detection of its dysfunction help to identify those patients who are most likely to benefit from an intensive respiratory rehabilitation programme. Such practice can contribute to reducing the adverse effects of critical illness and MV on the respiratory system; to restoring both physical and respiratory functions; preventing the need of MV and subsequent hospitalisation, resulting in improved patients' quality of life. An extensive body of evidence is

https://doi.org/10.1016/j.pulmoe.2019.11.003

already present in the literature, highlighting both appropriateness and feasibility of rehabilitation in acute respiratory settings.

Another exciting topic explored by Marchioni and Colleagues is the correlation between C-reactive protein (CRP) serum levels — and their increase during the first week of hospital admission —, and chronic CI.[3] Although specific inflammatory cytokines were not assessed, stratifying patients according to the level of CRP should help to identify those subjects most likely to benefit from rehabilitation.

On the other hand, high costs related to the hospitalisation of chronic CI patients in acute settings, have contributed and still are contributing, to the growth of the number of long-term acute care facilities — particularly in the United States.[7] Although these centres appear to offer a more favourable ratio between costs/quality and services/outcomes, Italy still seems to have a long way to go in this specific direction. In fact, due to the paucity of specialised post-acute facilities, chronic Italian CI patients are currently prone to the risk of prolonging their hospitalisation in acute settings. Prevalence of chronic CI — if related to rehabilitation — seems to be another factor that certainly deserves further analysis to plan appropriate and effective physiotherapeutic interventions.

Defining prevalence in chronic CI is a challenging effort because of varied definitions of the condition. Additionally, the increased risk of detrimental complications of chronic CI, even after stabilisation of the clinical conditions, necessitates the development of a universal and concordant definition.

The study by Marchioni and Colleagues[3] contributes to estimating the prevalence of chronic CI also encouraging rigorous randomised trials to explore further — through an interdisciplinary approach including physicians and physiotherapists — which are the specific interventions and the expected outcomes in patients with chronic CI.

Funding

None.

Conflicts of interest

The authors declare that they have no conflict of interest.

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14 November 2019
Available online 5 December 2019

Pulmonol. 2020;**26(3)**:121–122

PULMONOLOGY

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EDITORIAL

Hypersensitivity pneumonitis: Need for a better diagnostic evaluation

To the Editor

Given the variable prevalence of Hypersensitivity Pneumonitis (HP) across the world and the absence of widely accepted criteria for the definition of acute, subacute and chronic forms, characterization of different HP phenotypes is serious and a better classification of the disease stages is desirable, together with a better correlation of radiological and pathological features, to provide an adequate implementation of diagnostic and therapeutic approach.

Santos et al. retrospectively assessed clinical and diagnostic data of a cohort of HP patients from the North of Portugal.[1] 73,7% of patients had chronic form, agreeing with the literature data; in fact, the majority of patients with hypersensitivity pneumonitis who present to specialist centers have the chronic fibrotic form of the disease.[2] Chronic HP is characterized by a varied outcome and an accelerated rate of progression may be observed in a proportion of patients, similar to Idiopathic Pulmonary Fibrosis (IPF).[2] 12,9% of patients observed by Santos et al. had undetermined exposure and there was a significant association between chronic presentation and those patients with undetermined exposure. When a specific exposure cannot be clearly identified, differentiation of chronic HP can become more challenging; on the other hand, specific IgG signal merely reflects antigen exposure and a multidisciplinary diagnosis of chronic HP may not be associated with an improved outcome over patients diagnosed with IPF, as if they were the same disease.

Furthermore, in chronic HP sub-group, patients had most frequently ground glass, reticulation and honey combing patterns, while no differences were found regarding mosaic pattern and emphysema. It is worth pointing out this result as we know from the literature that computed tomography (CT) predictors of mortality in chronic HP includes reticular pattern, honeycombing and traction bronchiectasis; whereas mosaic attenuation (although the headcheese sign may be highly specific and moderately sensitive for a high-confidence diagnosis of fibrotic HP) is usually not predictive of outcome.[2] Chronic HP patients with more extensive fibrosis may progress to death with an IPF-like disease course.[2,3]

Finally, in an era in which the multidisciplinary team diagnosis is the accepted diagnostic standard, the number of patients with diffuse parenchymal lung diseases (DPLDs) undergoing lung biopsy is inevitably reduced as invasive complementary diagnostic tests are reserved only for cases in which the multidisciplinary evaluation is not enough to conclude a definitive diagnosis. In Santos's series, 29,2% of patients needed to perform histological analysis and a significant number of patients undergoing lung biopsy had chronic HP (for which differential diagnosis is considerably more challenging).[1] More than a third of lung biopsies were obtained by transbronchial lung cryobiopsy (TBLC); although additional research is needed to enhance knowledge regarding the role of TBLC in the diagnostic algorithm of chronic HP,[4] the recent Chest guidelines have further confirmed that TBLC contribution to the diagnosis of DPLDs obtained via multidisciplinary discussion appears to be good at least in experienced centers.[5]

In conclusion, Santos et al.[1] confirms the need for a more precise and complex diagnostic evaluation for hypersensitivity pneumonitis, especially the chronic fibrotic form, due to the differential diagnosis between this form and other fibrotic interstitial pneumonias with poor prognosis.

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1 January 2020
Available online 1 February 2020

Pulmonol. 2020;**26(3)**:123–129

PULMONOLOGY

www.journalpulmonology.org

REVIEW

Pneumonia mortality, comorbidities matter?

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Received 19 May 2019; accepted 24 October 2019

KEYWORDS
Hospitalized pneumonia mortality;
Comorbidities;
Risk model

Abstract Pneumonia remains one of the most important causes of mortality. In Portugal, it is the first cause of respiratory death, excluding lung cancer. This is a retrospective cohort study designed to seek for explanations, identifying the characteristics of patients and measure the impact of each one of them on the risk of dying from pneumonia. We analyzed demographic and clinical data of all patients (pts) with 18 years or older with pneumonia requiring hospitalization registered on the national health service registry of mainland Portugal over 2015. A total of 36366 patients corresponding to 40696 pneumonia hospital admissions in 2015 were analyzed. Most of the patients were very old (median age 80 years). Hospital mortality for pneumonia was higher among older (30,3% pts > 75 years). Pneumococcus is the more frequent bacterial isolate, reaching 41.2% of the isolates of total pneumonia cases. The frequency of pneumococcus decreases with aging; conversely, gram-negative bacteria and staphylococcus increase. Pneumococcus is more frequently identified in the winter, closely related to influenza outbreaks. Gram-negative bacteria are more prevalent during the summer months. Diabetes, obesity, COPD, and tobacco smoking are not associated with an increased risk of dying from pneumonia. Patients older than 75 years; living in a senior house; or with chronic renal disease, lung cancer, metastatic disease, mobility impairment, cachexia, dementia, cerebrovascular disease, and ischemic heart disease are at greater risk of dying from pneumonia. Comorbidities contribute decisively to the risk of dying from pneumonia in the hospital, regardless of their type or origin.

Introduction

Pneumonia has always been recognized as a terrifying disease. William Osler[1] in the nineteenth century made multiple descriptions of its different forms of presentation and

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https://doi.org/10.1016/j.pulmoe.2019.10.003

their relationship with frail, vulnerable, and elderly people.[1] Despite medical science, better health-care access, specialized units, sophisticated life-support systems, and even extracorporeal membrane oxygenation (ECMO), year after year, pneumonia mortality rates reach 30% of all respiratory causes of death in the Organization for Economic Cooperation and Development (OECD) countries report.[2] According to the last OECD report, Portugal had one of the highest rates of pneumonia mortality.[2] The figures on pneumonia occurrence and their distribution in the community (CAP) are almost impossible to determine accurately. Studying hospital admissions for moderate to severe pneumonias has been one way to understand the characteristics and the impact of this disease,[3,4] at least the most severe cases. In Portugal, pneumonia is certainly one of the main causes of death, representing 43.9% of all respiratory mortality, excluding lung cancer,[5] in 2015. This study was designed to seek explanations for these observations. We intend to analyze occurrence patterns, the distribution and the clinical characteristics of the patients with moderate and severe pneumonias requiring hospital admission to identify the main determinants of their morbimortality.

Objective

This study aims to evaluate the demographic distribution and clinical patterns associated with patients with pneumonia requiring hospitalization and their impact on mortality. Using all pneumonia cases admitted during 2015, we will create a risk model generating outcome estimates on the risk of dying for each individual patient.

Patients and methods

This is a joint project of the Portuguese Society of Pulmonology and the Priority Health Program for Respiratory Diseases of the Portuguese General Directorate of Health.

In this study, we retrospectively analyzed all patients requiring hospitalization for pneumonia in the National Health Service (NHS) hospitals in mainland Portugal in 2015. The Central Administration of the Portuguese Health System of the Ministry of Health (ACSS) keeps administrative, demographic, and clinical data of all admissions of NHS hospitals that cover the whole Portuguese resident population. The entirely anonymized patients' data, encoded from medical records by specialized codifiers, using the International Classification of Diseases, Ninth Revision Clinical Modification (ICD-9-CM),[6] was the data source for this study.

To make the study more patient-driven, a code number from each patient was generated, maintaining absolute anonymization, allowing us identify if a particular database entry, was a primary case or a readmission. The age, gender, geographical location of residence and hospital where the admission occurred, date of hospitalization up to the first five registered diagnoses by hospital admission, procedures, etiologic microbial information, previous surgery or traumatic event, readmission event, time of readmission, and outcome were the primary data we retrieved from the database. This database included up to 20 entries for diagnosis and procedures. We decided to include the first five registered diagnoses because the majority of the admissions had, at least, five entries for diagnosis.

To make diagnostic information usable, we developed a decision tree, creating diagnostic groups built from the diagnostic codes of the ICD-9-CM registered at each hospitalization (supplementary material). The diagnostic groups were constructed in a way that could easily be classified as comorbidities (diabetes, chronic renal failure, dementia, cachexia, cancer metastatic disease, obesity, alcohol/tobacco, etc.) or complications of the pneumonia if they were being classified as consequences of the disease (sepsis, respiratory failure, shock, empyema, acute renal failure, acid-base or hydroelectrolytic disturbances, etc.). After this classification procedure (supplementary material), the patients' characteristics (age group, gender, diabetes, asthma, COPD, cerebrovascular-disease sequels, dementia, mobility impairment of any cause, cachexia, ischemic heart disease, chronic renal failure, cancer of any site, metastatic cancer, lung cancer, tobacco smoking, obesity, living in a senior house, length of patient stay, airway aspiration, sepsis, sepsis and shock) and microbiologic data were analyzed.

Inclusion criteria

Patients aged 18 years or older admitted to hospital with a discharge primary diagnosis of pneumonia (ICD-9-CM 480–486, 487.0) or patients hospitalized with primary diagnosis of sepsis (ICD-9-CM 038-0389) if the diagnosis of pneumonia was, at least, present in the second of the five analyzed discharge diagnosis, were included.

Exclusion criteria

Patients younger than 18 years old or hospitalized with a discharge primary diagnosis other than pneumonia or a primary diagnosis of sepsis and other than pneumonia in the second diagnosis were excluded.

Statistical analysis

The clinical and demographic variables (see Table 1) were transformed into indicator variables and coded as0 if the characteristic was absent and 1if present. The median and age-group intervals presented age. The generated age-group variable ''age 75'' was dichotomous—1 if the patient was 75 or older and 0 if this condition was not met. Clinical variables (diabetes, chronic renal disease, shock, COPD, lung cancer, obesity, etc.) were coded 1 if present and 0 if absent. The outcome of the disease was coded 1 if death occurred and 0 if otherwise and the patient recovered from the disease. The interaction of all these variables and disease outcome was expressed as odds ratios (ORs). Some of them were also displayed as forest plots. A logistic regression was created to adjust the influence between the patient characteristics and the outcome.[7] The individual patient outcome estimates could be obtained by getting the sum of the products of the coefficient of each independent variable (β1-n) included in the logistic model (Fig. 2) and their code (X1-n)—1 if a particular characteristic was present and 0 if absent—and

Table 1 Patients & Characteristics & Case Fatalities.

Median Age 80 years	72.6 ± 22.4 (Mean ± STD)	Total (pts/adm) : 36.366/ 40,696
Age Groups (years)	Cases nº (%)	Hospital Mortality nº (%)
18–50	2,183 (6.0)	115 (5.3)
50–60	2,175 (6.0)	257 (11.8)
60–70	3,923 (10.8)	567 (14.5)
70–80	8,171 (22.5)	1,754 (21.5)
80–90	14,356 (39.)	4,291 (29.9)
> 90	5,558 (15.2)	2,140 (38.5)
Total	36,366	9,124 (25.1)
Characteristic (code)	Cases by Characteristic	Fatality by Characteristic nº (%)
Age 75 (1/0)	25,077 (69.0) / 11,298 (31.0)	7,608 (30.3) / 1,516 (13.4)
Gender (Male, 1 / Female, 2)	18,925 (52.0) / 17,441 (48.0)	4,909 (25.9) / 4,215 (24.2)
Readmission (1/0)	3,584 (9.9) / 32,782 (90.1)	1,093 (30.5) / 8,031 (24.5)
Diabetes (1/0)	5,892 (16.3) / 30,283 (83.7)	1,198 (20.3) / 7,901 (26.09)
Obesity (1/0)	912 (2.6) / 34,562 (97.4)	81 (9.0) / 8,915 (25.8)
Tobacco (1/0)	1,197 (3.3) / 34,978 (96.7)	100 (8.4) / 8,999 (25.7)
COPD (1/0)	4,337 (12.0) / 31,838 (88.0)	773 (17.8) / 8,326 (26.2)
Asthma (1/0)	727 (2.0) / 35,448 (98.0)	49 (6.7) / 9,050 (25.5)
Chronic Renal Disease (1/0)	4,106 (11.4) / 32,069 (88.6)	1,150 (28.0) / 7,949 (24.8)
Lung Cancer (1/0)	607 (1.7) / 35,568 (98.3)	244 (40.2) / 8,855 (24.9)
Cancer (1, yes / 0, no)	2,852 (7.8) / 33,514 (92.2)	1,060 (37.2) / 8,064 (24.0)
Metastatic Cancer (1/0)	644 (1.8) / 35,531 (98.2)	332 (51.6) / 8,767 (24.7)
Ischemic Heart Disease (1/0)	1,732 (4.8) / 34,443 (95.2)	499 (28.8) / 8,600 (25.0)
Cachexia (1/0)	410 (1.1) / 35,765 (98.9)	236 (57.6) / 8,863 (24.8)
Dementia (1/0)	3,410 (9.4) / 32,765 (90.6)	1,196 (35.1) / 7,903 (24.1)
Mobility impairment (1/0)	2,472 (6.8) / 33,703 (93.2)	928 (37.5) / 8,171 (24.2)
Senior House living (1/0)	925 (2.6) / 35,246 (97.4)	379 (41.0) / 8,720 (24.7)
Cerebrovascular disease (1/0)	2,388 (6.6) / 33,787 (93.4)	754 (31.6) / 8,345 (24.7)
Sepsis (1/0)	2,309 (6.4) / 33,865 (93.6)	1,008 (43.7) / 8,091 (23.9)
Sepsis and Shock (1/0)	400 (1.1) / 35,775 (98.9)	224 (56.0) / 8,875 (24.8)
Aspiration Pneumonia (1/0)	132 (0.4) / 36,234 (99.6)	81 (61.4) / 9,043 (25.0)
Hospital Stay ≤1 day/> 1 day (1/0)	2,207 (6.0) / 34,159 (94)	1,648 (74.7) / 74.76 (21.9)
Comorbidities (1/0)	30,565 (84.1) / 5,801 (15.9)	7,892 (25.8) / 1,232 (21.2)

Coded 1 if the characteristic is present, 0 if absent; pts (patients)/adm (admissions).

replacing this in the formula 1/1+e-(Constant-$\sum$(β1X1+ β2X2+... βnXn))obtain the individual probability of the risk of dying from pneumonia in a hospital. We used STATA Statistical Data Analysis 9.0[8] and BiostatXL MIX 2.0 (available at: http://www.meta-analysis-made-easy.com) to compute all these estimates.[9]

Results

A total of 36,366 patients corresponding to 40,696 pneumonia hospital admissions in 2015 (Table 1) were evaluable. Hospital mortality (case-fatality) was higher among patients with multiple admissions (30.6% vs. 24.5%).

Most of the patients were hospitalized from 1 to 15 days. Most of the patients (see Table1) were very old, 18,655 (47.1%) aged 80 or older. Hospital mortality increased sharply after the 60 s, reaching 38.5% after the 90 s.

Most of the patients admitted to hospital for pneumonia were male 18,925 (52%). Diabetes, COPD, and chronic renal disease (CRD) were present in 5892 (16.3%), 4337 (12%), and 4106 (11.4%) of the patients, respectively (Table 1). Cancer of any site was present in 2852 (7.8%) patients, including 607 (1.7%) with lung cancer and 644 (1.8%) with metastatic disease. Cardiac ischemic disease was an important comorbidity identified in 1732 (4.8%) patients. Dementia, serious mobility impairment, cerebrovascular disease, cachexia, and living in senior housing were identified in 3410 (9.4%), 2472 (6.8%), 2388 (6.6%), 410 (1.1%), and 925 (2.6%), respectively, of the pneumonia patients.

Only in 3220 (8.9%) of the pneumonia admissions was it possible to establish the etiology of the pneumonia (supplementary material). Pneumococcus was the microbiologic agent most frequently identified. Other bacteria such as miscellaneous gram-negative and multiresistant *Staphylococcus aureus* were also relevant isolates identified in the patients with pneumonia requiring hospitalization.

Most pneumonia admissions happened during the colder months, closely related to peak rates of influenza. However, the highest pneumonia hospital mortality rates occurred during the summer from July to September (supplementary material).

The distribution of microbiological isolates showed us the predominance of Pneumococcus during the entire year (sup-

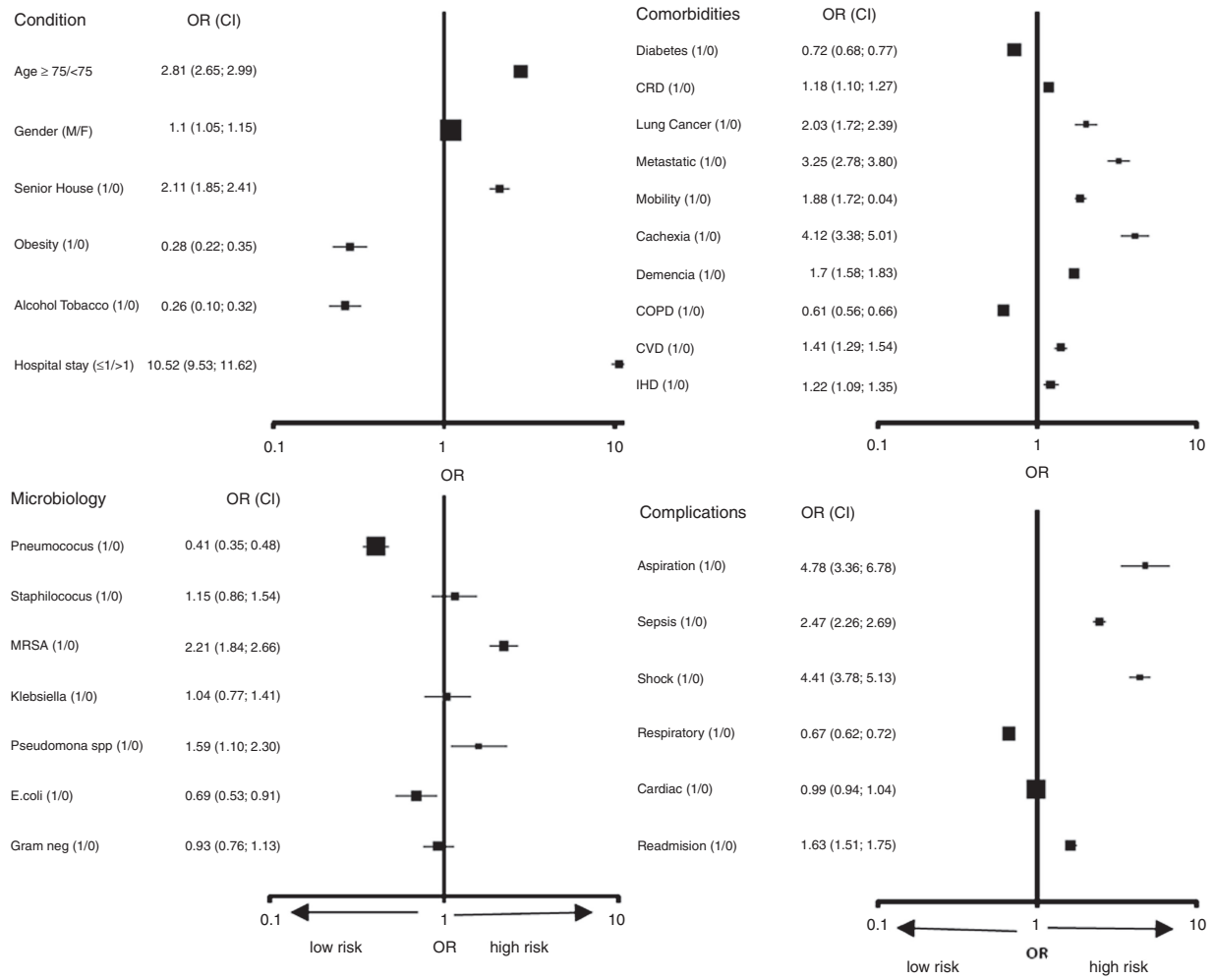


Fig. 1 Pneumonia Hospital Mortality – Sub-group analysis.
[OR (odds ratio), CRD (chronic renal disease), CVD (cerebrovascular disease), IHD (ischemic heart disease), MRSA (multiresistant *Staphylococcus aureus*), *E. coli* (*Escherichia coli*); hospital ≤1/>1 (days of hospital stay)]

plementary material), representing more than 40% of all virus and bacteria identifications. The microbiologic identifications had different distributions throughout the months of the year (supplementary material). There is a proportional increase of the identification rate of Staphylococcus and gram-negative bacteria species, from July to September (supplementary material). During winter (December, January, and February), most bacteria isolates were Pneumococcus. The microbiology seemed to be related to the age of the patients (supplementary material). Pneumococcus and atypical bacteria were more frequently identified in younger age groups. Conversely, among the elderly, apart from Pneumococcus, Staphylococcus, including multiresistant *Staphylococcus aureus* (MRSA) and miscellaneous gram-bacteria (supplementary material), plays the most important role. There is a clear downward trend of the frequency of pneumococcal isolates from the 50 s (supplementary material). The atypical bacteria were most frequently identified in the 18–50 age group, decreasing from there on. Older age groups were more prone to get pneumonia caused by gram-negative and Staphylococcus bacteria and be admitted to the hospital.

From all patients' characteristics, we selected the most frequent to evaluate their impact on pneumonia hospital mortality. These characteristics were classified into four groups: patient general condition, comorbidities, microbiology, and complications. We found a significant statistical association (Fig. 1) between the risk of dying and the condition of several of these patients, comorbidities, microbiology, or complications. Patients older than 75 years old; living in senior housing; with chronic renal disease, lung cancer, metastatic disease, mobility impairment, cachexia, dementia, cerebrovascular disease (CVD), or ischemic heart disease (IHD) are at greater risk of dying from pneumonia. Conversely, gender, obesity, tobacco consumption, diabetes, and COPD were not associated with an increased risk of dying for pneumonia patients admitted to hospital. The MRSA, Pseudomonas, aspiration pneumonia, shock, sepsis, hospital readmissions, and one day or even less of hospitalization are, on the contrary, significantly associated with an increased risk of dying from pneumonia. It is noteworthy that the identification of a Pneumococcus is associated with a lower risk of dying from pneumonia (Fig. 1).

Table 2 Pneumonia Hospital Mortality, Adjusted Risk Factors (nº cases - 35,470).

Characteristic	Odds ratio	P	IC (95%)
Gender (M-1/F-2)	0.79	0.00	0.73–0.83
Age $\geq$ 75/< 75	2.69	0.00	2.52–2.87
Metastatic D	4.15	0.00	3.49–4.93
Mobility Impairment	1.83	0.00	1.67–2.01
Cachexia	3.69	0.00	3.00–4.51
Dementia	1.43	0.00	1.32–1.55
Senior Housing	1.70	0.00	1-47–1.05
Tobacco	0.55	0.00	0.44–0.68
Sepsis	2.65	0.00	2.44–2.89
Shock	2.68	0.00	2.25–3.18
Obesity	0.46	0.00	0.36–0.58
COPD	0.69	0.00	0.63–0.75
Asthma	0.34	0.00	0.25-0.45
Chronic Renal D	1.20	0.00	1.11–1.29
Diabetes	0.84	0.00	0.78–0.90
Cerebrovascular D	1.31	0.00	1.19–1.44
Ischemic Heart D	1.28	0.00	1.14–1.43
Readmission	1.18	0.00	1.09–1.28

After adjusting for the risk of death from the patients' characteristics, we identified that individuals in the age group over, or equal to 75 years old, who were living in senior housing, or had previous cerebrovascular disease, cardiac or renal chronic disease, dementia, cachexia, mobility impair, neoplastic metastatic disease, or upfront sepsis were significantly associated with an increased risk of dying from pneumonia in the hospital (see Table 2).

Using these estimates it was possible to compute a predictive model for patients with pneumonia which required hospital admission, quantifying their individual risk of dying, from this disease (Fig. 2).

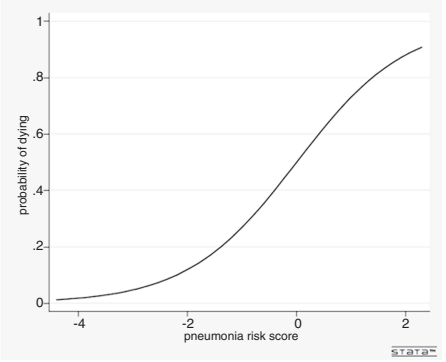
Discussion

The main findings of this study led us to understand that an overwhelming majority of patients dying from pneumonia had one or more severe chronic diseases, which certainly contributed to the outcome. According to a recent publication of health data in the OECD, the life expectancy at age 65 in Portugal is 17 years for males and 23 for females.[2] However, more than half of that time is spent struggling with some sort of disease.[2] Despite treatment conditions and full access to health services by the Portuguese population, we verified, year after year, an increase in mortality due to pneumonia in Portugal.[2] We are absolutely convinced that this entire chronic disease burden could affect the pneumonia figures, not only in incidence but also in mortality. This study's main motivation was to look for explanations of such a situation and try to prepare future strategies to minimize the impact of this disease on mortality. Portugal has a centralized system of registration for all hospital admissions involving all NHS hospitals. Beyond the administrative data, we have access to fully anonymized clinical data. Usually, these data are not patient-driven; only admission episodes are available for analysis. However, generating a code number from the real patient identification was possible, maintaining total patient anonymization and allowing us to identify the readmissions and, therefore, to study patients instead of admissions.

This study has several limitations. This is a retrospective database analysis, so there is the possibility of information bias and misclassification. Adjustments were made for demographics, clinic and microbiologic variables. However, some important patient characteristics (pneumonia severity PSI or CURB65 classification, mechanical ventilation-vasopressors; vaccinations; empiric antibiotics; suitability of antibiotic choice with bacterial isolates; co-infections; specimen isolates and bacteremia), could not be analyzed because they were not present in NHS database. Despite all these limitations, this study evaluates a large number of patients, representing all the NHS patients from an entire country, hospitalized for severe pneumonia, over the course of one year.

We studied 36,366 patients which corresponded to 40,696 hospital admissions in 2015. The main targets of this study were patient characteristics, particularly comorbidities and their influence on the outcome. The great majority of the patients with severe pneumonia were elderly, in total 30,565 (84.1%) (see Table 1), and had at least one chronic long-standing disease. Similar to other studies,[10,11] the elderly were more susceptible to getting sick with pneumonia and being hospitalized.

Most of the pneumonias occurred during winter; however, the deadliest happened from July to September. Host factors have been increasingly considered as decisive for the outcome of the patients with severe pneumonia.[11,12] In our study, advanced age, dementia, mobility impairment, metastatic cancer, and living in senior housing were some of the more important prehospitalization conditions and comorbidities of the patients hospitalized in 2015 with severe pneumonia in Portugal. As in other studies,[13–17] these patients' characteristics have been shown to be a determinant for the increased risk of dying from pneumonia. Pneumonia after viral infections[18] is a common occurrence,



Total Score	Death Risk	Impact
< –3	5%–10%	Low
≥ –3 – < –1	10%–30%	Medium
≥ –1	> 30%	High

Characteristic	Code	X	Coefficient	Total
Gender (M-1/F-2)	1/2		-0.22	
Age ≥ 75/< 75	1/0		0.99	
Metastatic Disease	1/0		1.42	
Mobility impairment	1/0		1.83	
Cachexia	1/0		1.30	
Dementia	1/0		0.36	
Senior House	1/0		0.53	
Tobacco	1/0		-0.59	
Sepsis	1/0		0.97	
Shock	1/0		0.98	
Obesity	1/0		-0.76	
COPD	1/0		-0.36	
Asthma			-1.07	
Chronic Renal D	1/0		0.18	
Diabetes	1/0		-0.17	
Cerebrovascular D			0.27	
Ischemic Heart D	1/0		0.24	
Readmission	1/0		0.16	
Constant				-1.73
Total Score				

Fig. 2 Hospital pneumonia mortality: risk model.

this is explained, although not completely understood, by association with the host biological factors,[19] disruption of the mucosal barrier, and ciliary malfunction caused by a previous viral infection impairing the viral or bacterial clearance, facilitating bacterial colonization and, subsequently, the pneumonia.[18,19]

We identified bacterial isolates in 3220 (8.9%) of our patients. The distribution of the microbiological isolates by age groups had shown that among the patients hospitalized for pneumonia, the frequency of pneumococcus decreased sharply with age; younger patients had more frequent pneumococcus isolates than older patients. This observation could explain the apparent ''protective effect'' of the association between pneumonia with pneumococcus identification and the lower risk of death.

A plausible explanation of this observation could be that pneumococcal pneumonia is in our series more frequent in younger patients and, therefore, less likely to have major comorbidities that can increase the risk of dying. The distribution of bacterial isolates throughout the year shows that during summer, there is proportionally, more frequent identification of gram-negatives and staphylococcus than in other months. This situation can be related to the excess mortality observed during those months. Regardless of the type, severe pneumonia determining hospitalization, the risk of dying seems to be more related to the comorbidities than with the etiology. On the contrary, probably the general condition of the patients and their susceptibilities also deeply influence the microbial agent. Diabetes, COPD, obesity, and tobacco smoking are usually related to pneumonia and the risk of hospitalization.[20,21] However, in our study, they are negatively associated with the risk of dying from pneumonia.

Chronic renal failure and ischemic heart disease are two important risk factors of pneumonia death. We observed from our data the association of the risk of death and the length of hospital stay. The risk of death for pneumonia was higher for short stays, decreasing afterward. This observation led us to consider that a significant number of patients might have been diagnosed and started the treatment later in the course of the disease. If we examine the age and the comorbidities of most of the patients and the possibility of many of them living alone, the latter hypothesis can be convincing.

Conclusion

Regardless of the importance of the etiology and the treatment, the characteristics of the host might be key to explaining the high mortality due to pneumonia we found in our data. Elderly patients and the high proportion of major comorbidities such as dementia, cachexia, severe mobility impairment, metastatic cancer, chronic renal disease, ischemic heart disease, cerebrovascular disease, sepsis on hospital admission and senior house living, are independent risk factors of dying from pneumonia in a hospital

Authors contribution

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Financial support: no financial support involved

Manuscript writing: All authors

Final approval of manuscript: All authors

Conflicts of interest

Authors have no conflicts of interest to declare, regarding the present work

Acknowledgments

The authors gratefully acknowledged Professor Fernando Araujo, Secretary of State of Health of Portuguese Government and Dr. Ricardo Mestre, Central Administration of

Health System (ACSS) by the encouragement and assistance in accessing national databases, making this work possible.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pulmoe.2019.10.003.

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Pulmonol. 2020;**26(3)**:130–137

PULMONOLOGY

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ORIGINAL ARTICLE

Hypersensitivity pneumonitis: Main features characterization in a Portuguese cohort

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Received 28 June 2019; accepted 15 September 2019
Available online 29 October 2019

KEYWORDS
Hypersensitivity pneumonitis;
Interstitial lung disease;
Antigens exposure

Abstract Hypersensitivity pneumonitis (HP) is an interstitial lung disease (ILD) which varies in prevalence across the world, depending on disease definition, diagnostic methods, exposure type and intensity, geographical environments, agricultural and industrial practices, and host risk factors. This study aimed to deepen knowledge about HP's clinical characteristics, diagnosis and functional and imaging features in a cohort of HP patients from the North of Portugal. To achieve this goal, a retrospective assessment of the clinical and diagnostic data was carried out, and patients were classified and compared according to disease presentation (acute, sub-acute and chronic HP forms).

Of the 209 HP patients included (mean age 58.3 ± 16.0 years), 52.6% were female and 73.7% presented a chronic form. Most patients had prior exposure to birds (76.6%). Dyspnoea and cough were the most frequently experienced symptoms, but no statistically significant differences were found between groups ($p = 0.089$, $p = 0.418$, respectively). Fever was most common in acute HP form ($p < 0.001$). The most common patterns found in Chest CT were ground glass

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https://doi.org/10.1016/j.pulmoe.2019.09.004

(p = 0.002) in acute/subacute presentation, and reticulation (p < 0.001) in chronic form, while mosaic attenuation, although was also frequently observed, no statistically significant differences were found between groups (p = 0.512). The most common functional pattern was restrictive (38% of patients, 73.7% with chronic HP form). Bronchoalveolar lavage lymphocytes were higher in acute and subacute forms although not reaching statistical significance (p = 0.072), with lowest CD4/CD8 ratio (p = 0.001) in acute forms.

Thus, given the significant disease heterogeneity, further studies with different populations and ambient exposures are needed to achieve a better stratification of the exposure risk, to provide proper implementation of avoidance methods and a precise diagnostic and therapeutic approach.

Introduction

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is an interstitial lung disease (ILD) triggered by an exaggerated immune response to the inhalation of a wide variety of antigenic particles found in the environment.[1,2] The most common antigens include moulds, bacteria, protozoa, animal (mostly bird) proteins and even low-molecular-weight chemical compounds. In addition, certain drugs may also cause HP, as a non-inhalational variant. It is worth mentioning that, HP may potentially arise in any work or home environment, where bacteria and moulds grow or birds are kept.[2]

HP prevalence varies considerably across the world, depending on the disease definition, diagnostic methods, type and intensity of exposure, geographical conditions, agricultural and industrial practices, and host risk factors. However, there is no consistent and standardized epidemiological approach to assessing the different HP forms.[3] Over the years, as the disease progresses, it may lead to chronic respiratory failure, resulting from well-established pulmonary fibrosis[4] or even pulmonary emphysema,[5] thereby conferring a potentially serious disease status on this entity.

HP is conventionally classified into acute, subacute and chronic forms, although, to date, there are no widely accepted criteria for distinguishing the various forms.[1,6,7] Moreover, there is little information on latency between exposure and symptoms onset, and it is uncertain whether they represent different stages of the disease.[6]

Current techniques, despite being in great demand and evolving, are not well standardized and, despite the efforts of experts to establish diagnosis based only on clinical data,[8] the definitive diagnosis must be supported by additional tests (i.e., laboratorial, radiologic and histology assays), some of them of an invasive nature. In fact, disease development and clinical presentation are influenced by several factors, such as the nature and number of inhaled antigens; intensity and frequency of exposure, and even the host's immune response, likely to be determined by a genetic background. Indeed, genetic susceptibility may explain how, after exposure, some individuals develop the disease, while others are only sensitized, but remain healthy, and others are not even sensitized.[2,6]

In this context, based on the above highlighted aspects, this study aims to deepen knowledge about HP's clinical characteristics, diagnosis and functional parameters in a Portuguese cohort of HP patients. To achieve this goal, patients were classified and compared based on the disease presentation status (acute, sub-acute and chronic HP forms).

Materials and methods

Study population

A retrospective analysis of medical records of patients diagnosed with HP followed in ILD outpatient clinic in Centro Hospitalar Universitário de São João, Porto - Portugal was performed over a period of 10 years (2007–2016). Individuals older than 18 years diagnosed with HP were included in this study, making a total of 209 patients. All patients were discussed and their diagnosis was established in the multidisciplinary team (MDT) meeting.

All relevant clinical data from the first appointment were collected, including patient demographics, medical history, smoking habits, occupational history, environmental antigen exposure history, pharmacological treatments, laboratory data, imagological features, pulmonary function data and thoracic pathology findings. This study had the approval of local Ethics Committee (Centro Hospitalar Universitário de São João, Porto – Portugal).

Diagnostic criteria

According to the clinical reports, exposure was classified into five categories: birds, moulds, cork, isocyanates and unknown (when the relevance of any antigen could not be established). HP diagnosis was considered in the presence of compatible exposure, clinical features (dyspnoea or cough, sputum production, asthenia, fever, weight loss, and absence of an alternative diagnosis), chest high-resolution computed tomography (HRCT) typical features, and high lymphocytic alveolitis in bronchoalveolar lavage (BAL). Patients without definitive diagnosis after the previous diagnostic approach were submitted to transbronchial lung cryobiopsy and/or surgical lung biopsy (SLB) after

an individualized assessment of the indication. As not all patients underwent the same complementary tests, the results are presented according to the number of patients who were submitted to each procedure.

HP subtypes considered were acute, subacute and chronic forms. Acute form was defined as symptoms within a few hours after antigen exposure, and regression within days after removal of exposure. The subacute form was considered when progressive symptoms occurred over days or weeks. Chronic form was characterized as persistent symptoms in patients with long or persistent exposure.[9] The imaging and histology data were also valuable, namely when fibrotic features were identified as their association with chronic presentation.

Thoracic high-resolution computed tomography

All scans were obtained using a high-spatial-resolution reconstruction algorithm and, for each patient, 1- and 3-mm thick slices were obtained. Two thoracic radiologists with experience in ILD evaluation reviewed all the images for the presence or absence of abnormalities: reticular pattern, traction bronchiectasis, honeycombing, centrilobular nodules, ground glass infiltration, mosaic attenuation and emphysema.[10–12] The combination of centrilobular nodules, ground-glass infiltration, mosaic attenuation, and middle and/or upper lobe distribution with or without fibrotic changes, were considered typical for HP.

Bronchoalveolar lavage fluid and flow cytometry

Bronchoalveolar Lavage (BAL) fluid was performed following the European Respiratory Society recommendations.[13] A total of 200 mL (four aliquots of 50 mL) of sterile saline solution were instilled into the bronchial tree and gently aspirated after each instillation.

The last three recovered samples were homogenized and analysed for total cellular counts (Neubauer chamber) and viability (trypan blue exclusion) was determined. A total of 500 cells were counted on Wright-Giemsa stained cytospin slides. The samples were processed by routine flow cytometry analysis with the following combinations of monoclonal antibodies: anti-CD45-PerCP-Cy5.5 (Clone2D1), anti-CD4-FITC (Clone SK3), anti-CD8-Pe (Clone SK1), anti-CD3-APC (CloneSK7) (all BD Biosciences, San Jose, CA, USA).[14] Samples were run through a BD FACS CaliburTM flow cytometer (BD Biosciences, San Jose, CA, USA) and analysed using BD CellQuest software (BD Biosciences, San Jose, CA, USA), with the acquisition of a minimum of 10,000 events. Lymphocytes were distinguished based on forward (FSC) versus side (SSC) scatters and additional gating was applied using SSC versus CD45, CD3, CD4 and CD8. All values were scored as percentages of lymphocytes.

Lung biopsies

Transbronchial cryobiopsy (TBLC) was performed using a combination of rigid (tracheoscope 14 mm, Karl Storz) and flexible (Olympus BF-XT40) bronchoscopy, under general anaesthesia with manual jet ventilation (working pressure of approximately 2 bar), using a flexible cryoprobe (90 cm with a 2.4 mm diameter; Erbokryo, Erbe, Germany). Biopsies were taken under fluoroscopic guidance from an optimal distance between the probe and the thoracic wall of 10–20 mm in different segments of the two different lobes of the same lung, usually three biopsies from lower lobe and two biopsies from the upper lobe. The probe was cooled to $-85\,^{\circ}C$ with nitrogen oxide for approximately 5–6 sec.

Transthoracic biopsies were performed by a senior interventional radiologist using CT fluoroscopy. The biopsies were taken with an 18-gauge automated cutting needle after administration of local anesthesia with a subcutaneous injection of 5 mL of 2% lidocaine. In most biopsies, only one specimen was obtained, with a maximum of 2 specimens/procedure.

Surgical lung biopsy was performed on patients intubated with a double lumen endobronchial tube under general anesthesia through video-assisted thoracoscopic surgery-Endopath ETS 45 mm endoscopic linear cutter (Ethicon Endosurgery, Cincinnati, OH- which allowed access to segments of different lobes in order to obtain multiple biopsy specimens. The biopsies sites were decided based on HRCT scan.

Biopsy samples were independently observed and evaluated by two pathologists. HP diagnosis was based on the presence of histology typical features such as centrilobular or/and perilobular fibrosis, bridging fibrosis, centrilobular fibroblastic foci, granulomas, mononuclear chronic interstitial inflammation and organizing pneumonia. If two or more findings were present, the biopsy was considered as high confidence for HP diagnosis; if only one feature was present, it was considered as low confidence.

Pulmonary function testing

Pulmonary function testing (PFT) was done according to a standardized protocol. Static lung volumes were measured using the plethysmography method, and the lung diffusion capacity of CO (DLCO) using the single breath-hold method.[13] A restrictive ventilatory pattern was defined as forced vital capacity (FVC) <80% of the predicted value with forced expiratory volume in the first second (FEV1)/FVC ratio >80% in the absence of a static lung volume study, together with a total lung capacity (TLC) <80% of the predicted value. An obstructive ventilatory pattern was established based on FEV1/FVC ratio <70%. The concomitant presence of characteristic functional criteria for both patterns was defined as a mixed ventilatory pattern. DLCO was considered to be decreased by <75% of the predicted value.

Determination of specific IgG antibodies

The presence of specific IgG antibodies (precipitins or enzyme-linked immunosorbent assay [ELISA]) was noted only when the relevance of a suspected exposure was uncertain. Precipitins were evaluated by Radial Immunodiffusion (RID) from Microgen Bioproducts Ltd. The test relies on the principle of double gel diffusion. When soluble antigens and homologous antibodies are placed in adjacent wells, cut into suitable diffusion media, they diffuse into each other

and produce visible precipitation lines along the interface of optimal relative concentrations.

Statistical analysis

Categorical variables were described as absolute values and relative frequencies and continuous variables as mean and standard deviation (SD), or minimum and maximum values, if appropriate. The relationship between categorical variables was determined using Pearson's chi-square. Comparisons among three or more groups were performed using one-way analysis of variance (ANOVA). All data were analysed using Statistical Package for the Social Sciences (SPSS, IBM Corp., USA) software, version 25.0, with alpha set at 0.05.

Results

Of the 209 HP patients included, with a mean age of 58.3 ± 16.0 years, 110 (52.6%) were females and 99 (47.4%) males (Table 1). A low percentage of smokers was found (4.9%), most of the patients were (n = 149, 73.4%) non-smokers. Median prior exposure time was 10 (range: 0.2–50.0) years. Exposure apparently exclusively to birds was found in 160 (76.6%) patients, of whom 89 (55.6%) were female. The main types of birds to which patients were exposed included pigeons (n = 91), canaries (n = 41), parakeets (n = 29), turtledoves (n = 13) and cockatiels (n = 9). Among patients with bird exposure (n = 160), 57.5% were only exposed to one type of bird and 42.5% to two or more. The rest had been exposed to moulds (n = 29, 13.9%), cork (n = 11, 5.3%), isocyanates (n = 4, 1.9%), and 27 (12.9%) patients had undetermined exposure (Tables 1 and 2).

In terms of clinical presentation, 154 (73.7%) patients (80 females, 74 males) presented chronic HP form, of whom 114 (71.2%) had exposure to birds (Tables 1 and 2). Acute form was found in 31 (14.8%) and subacute in 24 (11.5%) patients. There is a significant association between chronic presentation and those patients with undetermined exposure (p = 0.025), while the number of birds to which patients were exposed did not have any particular association with clinical presentation (p = 0.297).

Table 3 shows the data relating to respiratory symptoms. The most frequently reported respiratory complaints were dyspnoea (n = 179, 89.5%), followed by cough (n = 155, 77.5%), sputum (n = 69, 34.5%), asthenia (n = 50, 25.0%), weight loss (n = 32, 16.0%) and fever (n = 25, 12.5%). Fever was most common among those with the acute form (p < 0.001). No significant differences were found for other symptoms.

In the chest HRCT evaluation (Table 3), besides the association between ground glass with acute/subacute presentation (p = 0.002), chronic form HP patients most frequently had reticulation (p < 0.001) and honeycombing (p = 0.002) patterns. No statistically significant differences were observed when mosaic pattern (p = 0.512) and emphysema (p = 0.125) were considered.

The distribution of the various ventilatory patterns, as assessed by lung function tests, is shown in Table 4. Lung Function Tests were performed on all patients with clinical condition, most of them presenting a restrictive pattern (n = 76, 38.0%), 54 patients (27.0%) showed an obstructive pattern, 3 (1.5%) a mixed pattern, and 62 (31.0%) had normal lung volumes. Five patients with a severe form of the disease failed to perform the tests properly. In general, patients with chronic HP tended to have lower RV% (p = 0.013), DLCO% (p = 0.004) and DLCO/VA% (p = 0.039) values. Regarding the blood gases parameters, PaO_2 and $PaCO_2$, no statistically significant differences were found between groups (p = 0.322 and p = 0.261, respectively).

Patients with chronic HP presented lower BAL total cells count (p = 0.015), while eosinophils (p = 0.003) and CD4/CD8 ratio (p = 0.001) were lower in acute HP form. Moreover, although %lymphocytes were not statistically significant (p = 0.072) between groups, there was a slightly higher percentage in the acute and subacute forms. In fact, among chronic HP patients, only 79 (61.7%) of them had %lymphocytes >30% and 39 (25.3%) did not even have lymphocytosis.

IgG antibody analysis, a marker of exposure, was only used for patients without clear exposure. Thus, of the 59 patients tested, 86.4% were negative and 13.6% (n = 8) positive for birds.

Table 1 General characteristics of HP patients.

Characteristics	HP patients
Age (years), mean ± SD (min – max)	58.3 ± 16.0 (18–89)
Gender, n (%)	
Female	110 (52.6)
Male	99 (47.4)
Smoking status, n (%)	
Smokers	10 (4.9)
Non-smokers	149 (73.4)
Ex-smokers	44 (21.7)
Prior exposure (years), mean±SD	13.3 ± 11.6
Types of exposure, n (%)	Birds: 160 (76.6)
	Moulds: 29 (13.9)
	Cork: 11 (5.3)
	Isocyanates: 4 (1.9)
	Undetermined: 27 (12.9)
Patients who reported bird exposure, n	Pigeons: 91
	Canaries: 41
	Parakeets: 29
	Turtledoves: 13
	Blackbird: 8
	Goldfinches: 7
	Parrots: 8
	Cockatoos: 5
	Chickens: 17
	Mandarin duck: 2
	Cockatiels: 9
	Lovebirds: 6
Clinical presentation, n (%)	
Acute form	31 (14.8)
Subacute	24 (11.5)
Chronic form	154 (73.7)

Table 2 Clinical presentation based on exposure types.

Types of exposure	HP patients	Clinical presentation			*p* value
		Acute	Subacute	Chronic	
Overall exposure					**0.025**
Birds	160 (76.6)	24 (77.4)	22 (78.6)	114 (66.3)	
Cork	11 (5.3)	5 (16.1)	0	6 (3.5)	
Moulds	29 (13.9)	1 (3.2)	3 (10.7)	25 (14.5)	
Isocyanates	4 (1.9)	1 (3.2)	1 (3.6)	2 (1.2)	
Undefined	27 (12.9)	0	2 (7.1)	25 (14.5)	
Birds exposure					0.297
1	92 (57.5)	13 (54.2)	16 (72.7)	63 (55.3)	
≥2	68 (42.5)	11 (45.8)	6 (27.3)	51 (44.7)	

Bold: $p < 0.05$

Table 3 Patients' symptomatology and chest CT findings, according to clinical presentation.

	Acute	Sub-acute	Chronic	Total	*p* value
Symptoms, n (%)					
Dyspnoea	30 (96.8)	18 (78.3)	131 (89.7)	179 (89.5)	0.089
Cough	25 (80.6)	20 (87.0)	110 (75.3)	155 (77.5)	0.418
Fever	16 (51.6)	2 (8.7)	7 (4.8)	25 (12.5)	**<0.001**
Asthenia	12 (38.7)	5 (21.7)	33 (22.6)	50 (25.0)	0.158
Sputum	11 (35.5)	11 (47.8)	47 (32.2)	69 (34.5)	0.339
Weigh loss	6 (19.4)	3 (13.0)	23 (15.8)	32 (16.0)	0.812
Chest CT findings, n (%)					
Ground glass	23 (76.7)	22 (91.7)	89 (58.6)	134 (65.0)	**0.002**
Mosaic pattern	8 (42.3)	8 (63.2)	57 (37.5)	73 (40.6)	0.512
Reticulation	2 (6.7)	3 (12.5)	83 (54.6)	88 (42.7)	**<0.001**
Emphysema	1 (3.2)	0	16 (10.5)	17 (8.3)	0.125
Honeycombing	0	0	31 (20.4)	31 (15.0)	**0.002**

Bold: $p < 0.05$

Of all patients included, 61 (29.2%) needed to perform histological analysis to obtain a clear diagnosis, of whom 33 (54.1%) needed surgical lung biopsy, 20 (32.8%) TBLC and 8 (13.1%) transthoracic biopsy. These procedures were performed only on chronic patients with overlap imaging features with other fibrotic ILDs. Thus, according to the criteria described above, of the 33 SLB, 30 (90.9%) were considered as high confidence for HP diagnosis, while in TBLC, 14 (70%) were also considered as high confidence, and with respect to transthoracic biopsy, only 1 (12.5%) met the high confidence criteria.

Discussion

Since HP results from the inhalation of an antigen to which a patient has been previously sensitized, and there is considerable discrepancy in the prevalence worldwide and limited epidemiology-related data on our population, we felt impelled to conduct this study. In this cohort, which comprises patients from the North of Portugal, a significant proportion of HP chronic presentation and a remarkable association with bird exposure were clearly evident. The high proportion of patients with chronic HP was associated with a relevant proportion of restrictive functional pattern and a significant number of patients undergoing lung biopsy, because in this form the differential diagnosis is considerably more challenging.

Although evidence has shown that the HP diagnosis has increased in recent years, there are significant geographical dissimilarities, expressed in the various ILD registries from different countries. According to these data, HP incidence is as low as 2.7% in Greece, 7.0% in Denmark or surprisingly 5.1% in Spain, and is significantly higher in other countries, such as 47.3% in India.[14–17] Although we do not have data from national registries, from our experience, we believe that our situation is analogous to those countries with higher incidence of HP, and chronic HP is probably the most frequent fibrotic ILD here. In the last five years of this study (2012–2016), 634 patients were diagnosed with ILD at our centre, of which 123 (19.4%) had HP. If we remove 117 patients with sarcoidosis, which are usually included in an independent registry, HP patients account for almost a quarter of all patients, more precisely 23.7%, and were always the most frequently diagnosed ILD in all the years considered. In contrast to our findings related to gender, most of the studies found a higher proportion of men. These differences seem to be explained by the type of exposure considered and even by social, economic and cultural dif-

Table 4 Lung function tests, blood gases and BAL parameters in HP patients.

	HP Patients	Clinical presentation			*p* value
		Acute	Subacute	Chronic	
Pulmonary function test and blood gases parameters					
Spirometry pattern, n (%)					0.251
Normal	62 (31.0)	8 (28.6)	5 (22.7)	49 (32.7)	
Restrictive	76 (38.0)	11 (39.3)	9 (40.9)	56 (37.3)	
Obstructive	54 (27.0)	7 (25.0)	8 (36.4)	39 (26.0)	
Mixed	3 (1.5)	2 (7.1)	0	1 (0.7)	
Deficient cooperation	5 (2.5)	0	0	5 (3.3)	
FVC, % predicted	81.4±23.9	78.3±26.0	82.1±21.6	81.9±23.3	0.762
FEV1, % predicted	80.8±23.9	73.4±24.1	80.8±25.3	82.3±23.6	0.191
FEV1/FVC	81.7±10.1	81.0±10.9	82.0±6.7	81.8±10.4	0.906
RV, % predicted	95.9±37.4	114.0±49.0[a]	101.0±31.4[a,b]	91.2±34.2[b]	**0.013**
TLC, % predicted	84.1±20.4	87.6±20.7	90.0±21.1	82.5±20.2	0.223
DLCO, % predicted	57.3±23.9	71.0±33.0 [a]	62.5±18.4 [a,b]	53.8±21.6[b]	**0.004**
DLCO/VA, % predicted	72.9±23.7	84.8±23.1[a]	71.3±17.3[b]	70.9±24.1[b]	**0.039**
PaO_2, mmHg	72.7±13.8	70.1±15.1	76.8±12.7	72.6±13.7	0.322
$PaCO_2$ mmHg	37.7±6.2	36.6±6.6	35.6±4.7	38.1±6.2	0.261
Bronchoalveolar lavage					
Total cells count, 10^5 ml^{-1}	4.2±5.7	7.2±6.8[a]	5.4±5.4 [a,b]	3.4±5.3[b]	**0.015**
Macrophages, %	44.8±22.7	31.2±17.4	31.1±20.3	50.0±22.1	0.127
Lymphocytes, %	43.4±24.1	58.6±16.7	58.8±21.5	37.3±23.3	0.072
Neutrophils, %	7.0±7.5	6.4±8.3	6.3±5.6	7.3±7.6	0.539
Eosinophils, %	3.1±4.0	1.7±1.7 [a]	2.2±3.0 [a,b]	3.5±4.5 [b]	**0.003**
Mast cells, %	0.3±0.5	0.4±0.6	0.2±0.3	0.2±0.5	0.084
CD4+ lymphocytes, %	50.4±23.2	43.1±19.5	52.4±27.0	52.0±23.1	0.169
CD8+ lymphocytes, %	40.1±21.9	47.1±18.7	35.4±24.7	39.2±21.9	0.451
CD4/CD8 ratio	2.5±3.0	1.3±1.1[a]	3.5±3.8[b,c]	2.6±3.2[c]	**0.001**

*different letters mean statistically significant differences between groups.
DLCO, CO Pulmonary Diffusion Capacity; FEV1, Forced Expiratory Volume in 1 s; FVC, Forced Vital Capacity; RV, Residual Volume; TLC, Total Lung Capacity; VA, Alveolar Volume.
Bold: $p < 0.05$

ferences. However, it is not yet clear whether this finding really represents differences in exposures or different susceptibilities of the disease.[18] Another epidemiologic aspect found in this study was the large proportion of non-smokers (73.4%), as often described in the literature.[19] In fact, several reports pointed to a lower HP prevalence in smokers due to the immune impairment induced by smoking.[1,6,8]

On the other hand, as previously highlighted in other reports, a broad spectrum of antigens may trigger the disease, and the inciting antigen may not be identifiable in many patients with HP. Exposure to birds was the most frequently identified antigen in our setting (76.6%), which agrees with literature data.[2,19] In Portugal, there are many colombophiles, and pigeon racing is one of the most popular sports. Moreover, there is a tradition of keeping pets, and birds are among the most popular. However, many other antigens may also be involved, as demonstrated in our results (moulds, cork, isocyanates), while some cases remain unknown. We believe that fungal exposure is significantly higher in our region, and the proportion of patients with this association is clearly underestimated, which is related to the difficulty in obtaining this information, since patients generally do not recognise this source, and no evaluation of the environment in their homes or workplaces has been achieved. The association between HP patients with no antigen identified with chronic forms and even with a worse prognosis has been previously described and we suggest that this is probably due to persistent exposure to the unidentified causal antigen.[20,21] The hypothesis that some of these cases correspond to unidentified IPF needs to be considered, although the diagnostic accuracy achieved gives this assumption a low probability, at least in a significant number of these patients, which may be a major influence on this association.

HP clinical presentation has been classically defined in the last three decades as acute, subacute and chronic.[1,6,8,9] However, there is a long controversy on this classification, namely around the subacute concept.[6,7,22] In 2009, the HP study group, in an investigation involving a cohort of 165 HP patients, defined two main clusters, one had recurrent systemic symptoms and chest tightness occurring few hours after antigen exposure and chest X-rays with no relevant change in almost 30% of cases, and a second cluster with features of advanced ILD, inspiratory crackles, clubbing in one third of cases and a restrictive pattern on pulmonary function tests.[22] Although these features fit in what we call acute and chronic forms, they advocate another nomenclature, especially because of the misconception these terms

may suggest, as they imply that chronic HP follows acute HP, which is, at least, uncertain. One of the authors' main conclusions is that subacute HP is particularly difficult to define.[22] More recently, Vasakova et al., based on the idea that the classical classification is outdated and has no prognostic value, proposed an alternative nomenclature with two main categories based on clinical–radiologic–pathologic correlation: acute/inflammatory HP with symptoms duration of 6 months, often reversible, characterized by typical radiologic and histopathologic patterns, and chronic/fibrotic with the presence of fibrotic changes in HRCT images and/or lung tissue.[7] Although this controversy is ongoing, the classic classification persists and we need more data and discussion to obtain a clear and sustainable classification in a guideline that should be the result of a consensus of leading HP experts. In our study, we decided to maintain the classic presentation, because no other alternative has been validated so far. Moreover, as this is an observational study, with no prognostic factor-related investigation, the classic classification in our opinion seems to be appropriate. In contrast to the findings of Morell et al.,[19] our results showed that the most frequent form was chronic (73.7%). These findings may be due mainly to the type of antigens that are usually associated with low but persistent exposure, which is usually linked to chronic forms. A different genetic susceptibility in this specific population may also explain this high burden of chronic forms.

Following clinical evaluation, diagnosis relies on typical chest CT scan findings, high lymphocytosis in BAL, and, in some cases, histological evaluation.[1,6,23] For these patients, the predominance of dyspnoea and cough was mainly found in chronic HP form and fever in the acute presentation, which fits in with established concepts.[19,24] In this study, as observed in other series, the most common imaging findings were ground-glass opacification, mosaic pattern and reticulation.[7,11,12,24,25] CT patterns showed acute and subacute HP forms are usually characterized by ground-glass opacities,[11,25,26] and chronic form by reticulation, traction bronchiectasis and volume loss, with or without evidence of honeycombing.[11,26]

With BAL, lymphocytosis was most commonly documented in acute and subacute forms, like other series.[1,4,6–9] Chronic forms, especially those with fibrotic features overlapping fibrotic pneumonias as common interstitial pneumonia, usually have lower or even no lymphocytosis, as we observed in this cohort where 25.3% of the patients had a normal range of lymphocytes. In addition, a trend towards a lower CD4/CD8 ratio was observed in HP patients with acute form, in contrast to subacute and chronic presentations, showing a $CD4^+$ predominance. In fact, $CD8^+$ lymphocyte alveolitis is frequently an acute HP feature, which decreases with exposure withdrawal, while chronic form HP patients tend to have more $CD4^+$ than $CD8^+$ cells.[27,28]

HP patients generally present restrictive ventilatory impairment[29] in pulmonary function tests, although an obstructive pattern may also be present,[23] which matches our findings. Most of our patients also presented a decrease in %DLCO, mainly in the chronic form. Indeed, it is clear that functional deterioration can be very serious in these patients, and may even lead to an irreversible clinical situation, in which the only therapeutic option is lung transplantation.

Although there are false positive and false negative pitfalls, it is well recognized that specific IgG antibodies against avian antigens may be useful as support evidence for HP, as a marker of exposure, but not of the disease.[1,6,9] Accordingly, measurement of these IgG antibodies is only carried out in cases in which exposure to these avian antigens was not entirely clear. In addition, specific IgG antibodies may also help to establish a relationship between exposure and disease, to track possible inducers when these have not been identified or to reduce the likelihood of aetiology by feathers or fungus, if negative.[1,6,9] For histological evaluation, it should be emphasized that the use of invasive complementary diagnostic tests for lung biopsy should be reserved only for cases in which the MDT evaluation, including clinical, imaging and BAL data, is not enough to conclude a definitive diagnosis. In the current series, as observed in other reports,[19] a low percentage of patients required more invasive diagnostic methods. More recently, transbronchial lung cryobiopsy has enabled an accurate and less invasive diagnosis,[30] certainly leading to an increase in the number of patients undergoing lung biopsy. In this cohort, despite the predominance of surgical lung biopsy (n = 33, 54.1%), these patients are mainly prior to 2014, when the cryobiopsy became available in our centre, after which almost all cases with histology in their diagnostic work-up underwent this new bronchoscopy procedure. Although 70% of TBLC was considered as high confidence in a lesser extent than the 90% within SLB, the remain 30% of TBLC considered as low confidence allowed, together with data from thoracic HRCT, to reach a confident diagnosis in MDT evaluation. The low level of confidence of transthoracic biopsy was expected, since this procedure is not considered as the most adequate in this scenario; however, all patients submitted to this intervention does not have clinical conditions for a more invasive procedure. After histological analysis, classified as high or low confidence, the final diagnosis is always established at the MDT meeting. HP is considered a difficult diagnosis, with low agreement. This was clearly demonstrated by Walsh et al. in a case-cohort study, involving 113 ILD patients evaluated by 7 MDT specialists from different regions of the world. They found a general agreement of a satisfactory Weighted-kappa coefficient (K) of 0.50 between the different MDTs, but when the different ILD diagnosis were stratified, they found in HP only a poor K of 0.25, the lowest observed.[31] In fact, the chronic forms often have imaging overlaps with other fibrotic ILDs, frequently with UIP or NSIP-like features. This may also reflect the disparity in regional methodology, along with the lack of consensus guidelines for HP diagnosis. There is an urgent need for an international consensus towards a clear HP definition, diagnosis and classification.[32] Our MDT is composed of 4 pulmonologists, 3 radiologists and 2 pathologists with many years of experience dedicated to ILDs and the large number of HP diagnoses discussed in this study shows at very least substantial expertise in this area.

Besides the general disagreement about HP diagnosis, specifically in chronic forms, the monocentric and retrospective design of this study is a clear limitation. The lack of antigen investigation in the patient environment clearly limits the accuracy of causal antigen recognition.

In conclusion, this study highlights the relevance of HP, particularly in chronic forms, which require a more precise and complex diagnostic assessment, due to the differential

diagnosis between this form and other interstitial pneumonias. Due to chronicity and poor prognosis in a significant number of cases, there is an unmet need for meticulous research on genetic polymorphisms associated with disease predisposition and the indoor environment that may account for a significant number of cases, in order to take the first steps towards the implementation of an antigen avoidance plan.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Martins N. would like to thank the Portuguese Foundation for Science and Technology (FCT–Portugal) for the Strategic project ref. UID/BIM/04293/2013 and ''NORTE2020—Programa Operacional Regional do Norte'' (NORTE-01-0145-FEDER-000012).

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Pulmonol. 2020;**26(3)**:138–144

PULMONOLOGY

www.journalpulmonology.org

ORIGINAL ARTICLE

A negative screening of rare genetic variants in the *ADIPOQ* and *STATH* genes in cystic fibrosis

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Received 29 June 2019; accepted 2 September 2019
Available online 9 October 2019

KEYWORDS
ADIPOQ;
Cystic fibrosis;
Genotype;
Modifier gene;
Phenotype;
STATH

Abstract
Background: The phenotypic variability in cystic fibrosis (CF) is widely recognized and modulated by environmental and genetic factors, including *CFTR* pathogenic variants and modifier genes genetic variants. In this context, determining the presence of variants in genes involved in immune response may allow a better understanding of CF variability, mainly in lung disease. Thus, *ADIPOQ* and *STATH* genes were selected and the analysis of exons and exon/intron junctions was performed for the determination of variations in its sequence, to determine the possible genetic modulation.
Methods: A total of 49 patients with CF, diagnosed for showing abnormal [chloride] levels in the sweat test, and identification of two pathogenic variants in *CFTR* categorized as class I and II were included. Genetic sequencing was performed for the identification of variants in the modifier genes.
Results: In our analysis, there was absence of rare genetic variants in *STATH* and *ADIPOQ* genes associated with the clinical variability. Thus, we are not able to establish an association between the disease severity and rare genetic variants in *STATH* and *ADIPOQ* genes, considering exons and exon/intron junctions.

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https://doi.org/10.1016/j.pulmoe.2019.09.003

Conclusions: Considering the negative screening for rare genetic variants in *ADIPOQ* and *STATH* genes, it may be concluded that these genes are not associated with phenotypic modulation of CF in our population. To understand the modifier genes and its action at CF variability it is essential to promote a better overview of the disease. Also, negative reports can help to direct new studies without the use of unnecessary financial support.

Introduction

The *ADIPOQ* (Adiponectin, C1Q And Collagen Domain Containing) is located in the 3q27 region, has three exons and encodes the adiponectin protein (GBP38, adipoQ, apM1 or Acpr30).[1] Adiponectin belongs to the group of adipokines, which are produced by adipocytes, they are important in insulin sensitivity,[2] energy metabolism and glucose sensitivity,[3] vascular disease and immune response, acting as an anti-inflammatory factor.[4,5] Adiponectin is expressed almost exclusively in adipose tissue, but a low expression occurs in other tissues.[6,7] Also, adiponectin can modulate cytokine production in different types of myeloid cells and induce the production of the IL-10 mediator, having an anti-inflammatory and immunosuppressive effect in hematopoietic cells.[8]

Adiponectin occurs in its complete form as well as in fragments, and consists of the C-terminal globular, known as the globular domain of adiponectin. In its basic structure, adiponectin has 244 amino acids distributed in four domains: N-terminal sequence, variable domain, collagen domain and C-terminal globular domain.[9] However, in its complete form, it can acquire different properties, such as monomers and trimers that can still associate with each other via collagen domains in groups of four to six, and thus result in oligomers with high molecular weight.[7,10]

Functional variants in *ADIPOQ* affect the levels of circulating adiponectin.[11] These variants result in high levels of adiponectin in patients with CF and may modulate the disease phenotype by suppressing inflammation and improving the nutritional status.[2] Corroborating the previous idea, it was shown that in the nasal epithelium of homozygous p.Phe508del, the most frequently expressed *ADIPOQ* occurred in mild lung disease.[12]

The *STATH* (Statherin) located in region 4q13.3 has five exons, which encode the statherin protein[13] which is a peptide with antimicrobial properties expressed in the saliva, upper airways and nasal secretions, which participates in the development of biofilms of the oral cavity, mediating bacterial adhesion.[2] Statherin is important in the saliva's interaction with atmospheric air,[12,14] having the role of maintaining oral health, working in conjunction with calcium and inhibiting crystal growth, showing high affinity for surfaces with hydroxyapatite. As a mediator of bacteria adhesion, statherin has epitopes that promote the growth and adhesion of certain microorganisms in the oral cavity (*Porphyromonas gingivalis*) while inhibiting the growth of others (*Staphylococcus aureus*).[15] Although its antibacterial activity against *Pseudomonas aeruginosa* has not been investigated, it is known that infection with this bacterium accelerates the decline in the lung function.[16] Also, statherin with high activity levels was found in homozygous p.Phe508del and with moderate and severe lung disease.[12,17]

Considering that the genetic profile of patients with CF for rare variants in *ADIPOQ* and *STATH* genes are still controversial and poorly understood, we aimed to identify sequence changes in exons and exon/intron junctions of *ADIPOQ* and *STATH* genes and to verify the existence of an association between its variants and CF severity.

Methods

Participants

A total of 49 patients with CF diagnosed by (having shown [chloride] ($\geq$60 mEq/L)) the sweat test were included. All participants were homozygous or compound heterozygous for *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) pathogenic variants (class I or II). No patient was diagnosed via the neonatal screening test. The study was approved by the Institutional Ethics Committee of the University of Campinas (#570/2004). All participants or their parents signed a consent form before the beginning of the study.

DNA extraction

The DNA was obtained via phenol-chloroform extraction. The [DNA] used for analysis was 50 ng/mL, evaluated using a GE NanoVue™ Spectrophotometer (GE Healthcare Biosciences, Pittsburgh, USA).

ADIPOQ and *STATH* screening

The polymerase chain reaction (PCR) for amplification of *ADIPOQ* and *STATH* genes was performed with bidistilled water, 10$\times$ Taq buffer with $(NH_4)_2SO_4$, $MgCl_2$ (25 mM), dNTP (25 mM of each nitrogenous base), primers (0.2 ρmol), Taq polymerase (5 U) and genomic DNA (50 ng/mL). The primers used in the analyses are shown in Table 1. The PCR conditions for *ADIPOQ* and *STATH* genes were 94 °C/5 min; 35 cycles of 94 °C/1 min, annealing temperature/1 min and 72 °C/2 min;

Table 1 Sequence of primers, size of the fragments and annealing temperature of *ADIPOQ* and *STATH* genes.

Gene	Location	Sequence 5ᐟ3´	Size of fragments (basis pair)	Annealing temperature (ºC)
ADIPOQ	Exon 1	TGAGTACCAGGCTGTTGAG GGAGAACGGAGGAAGAAG	252	63
	Exon 2	AACTGGGTGTGTGTGTGG GTAGGAGGTCTGTGATGAAAG	322	64.5
	Exon 3a	GGCAGGAGTTCTGTTCTTTG CAGGAATGTTGCAGTGGAAT	300	58
	Exon 3b	AACATGCCCATTCGCTTTAC TGTAATCCCTCAAGCAACCAC	525	
STATH	Exon 2	GCTTTGGAGCGTAGTATAATC AACACAAGGAATAGAGAGACTC	222	60
	Exon 3 e 4	GGACTACACAGCATTATCAG GTGTCTATCGATGATTTGC	299	63.5
	Exon 5	ACATTTCAAGGAGCTATACAGC AAACGCTTGCACTGTCATTATC	411	58

ADIPOQ, Adiponectin; *STATH*, Statherin.

and 72 °C/7 min. The annealing temperature for each fragment analyzed is shown in Table 1.

Genetic sequencing

The sequencing of exons and exon/intron junctions of *STATH* and *ADIPOQ* genes was performed in MegaBACE® 1000 (GE Healthcare, Pittsburgh, PA, USA) using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (with Thermo Sequenase™ II DNA Polymerase) (GE Healthcare, Pittsburgh, PA, USA) following the manufacturer's recommendations. The sequences were analyzed using the Chromas Lite software, version 2.3.3.0 Chromas MFC application.[18]

Clinical markers

The clinical markers included were: *CFTR* pathogenic variants, age at the time of diagnosis, age at the onset of pulmonary and digestive symptoms, first infection by *P. aeruginosa*, spirometry, Shwachman-Kulczycki score, Kanga score, and transcutaneous oxygen saturation of hemoglobin.

For age at the time of diagnosis, age at the onset of pulmonary and gastrointestinal symptoms, and time until the first isolation of *P. aeruginosa*, the following groups were used: up to two months old, 13–36 months old, above 36 months old. In the case of digestive symptoms, meconium ileus was considered as an additional clinical marker. For *P. aeruginosa*, some patients were decolonized before the beginning of the study and were not included in the descriptive analysis.

Spirometric values were described considering forced expiratory volume in one second (FEV_1%) as: (i) severe obstruction: <40%; (ii) moderate obstruction: ≥40% to <60%; (iii) mild obstruction: ≥60% to <80%; (iv) normal lung function: ≥80%.

Spirometry was performed using a speedometer CPFS/D model (Med Graphics, Saint Paul, Minnesota, USA). Data were recorded using the BREEZE PF software Version 3.8 B for Windows 95/98/NT.

The Shwachman-Kulczycki score was classified as excellent (86–100), good (71–85), mild (56–70), moderate (41–55) and severe (40 or less). The score was evaluated by two professionals, because it was a subjective analysis. In case of disparity between evaluators, a third evaluation was performed. The Kanga score was analyzed considering the presence or absence of exacerbation in the points obtained.

The transcutaneous oxygen saturation of hemoglobin was categorized as: (i) normal: ≥95%; (ii) mild hypoxemia: ≥91 to <95; (iii) moderate hypoxemia: ≥85 to <90; (iv) severe hypoxemia <85%.

Results

Population analyzed

Of the 49 patients with CF included in the study, 25/49 (51.02%) were female. Ages ranged from one to 26 years old, with the mean age being 9.71 ± 6.06 years old. The mean age at the time of diagnosis was 29.70 ± 2.47 months old. For the first *P. aeruginosa*, the mean age was 38.11 ± 3.17 months old (Tables 2 and 3).

In relation to the *CFTR* genotype, there was a high prevalence of homozygous p.Phe508del (63.26%). The p.Phe508del allele was the most prevalent (79.59%), followed by p.Gly542X (9.18%), p.Arg1162X (6.12%), p.Asn1303Lys (3.06%) and p.Arg553X (2.04%) (Table 2).

Sequencing

The three exons of *ADIPOQ* gene and four of the five exons of *STATH* were analyzed. Exon 1 of the *STATH* gene was not analyzed being considered a non-translated region. The three and four exons were analyzed with a single primer pair. No changes (rare genetic variants) were found in all patients with CF for all fragments. Also, there is no need to compare among the homozygous subjects for *CFTR* and compound heterozygous subjects for *CFTR* because all *CFTR* variants included are severe variants.

Table 2 Characterization of the patients with cystic fibrosis for cystic fibrosis transmembrane regulator (*CFTR*) variants, Shwachman-Kulczycki score, Kanga score, transcutaneous oxygen saturation of hemoglobin (SpO_2) and forced expiratory volume in the first second (FEV_1%).

CFTR genotype	Shwachman-Kulczycki	Kanga	SpO_2	FEV_1%
F508del/F508del	Moderate	Normal	Normal	Normal
F508del/F508del	*	Normal	Normal	Mild
F508del/F508del	Excellent	Normal	Mild	Normal
F508del/R1162X	Excellent	Normal	Mild	Severe
F508del/F508del	*	*	*	*
F508del/F508del	*	Exacerbate	Normal	Moderate
F508del/F508del	Moderate	Normal	Normal	Normal
F508del/F508del	Severe	Exacerbate	Mild	Severe
F508del/F508del	*	Normal	Normal	Mild
F508del/F508del	Moderate	Normal	Normal	Normal
F508del/G542X	*	Normal	Severe	Severe
F508del/G542X	Moderate	Normal	Mild	Severe
F508del/F508del	*	*	*	*
F508del/N1303K	*	*	*	*
F508del/F508del	*	Exacerbate	Mild	Mild
F508del/F508del	Excellent	Normal	Normal	Moderate
F508del/F508del	Good	Normal	Mild	Mild
F508del/F508del	*	Normal	Mild	Mild
F508del/R553X	Excellent	Normal	Normal	Normal
F508del/G542X	*	Normal	Normal	Mild
F508del/F508del	*	Normal	Normal	Normal
F508del/F508del	Moderate	Normal	Normal	Normal
F508del/N1303K	*	Normal	Normal	*
F508del/F508del	*	*	*	*
F508del/F508del	Good	Normal	Normal	Normal
F508del/N1303K	Good	Normal	Normal	*
F508del/R1162X	Good	Normal	Normal	Normal
F508del/R553X	*	Normal	Mild	Severe
F508del/G542X	Excellent	Normal	Normal	Normal
F508del/F508del	*	Exacerbate	Moderate	*
F508del/F508del	*	Normal	Normal	*
F508del/G542X	Moderate	Normal	Normal	Moderate
F508del/F508del	*	Normal	Normal	*
F508del/G542X	*	Normal	Normal	*
F508del/F508del	*	Normal	Mild	*
F508del/G542X	Excellent	Normal	Normal	Normal
G542X/R1162X	Good	Exacerbate	Mild	Mild
F508del/F508del	*	Normal	Normal	*
F508del/G542X	Good	Normal	Normal	Normal
F508del/F508del	Moderate	Normal	Normal	*
F508del/F508del	Good	Normal	Normal	Normal
F508del/F508del	*	Normal	Normal	Normal
F508del/F508del	Mild	Normal	Normal	*
F508del/F508del	Excellent	Normal	Normal	Normal
F508del/R1162X	*	*	*	*
F508del/F508del	*	*	*	*
F508del/F508del	*	*	*	*
R1162X/R1162X	*	*	*	*
F508del/F508del	Moderate	Normal	Normal	Mild

*, absence of data; F508del → c.1521_1523delCTT (p.Phe508del)], rs113993960; G542X → c.1624 G > T (p.Gly542Ter), rs113993959; N1303 K → c.3909C > G (p.Asn1303Lys), rs80034486; R553X → c.1657C > T (p.Arg553Ter), rs74597325; R1162X → c.3484C > T (p.Arg1162Ter), rs74767530.

Table 3 Distribution of the patients with cystic fibrosis according the clinical characteristics.

Clinical variable	Category	Number of participants (%)
Age at the time of diagnosis	<12 months old	27 (56.25)
	13 to 36 months old	8 (16.66)
	>36 months old	13 (27.08)
Onset of pulmonary symptoms	<12 months old	33 (70.21)
	13 to 36 months old	7 (14.89)
	>36 months old	5 (10.64)
	No clinical symptom	2 (4.25)
Onset of digestive symptoms	<12 months old	32 (68.08)
	13 to 36 months old	4 (8.51)
	>36 months old	4 (8.51)
	Meconium ileus	7 (14.89)
	No clinical symptoms	1 (2.12)
Age at the time of the first infection by *Pseudomonas aeruginosa*	<12 months old	13 (27.66)
	13 to 36 months old	14 (29.79)
	>36 months old	15 (31.91)
	Without bacteria	5 (10.64)
Kanga score	Non-exacerbation	38 (88.37)
	Exacerbation	5 (11.36)
Shwachman-Kulczycki score	Excellent	7 (29.16)
	Good	7 (29.16)
	Moderate	8 (33.33)
	Mild	1 (4.16)
	Severe	1 (4.16)
Transcutaneous oxygen saturation of hemoglobin	Normal	32 (72.72)
	Mild	10 (22.72)
	Moderate	1 (2.27)
	Severe	1 (2.27)
Forced expiratory volume in the first second of the forced vital capacity	Normal	15 (51.72)
	Mild	8 (27.59)
	Moderate	3 (10.34)
	Severe	3 (10.34)

Discussion

Population analyzed

According to age range, most patients were included in the range between zero and ten years old (69.38%). Due to the inclusion of patients with pathogenic mutations in the class I and II *CFTR* group, these data may be associated with severe prognosis and low life expectancy considering the presence of severe mutations. However, the survival rates and prognosis of patients with CF have improved. One of the factors that has been associated with this fact is the systematic care of patients in specialized centers.[19]

Regarding sex, a uniform distribution was observed, a fact associated with autosomal recessive inheritance. However, the literature reports a slight predominance of males compared to females, increasing with the patients' age.[20,21] The lower prevalence in females may occur due to the vulnerability of females to certain clinical characteristics, such as the occurrence of diabetes mellitus.

The mean age at the time of diagnosis (2.47 years old) was higher than that found by Dorfman et al. (2008)[22] (0.36 years old) in a group of 611 homozygous p.Phe508del. The average age at the time of the first infection by *P. aeruginosa* (3.17 years old) was lower than the one reported in the same study (7.5 years old). This difference can be explained by the lower age at the time of diagnosis of the previous study, compared to ours, suggesting that the early treatment of these patients can delay the colonization by *P. aeruginosa*.

ADIPOQ

Low adiponectin levels promote inflammation and are associated with increased insulin resistance and high risk of cardiovascular disease.[23] Furthermore, adiponectin is associated with the regulation of energy balance, and in CF, chronic energy deficiency and thus higher [adiponectin] levels occur.[24]

Adiponectin has anti-inflammatory properties, mainly inhibiting the production of pro-inflammatory cytokines and inducting anti-inflammatory factors. However, higher levels of adiponectin were found in patients with CF compared to healthy subjects and as explanation, the presence of deficiency in the energy balance was considered[24]; moreover, the absence of correlation between inflammation markers [e.g. C-reactive protein (CRP) and fibrinogen] and adiponectin was reported, suggesting that adiponectin lev-

els are not reduced in CF, even in the presence of low-grade inflammation or chronic infection/inflammation.[23,25]

In addition, functional variants in *ADIPOQ* have been associated with levels of circulating adiponectin.[8,12] Some of these variants result in high levels of adiponectin, which could support a better clinical outcome of patients with CF, since adiponectin acts in suppressing inflammation-related diseases and improving nutritional status.[2] In this context, two variants (exon 2 and 3) that decrease the level of circulating adiponectin were described.[26] However, in our study, the presence of these variants was not supported.

In our study, it was not possible to establish the relationship between variants in *ADIPOQ* and CF severity, since no variants were found.

STATH

Studies relating *STATH* and CF are scarce. However, it is known that statherin is an antimicrobial peptide expressed in the upper airways and nasal secretions involved in the development of biofilm in the oral cavity, mediating bacterial adhesion.[2] It has recently been identified as the most prominent protein in the saliva's interaction with atmospheric air.[12,14]

As a mediator of adhesion of bacteria, statherin has epitopes that promote the growth and adhesion of certain microorganisms in the oral cavity (*P. gingivalis*) while inhibiting the growth of others (*S. aureus*),[15] although their antibacterial activity against *P. aeruginosa* has not yet been investigated. It is known that the infection by *P. aeruginosa* accelerates the decline in lung function.[16] Thus, a protein that acts in bacterial adhesion in the oral cavity and upper airways is of extreme interest to studies related to the CF phenotype.

Statherin with high activity levels was found in homozygous p.Phe508del in moderate lung disease.[12] This increase in expression was confirmed by an analysis of the mRNA produced by *STATH* in a sample of 12 patients with CF and moderate and severe pulmonary disease.[17]

In our study, it was not possible to establish a relation between variants in *STATH* and CF severity, since no variants were found.

Conclusion

No rare sequence alteration in the exon and exon/intron junctions of *STATH* and *ADIPOQ* genes were found. It was not possible to establish an association between CF and *STATH* and *ADIPOQ* genes for the regions analyzed in our study. It should be noted that the analyzed population is admixed and should have had greater polymorphic variability than other previously studied populations, which did not occur in our data.

Conflict of interests

The authors declare no conflict of interests.

Authors' contribution

CAACC/FALM/JDR/CSB contributed to the study's conception and design, acquired, analyzed and interpreted the data, drafted the manuscript and revised its intellectual contents, and approved the manuscript for publication.

Acknowledgements

We thank Luciana Cardoso Bonadia, Taís Daiene Russo Hortencio, Kátia Cristina Alberto Aguiar, Aline Cristina Gonçalves and Antônio Fernando Ribeiro for assistance in data collection and organization of ideas, Maria Angela Ribeiro for spirometry analysis.

[FALM] thanks the São Paulo Research Foundation (FAPESP, acronym in portuguese) for sponsoring #2011/12939-4, #2011/18845-1, #2015/12183-8 and #2015/12858-5; the Research, Teaching and Extension Support Fund (FAEPEX, acronym in portuguese) of the University of Campinas for sponsoring #0648/2015; [JDR] thanks FAPESP for sponsoring #2011/18845-1 and #2015/12183-8.

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Pulmonol. 2020;**26(3)**:145–150

PULMONOLOGY

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ORIGINAL ARTICLE

Can environmental determinants explain Nontuberculous Mycobacteria geographic incidence?

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Received 24 April 2019; accepted 10 December 2019
Available online 24 December 2019

KEYWORDS
Nontuberculous mycobacteria;
Environmental determinant;
Geographic distribution;
Portugal

Abstract
Introduction and Objectives: Nontuberculous mycobacteria (NTM) are opportunistic agents that have gained importance during the last decades due to their increasing incidence in high-risk populations. Their modes of transmission differ from person-toperson contact commonly described in Mycobacterium tuberculosis (MTB). In fact, NTM are frequently found in soil, natural waters and drinking-water distributions systems, emphasizing the contribution of environmental factors when discussing this disease's susceptibility. Our aim is to evaluate the incidence of NTM in Portugal and to identify the main environmental variables related to it.
Material and Methods: We performed a cross-sectional study centred on 2011 (date of the latest Portuguese census) from collected personal features and environmental data available in public databases. Environmental values when only known at the district level were interpolated using inverse distance weighting. A semiparametric poisson model was used to estimate NTM incidence. The non-parametric part of the model was obtained by using thin plate smoothing splines defined on the spatial component of the data.
Results: 359 new NTM cases were notified during a five-year period. None of the environmental determinants studied was strong enough to predict NTM geographical incidence in Portugal ($p > 0.05$), except for population density ($p < 0.001$). Personal characteristics such as female sex ($p < 0.001$), age ($p < 0.001$) and Human Immunodeficiency Virus infection and Acquired Immune Deficiency Syndrome (HIV/AIDS) incidence ($p < 0.001$) are associated with an increase of NTM disease incidence.

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https://doi.org/10.1016/j.pulmoe.2019.12.003

Conclusions: NTM appears to be more common in elderly women, especially if they have HIV/AIDS disease or if they live in urban, highly populated areas. Overall, female sex seems to assume the most relevant role when discussing predisposition to NTM disease. However, further studies are needed to evaluate the impact on NTM geographical incidence by other environmental and personal variables not included in this one.

Introduction

Nontuberculous mycobacteria (NTM) are opportunistic human pathogens known to be an important cause of morbidity and mortality in immunocompromised individuals.[1] NTM epidemics have changed since the emergence of Human Immunodeficiency Virus infection and Acquired Immune Deficiency Syndrome (HIV/AIDS), transforming this rare infection into a common diagnosis in high-risk populations.[2–4] Despite still being underdiagnosed, NTM prevalence has exponentially grown during recent decades and it will continue to increase given the greater life expectancy and the lengthened survival of risk groups in developed countries.[1,5,6]

It is now known whether NTM are transmitted via aerosols.[2,4–6] A great influencer in NTM epidemiology is its lipid-rich outer membrane constituted by long chain mycolic acids which makes them more impermeable with a slower growth rate.[6,7] The characteristics of this membrane explain not only the preferential attachment to surfaces but also why NTM are so widespread despite low rate division and impenetrability to hydrophobic nutrients.[2,5,6,8]

Originally, NTM were identified in natural waters and soils and unusual places such as acid, brown water swaps, boreal and peat rich soils and metal working fluids.[2,6] These findings show that NTM are able to thrive in a diverse range of organic compounds but mostly acidic waters and soils and areas with air:water interfaces; they also prove that its growth is not influenced by temperature range, degree of salinity or oxygen tension.[2,6,8,9] In addition to their presence in natural environments, NTM are frequently isolated from household plumbing, drinking-water distribution systems and hospital water systems where they proliferate as a biofilm which contributes to their intrinsic resistance to pollutants and heavy metals.[2,5–8] Also, human activities such as disinfection, promotion of polluted environments and injudicious use of antibiotics help to select bacteria progressively more resistant and accustomed to human environments, specially urban areas and most densely populated places.[2,6,8]

Worldwide, NTM appear to be more frequent in tropical and subtropical regions; however, it is now recognized that epidemiological data varies according to geographical areas which in turn correlates with local clinical settings.[8,10] So, it is necessary to conduct epidemiological surveillance in order to provide local epidemiological data that is useful in the management of patients.[10] In this study, we will evaluate the incidence on NTM in Portugal and its association with some environmental variables (temperature, soil, pH, humidity and precipitation).

Material and methods

We conducted a cross-sectional study centred on 2011 (date of the latest nationwide census performed in Portugal).

The new NTM cases reported between 2009 and 2013 were extracted from Sistema de Vigilância Intrínseco do Programa Nacional de Luta Contra a Tuberculose (SVIG-TB),[11] a public health surveillance system for tuberculosis cases in Portugal which also includes nontuberculous mycobacteria data (i.e. a database for both tuberculous and nontuberculous mycobacteria). Population density data and personal features of the Portuguese population such as age, sex, HIV/AIDS incidence and migrants' proportion were collected from the 2011 nationwide census organized by Instituto National de Estatística (INE),[12] an institute responsible for producing official statistical data for the Portuguese government. Environmental determinants such as temperature, humidity and precipitation in the period between 2009 and 2013 were obtained from Instituto Português do Mar e da Atmosfera (IPMA),[13] a public institute with nationwide research responsibilities concerning the ocean and the atmosphere. Soil pH data were collected from the European Soil Data Centre (ESDAC) dataset,[14,15] a project from the Joint Research Centre (JRC) organised by the European Commission.[16] To simplify our geographical analyses, we excluded the archipelagos of Azores and Madeira (Azores and Madeira inhabitants represent approximately 5% of the total Portuguese population) and considered the 278 Portuguese municipalities as the spatial reference units. All data were collected according to this geographical division, except for IPMA's data which were only available for districts.

Microsoft EXCEL (2013) and R (version 3.5.2) were the data analyses tools used. Values collected from IPMA are monthly averages over a 5-year period and were interpolated using the inverse distance weighting method in order to obtain values for each municipality. Semiparametric poisson models were used to estimate NTM incidence. The non-parametric part of the models was obtained by using thin plate smoothing splines defined on the spatial component of the data. All models include the spatial component so that each explanatory variable is adjusted for geographical localization. Selection of variables in order to obtain a final model was made using the backward stepwise method. The level of significance was set at a $p < 0.05$.

Table 1 Effect of each environmental variable adjusted to NTM geographic distribution.

Environmental determinants	Expected effect (EE)	Confidence Interval [95% CI]	p Value
Humidity (%)	1.210	0.802–1.825	0.363
Mean temperature (ºC)	0.399	0.077–2.076	0.275
pH	0.859	0.575–1.283	0.457
Population density (thousands/km^2)	1.123	1.054–1.198	<0.001
Precipitation (mm or kg/m^2)	1.044	0.964–1.130	0.288

The value of expected effect translates how much more effect that variable will have on NTM geographical incidence. For example, considering humidity, for each increase of one percent in humidity there will be a 21% increase in expected NTM incidence. However, this increase does not inform us about the impact of this variable on NTM geographical incidence. For that, we must analyse the confidence interval from narrowest and farthest intervals of 1 translated into a greater overall impact on NTM incidence.
Note: For each environmental determinant the mean values from period 2009–2013 was considered, except for population density and pH.

Results

According to 2011 nationwide census, 10,562,178 people lived in Portugal during that year. Between 2009 and 2013, 359 new NTM cases were reported to SVIG-TB (mean = 71.8 cases/year). The majority came from Lisbon (mean = 8.0 cases/year) and Oporto (mean = 6.8 cases/year) municipalities, the most populated areas in Portugal.

As for the environmental variables, we found a positive effect of population density on the NTM geographical incidence (Expected effect (EE) = 1.123, CI = 1.054–1.198). Humidity (EE = 1.21, CI = 0.802–1.825) and precipitation (EE = 1.044, CI = 0.964–1.130) had the same effect but in a smaller magnitude. Conversely, mean temperature (EE = 0.399, CI = 0.077–2.076) and pH (EE = 0.859, CI = 0.575–1.283) were associated with a negative effect on NTM geographical incidence. Except for population density, none of those associations were statistically significant ($p > 0.05$) (Table 1).

Regarding personal features, sex has shown to have the greatest effect on NTM geographical incidence (EE = 0.695, CI = 0.612-0.788), in that male sex appears to act as a protective factor on NTM infection. Age older than 45 years old had the second greatest effect on NTM geographical incidence (EE = 1.091, CI = 1.049–1.134). HIV/AIDS incidence (EE = 1.019, CI = 1.010–1.028) was also positively associated to NTM geographical incidence. The migrant proportion was another variable positively related to NTM geographical incidence but not as strong as others (EE = 1.095, CI = 1.029–1.164) (Table 2).

Discussion

We studied all patients notified with NTM in a period of 5 years (n = 359) and calculated an annual incidence of 71.8 cases/year. Of all environmental determinants analysed, population density ($p < 0.001$) is the only environmental variable that has been shown to influence NTM epidemiology. Personal characteristics such as female sex ($p < 0.001$), age ($p < 0.001$) and HIV/AIDS incidence ($p < 0.001$) demonstrated a positive effect on the NTM geographical incidence.

Cassidy et al. described the microbiologic and demographic features of NTM disease when estimating the population-based prevalence of pulmonary and extrapulmonary NTM cases in Oregon. They were capable of associating the higher prevalence of disease in the western, more urban portion of Oregon to a wetter and more temperate climate relative to what was observed in rural areas of the same state.[1] In a study conducted by *Iivanainen et al.*, to evaluate the impact of environmental factors on the occurrence of environmental mycobacteria, they found that the number of total NTM in Finnish brook waters also correlated positively with precipitation.[2,17] They proved that in rainy periods there is an increase in chemical oxygen demand, acidity and counts of mycobacteria in the waters.[17]

In our population, we also found a positive effect of humidity and precipitation and a negative one by pH and temperature on NTM geographical incidence, albeit not being statically significant. In part, these results can be explained by our small sample size.

We found, as well, that the population density positively influences NTM incidence. *Cassidy et al*. also confirmed this when they studied NTM cases in Oregon residents.[1] They found a higher prevalence of disease in the western, more urban portion of Oregon that they attributed to the use of municipal water systems, since it had already been demonstrated in urban communities of Boston in 1970s/80s that water stored in reservoirs for long periods of time promotes biofilm formation.[1,18,19] However, as *Cassidy et al*. admitted, it is possible that these results are influenced by a diagnostic bias since patients with chronic lung diseases might more commonly live in urban areas and therefore more easily seek medical care.[1] In addition, another diagnostic bias may be present in these patients as NTM infection is higher in individuals with chronic lung diseases.[4,5,10,20,21]

'Windermere syndrome' corresponds to a single case of an elderly, non-smoking, thin woman where pulmonary disease was for the first time attributed to NTM.[1,5,22] Subsequent studies confirmed that NTM pulmonary disease is more common in previously healthy elderly women.[7,9,10,19,23–25] This is similar to our findings that female sex and age greater than 45 years old are, respectively, the first and second best NTM geographical incidence influencers when analysed individually. But when creating a model starting with all significant variables together with geographic variation and applying a selection of variables procedure, sex is the only one to appear in the final model (see Figs. 1 and 2). Although *Stout et al*. confirmed this increase in NTM incidence with age, they argued that gender predominance in NTM could

Table 2 Effect of each personal feature adjusted to NTM geographic distribution.

Personal features	Expected effect (EE)	Confidence Interval [95% CI]	p Value
Age >45 years old	1.091	1.049–1.134	<0.001
HIV/AIDS incidence (per 100.000 hab)	1.019	1.010–1.028	<0.001
Male sex (%)	0.695	0.612–0.788	<0.001
Proportion of migrants (per 1000 hab)	1.095	1.029–1.164	0.004

The value of expected effect translates how much more effect that variable will have on NTM geographical incidence. For example, considering age older than 45 years, for an increase of a percentage point in the proportion of older people in the population there will be a 9.1% increase in expected NTM incidence. However, this increase does not inform us of the impact of this variable on NTM geographical incidence. For that, we must analyse the confidence interval since narrowest and farthest intervals of 1 translate into a greater overall impact on NTM incidence.
Note: For each personal feature, values from the 2011 portuguese census were considered.

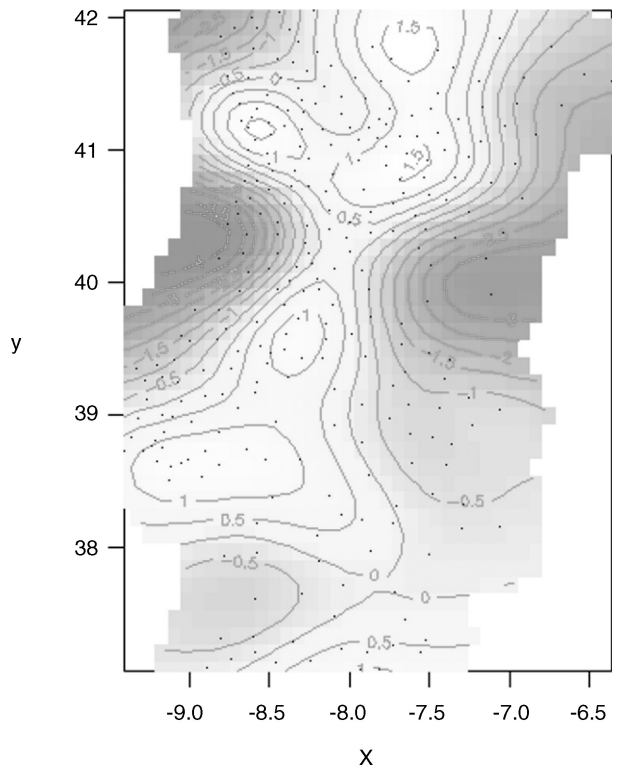

Figure 1 NTM incidence model using geographical distribution as a single influencer. This model does not represent actual NTM cases distribution, but it is rather predicted NTM incidence variation solely by geographical distribution. White areas represent places with highest predictable NTM incidences. Red areas represent places with lowest predictable NTM incidences. Green lines are log-scale level curves that compare predicted NTM local incidences to the mean value of NTM incidence considered for mainland Portugal.
x = latitude, y = longitude

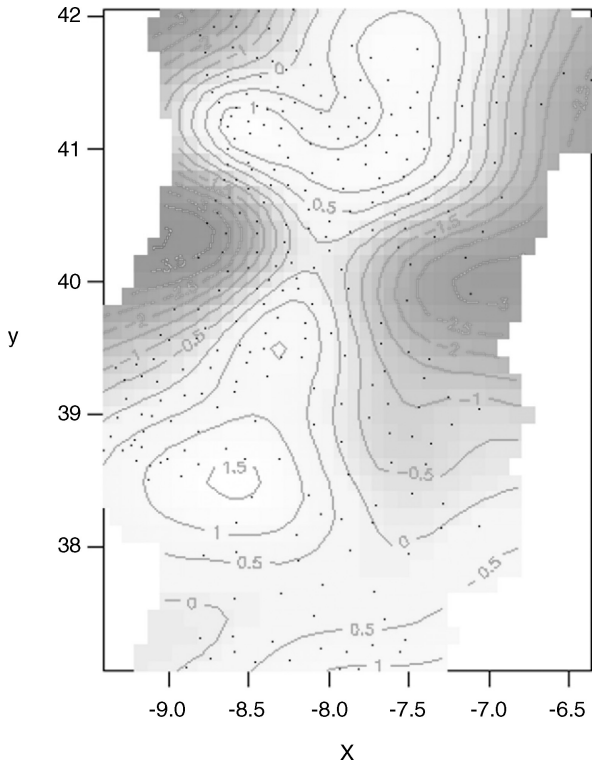

Figure 2 Effect of geographic distribution on NTM incidence when adjusted to male proportion. In this model, we subtract the impact of sex on NTM incidence, considering that male proportion is the same for all municipalities. By doing this, we can see how much more geographical variation influences NTM incidence (comparatively to what was already explained by sex). This model predicts better actual NTM cases distribution because it matches geographical variation with male proportion.
x = latitude, y = longitude.

be wrongly overestimated in men by the different prevalence of smoking-associated lung damage between males and females.[5,9]

Our study similarly found a positive influence of HIV/AIDS incidence on NTM geographical incidence. This agrees with the current evidence that NTM cases are highest among immunosuppressed groups.[1,3–5,26] When considering the Portuguese landscape, the relationship between HIV/AIDS incidence and NTM geographical incidence was strongest in Lisbon and Oporto regions, areas where HIV/AIDS incidence is higher.[27]

Finally, our study still found that migrant proportion has a small effect on NTM geographical incidence. A plausible theory is that migrants usually live in suburban and urban areas of the country's most populated cities but with worse access to health care facilities and diminished hygienic-sanitary

conditions compared to the native population.[1,2,6,28,29] Howsoever, the idea that NTM disease is more common in the migrant population has been debated in more recent works. In a study carried out in Canada by *Hernandez-Garduno et al*. to determine the risk factors for pulmonary colonization by NTM, they concluded that NTM colonization risk is not so different between Canadian-born people and foreign-born people residing in Canada for at least 10 years.[30] This underlines the cumulative risk of exposure to environmental sources.

Other authors have already identified others personal features as risk factors for NTM disease. Commonly, NTM infection is greater in individuals with prior or current lung diseases, patients exposed to the aspiration of gastric contents, individuals with deformations of the chest or individuals immunosuppressed for other reasons than HIV/AIDS.[4,5,9,10,20,21] Genetic causes were also implicated in the pathophysiology of NTM infection.[5,7] For example, *Kotilainen et al*. proposed C4 deficiency as a risk factor for NTM pulmonary infection in elderly female patients.[7] Other research identified defects in the interleukin-12/interferon-gamma axis in families predisposed to rare disseminated NTM disease.[5,7,20,31,32] Although the available evidence is limited, there is some suggestion that tobacco and alcohol may be cofactors in the development of NTM pulmonary involvement.[4–6] Lastly, race appears to play a part in NTM disease susceptibility.[19,30] In their works, *Bodle et al*. identified a higher prevalence of NTM positive cultures in white people from a sample of patients in New York City.[19] Given the large number of personal features highlighted here, it is not surprising that those were the most promising factors in our study.

The strength of this study is that it is a nationwide longitudinal study with NTM cases routinely notified by family physicians. One of the limitations associated is related to the few environmental variables analysed. This was limited by the scarce data collected by the meteorological stations that are property of IPMA. Another limitation is the fact that our study used data from 2011, so it could be argued that the conclusions found are valid only for that period and no further. Also, the small number of NTM cases identified per year made it more difficult to find any statically significant result between environmental determinants and NTM incidence. Moreover, local differences in health services assessment and inequality expertise in NTM diagnosis by regional hospitals could have negatively influenced the relations studied.[5,9,21] Additionally, the mathematical approach applied to the IPMA data to derive values from districts to municipalities could similarly influence the results encountered. So, a more judicious study design should be implanted in future researches to minimize these glitches.

Conclusion

Although we had found some correlations between environmental determinants and NTM geographical incidence in mainland Portugal, population density is the only environmental variable significantly associated to the incidence of NTM. However, personal variables such as sex, age and HIV/AIDS incidence were revealed to have a greater effect on this outcome. Further studies are needed with a larger sample and with inclusion of other environmental determinants not routinely measured.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to thank ISPUP (Instituto de Saúde Pública da Universidade do Porto) for helping collect all data from SVIG-TB and ESDAC datasets and, importantly, for making a financial effort to acquire meteorological data from IPMA.

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Pulmonol. 2020;**26(3)**:151–158

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ORIGINAL ARTICLE

Prevalence and development of chronic critical illness in acute patients admitted to a respiratory intensive care setting

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Received 26 August 2019; accepted 23 September 2019
Available online 28 October 2019

KEYWORDS
Chronic critical illness;
Acute respiratory distress syndrome;
Respiratory failure;
Mechanical ventilation;
Respiratory intensive care unit;
Tracheostomy

Abstract
Introduction: Chronic Critical Illness (chronic CI) is a condition associated to patients surviving an episode of acute respiratory failure (ARF). The prevalence and the factors associated with the development of chronic CI in the population admitted to a Respiratory Intensive Care Unit (RICU) have not yet been clarified.
Methods: An observational prospective cohort study was undertaken at the RICU of the University Hospital of Modena (Italy). Patients mechanically ventilated with ARF in RICU were enrolled. Demographics, severity scores (APACHEII, SOFA, SAPSII), and clinical condition (septic shock, pneumonia, ARDS) were recorded on admission. Respiratory mechanics and inflammatory-metabolic blood parameters were measured both on admission and over the first week of stay. All variables were tested as predictors of chronic CI through univariate and multivariate analysis.
Results: Chronic CI occurred in 33 out of 100 patients observed. Higher APACHEII, the presence of septic shock, diaphragmatic dysfunction (DD) at sonography, multidrug-resistant (MDR) bacterial infection, the occurrence of a second infection during stay, and a C-reactive protein

Abbreviations: ARDS, acute respiratory distress syndrome; ARF, acute respiratory failure; Chronic CI, chronic critical illness; CRP, C reactive protein; MDR, multidrug-resistant; MV, mechanical ventilation; NIV, non-invasive mechanical ventilation; RICU, respiratory intensive care unit; RR, relative risk; DD, diaphragm dysfunction.

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https://doi.org/10.1016/j.pulmoe.2019.09.006

(CRP) serum level inceasing 7 days over admission were associated with chronic CI. Septic shock was the strongest predictor of chronic CI (AUC = 0.92 p < 0.0001).
Conclusions: Chronic CI is frequent in patients admitted to RICU and mechanically ventilated due to ARF. Infection-related factors seem to play a major role as predictors of this syndrome.

Introduction

In recent years, life support techniques have improved the outcomes of patients with acute respiratory disorders, but have also led to the emergence of a population of survivors even though dependent on mechanical ventilation (MV), the so called ''chronically critical ill-chronic CI''.[1]

In western countries, chronic CI is rapidly growing and it is associated with increased number of days in the intensive care unit (ICU), prolonged hospitalization in post-acute (weaning) centers, and poor prognosis in the long term.[2] Around 50% of patients developing chronic CI die within 6 months following discharge from ICU, while only 10% of survivors return home with autonomy.[3,4]

The clinical features of these patients are consistent enough to define chronic CI as a syndrome characterized by neuroendocrine changes, alteration of body composition, neurological modifications, malnutrition and muscle wasting.[5–7] However, an exhaustive definition of chronic CI is still not available. A recent consensus definition resulted from the combination of two factors: care received in ICU for at least 8 days, and at least one out of five eligible conditions (*MV prolonged* >96 h*; tracheostomy; sepsis or other severe infections; severe wounds and/or multiple organ failure; ischemic stroke, intercerebral hemorrhage or traumatic brain injury*), prolonged MV and tracheostomy were the two reliable indicators of chronic CI occurring in patients with acute respiratory failure (ARF).[8,9]

Although chronic CI is described in the whole population of patients admitted to ICUs,[10–12] there are still no studies focusing on those patients suffering from *de novo* ARF following conditions with a vigorous local and systemic inflammatory response, such as pneumonia and Acute Respiratory Distress Syndrome (ARDS).[13,14]

Primary aim of this study was to describe the prevalence and the development of chronic CI in a population of patients with *de novo* ARF admitted to a specialized RICU. The association between clinical and mechanical features and the development of chronic CI was investigated as secondary outcome.

Materials and methods

Study population

This prospective observational cohort study was carried out in a single 6-bed RICU at the University Hospital of Modena (Italy) over a 24-month period (January 2016–January 2018). Written informed consent to participate was obtained from all patients enrolled or their relatives. Approval was obtained from the local ethics committee of Modena (protocol 839/C.E. and 266/16 C.E.), and the trial was registered at clinicaltrials.gov (NCT03851822).

Eligible patients >18 years of age were those consecutively admitted to the RICU due to ARF (hypoxemia or hypoxemia with hypercapnia) requiring MV, and with a stay >8 day. Exclusion criteria were: 1) refractory shock, 2) patient goals of care not consistent with aggressive management, 3) end-stage chronic obstructive pulmonary disease (COPD) requiring home oxygen and/or ventilatory support, 4) interstitial lung disease, 5) neuromuscular disease, 6) chest wall deformities, 7) pregnancy, 8) chemotherapy or radiotherapy during the past 30 days, 9) brain injury on CT scan and/or Glasgow Coma Scale (GCS) score <8, 10) tracheostomy. All patients were treated according to the best current clinical practice by the attending staff, unaware of the study purpose. All patients were provided with mobilization and rotation on a daily basis.

General measurements

Demographics, severity scores, namely the Kelly-Matthay Scale, the Acute Physiology and Chronic Health Evaluation II (APACHE II), the Simplified Acute Physiology Score (SAPS II), and the Subsequent Organ Failure Assessment (SOFA) were recorded at admission. Presence of ARDS, pneumonia or septic shock was assessed at baseline whereas pre-existing comorbidities were reported through the Charlson Comorbidity Index; concomitant COPD had to be confirmed by both smoking habit, past medical history, non-reversible obstruction at spirometry performed in the year before. Hospitalizations in the previous 6-month were also reported.

Arterial blood gases (PaO_2, $PaCO_2$), pH, and PaO_2/FiO_2 ratio were recorded at baseline; blood procalcitonin, glucose, albumin, creatinine and lactate were measured at baseline and after 1, 2 and 7 days following admission. Multidrug-resistant (MDR) microorganism colonization and infection, and the onset of a second infection during RICU stay were recorded.

Lung mechanics

A subset of patients under MV as divided into two groups according to the ventilatory mode (assisted spontaneous breathing — ASB, and controlled ventilation-CMV) was subjected to measurement of lung mechanics.

Patients in the ASB group underwent esophageal pressure (Pes) and transpulmonary pressure (PL) monitoring performed through a nasogastric tube (NutriVent nasogastric polyfunctional catheter; SIDAM, Mirandola, Italy) with a pressure transducer (OptiVent monitor, SIDAM) and according to a standard procedure.[15,16] PL was calculated as airway pressure (Paw) − Pes. In order to avoid using absolute values for Pes and PL, we always referred to ΔPes and ΔPL from the end-expiratory level, respectively. The waveforms of Paw, Pes and airflow were continuously recorded using a data acquisition system (PowerLab; AD Instruments, Colorado Springs, CO, USA) at a sampling frequency of 100 Hz for offline data analysis.[17]

Patients in the CMV group, underwent ventilation set in control mode with a Vt = 6 ml/kg and positive end-expiratory pressure (PEEP) adjusted on the basis of the incremental FiO_2/PEEP combinations.[18] Measurements of static respiratory mechanics were performed after 30 min of constant flow during MV. The values of pressure were obtained during baseline ventilation with an airway occlusion at the end of inspiration pressing the end-inspiratory hold, until reaching a plateau pressure, and subsequently performing a similar procedure with an end-expiratory occlusion. During the procedure, occlusions that did not produce a clear plateau were discarded. Airway driving pressure (ΔPaw) was defined as the end-inspiratory plateau pressure (Pplat) — positive end-expiratory airway pressure (PEEPtot). All these measurements were performed within 24 h from MV start.

Diaphragmatic function

Ultrasound (US) of the diaphragm was assessed on both sides at admission during spontaneous breathing. Motility of the diaphragm was assessed at the bedside in the semi-recumbent position by a B-mode US device (GE Vivid 7; GE Healthcare Life Sciences, Helsinki, Finland) connected to a 7−12 MHz linear probe as previously reported.[19] Thickening fraction (TF) during spontaneous breathing[19] was calculated as: $\Delta Tdi = (\text{end-inspiration } Tdi - \text{end-expiration } Tdi / \text{end-expiration } Tdi) \times 100$. The best value of three measurements was taken as representative of the diaphragm function and then recorded for analysis.

Definitions and outcomes

Diaphragm dysfunction (DD) was defined according to the presence of TF bilaterally lower than 20%.[19]

Survivors with both hospital stay >8 days and with tracheostomy due to a need of MV > 21 consecutive days for at least 6 h/day were defined as having chronic CI.[1] The prevalence of chronic CI was the primary aim of this study.

According to the Berlin definition, patients were considered as having ARDS if they had: [1] acute respiratory failure not fully explained by cardiac failure or fluid overload; [2] bilateral opacities consistent with pulmonary edema on the chest radiograph or the computed tomography scan; and [3] onset within 1 week after a known clinical insult or new/worsening respiratory symptoms.[20] Septic shock was present according to the onset of sepsis with requirement of vasopressor to maintain a mean arterial pressure ≥65 mmHg and serum lactate level >2 mmol/L (>18 mg/dL) in the absence of hypovolemia.[21] Multi-Drug-Resistant (MDR) infection was defined as the presence of sepsis with blood isolation of a MDR bacterial agent. The onset of a second infection was defined by the occurrence of clinical, radiological and microbiological signs of infection during RICU stay (after at least 2 days of stability when recovering from the first episode). The association between chronic CI and demographics, inflammatory and metabolic blood parameters (C-reactive protein-CRP, sepsis and septic shock, infection and MDR bacterial infection), and respiratory mechanics (transpulmonary and driving pressure) on admission and/or their trends between days 1 and 7, were considered as secondary outcomes of the study.

Statistical analysis

The statistical package GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. Initially, we performed a power test ($\alpha = 0.05$, power 80%) considering a prevalence of up to 20% of patients undergoing MV for >48 h and/or tracheostomy in the critical care area,[7] and an average annual mortality rate of 24% among patients admitted to RICU and requiring MV. A sample size of 127 patients was required to confidently perform analysis on the pre-specified primary scope of our study.

The considered variables were investigated through univariate analysis and contingency table analysis for relative risks to detect the possible risk factors associated with chronic CI once in RICU.

Significant variables then entered a multivariate regression analysis, with a backward stepwise method to exclude non-significant variables from the model. Independent variables able to predict chronic CI at the multivariate test were then entered intoa ROC analysis.

A p-value lower than 0.05 was considered to be statistically significant.

Results

In the period considered, 127 patients were found to be eligible; 27 out of the 127 subjects died in RICU and were excluded from the analysis (see Fig. 1).

Thirty-three patients (33%) developed chronic CI during stay in RICU. Table 1 shows demographics, biochemistry, clinical and physiological characteristics of patients developing chronic CI (n = 33) as compared with others (n = 67). Causes of de novo ARF included acute exacerbation of COPD (n = 45), septic shock (n = 19), pneumonia (n = 10), pulmonary embolism (n = 10), ARDS (n = 7 [pulmonary infection driven n = 5, extra-pulmonary infection driven = 2]), acute exacerbation of asthma (n = 5), lung inhalation (n = 4). In the subset of patients undergoing lung mechanical assessment, ΔPL and ΔPaw were not associated with chronic CI. The trend of CRP levels, but not other blood parameters, over 1-week from admission was different when comparing patients with or without chronic CI (p = 0.037, Fig. 2).

Table 1 also shows the results from the univariate and the multivariate analysis performed to identify potential predictors of chronic CI development in the study cohort.

Higher APACHE II score on admission, the presence of septic shock, DD, and MDR infection, the onset of a sec-

Table 1 Demographics, clinical and physiological variables in the study population, univariate and multivariate analysis of variables as potential risk factors for developing critical care illness in the population in this study.

Outcome	Total = 100 (100)	Chronic CI = 33 (33)	No-CI = 67 (67)	Univariate analysis		Multivariate analysis	
				Relative Risk (95%CI)	p value	Relative Risk (95%CI)	p value
Age (years)	69. 8 (8.6)	66.1 (6.9)	72 (10.2)	0.76 (0.34–1.29)	n.s (0.09)		
Male sex (n)	51 (51)	20 (61)	31 (46)	1.2 (0.43–1.9)	n.s. (0.76)		
BMI	24.5 (6.5)	25.6 (4.5)	24.2 (6.9)	1.4 (0.65–2.1)	n.s. (0.54)		
Kelly score	2.3 (1.5)	2.5 (1.3)	2.2 (1.6)	1.67 (0.87–2.3)	n.s (0.08)		
APACHE II score	23.4 (9.7)	30.1 (12.6)	19.9 (9)	3.5 (1.6–8.2)	<0.001	3.5 (1.2–7.4)	0.002
SAPS II score	39.5 (16.5)	47.3 (18.6)	35 (14.5)	3.9 (1.1–10.3)	0.002		
SOFA score	6.1 (2.5)	7.36 (1.6)	5.4 (2.7)	2.9 (1.2–5.3)	0.01		
Charlson index	2.7 (2)	3.1 (2.5)	2.4 (1.8)	1.5 (0.67–3.5)	n.s. (0.18)		
Presence of COPD (n)	61 (61)	21 (63)	40 (60)	1.5 (0.85–2.6)	n.s. (0.24)		
Pneumonia (n)	10 (10)	4 (12)	6 (9)	1.1 (0.35–3.3)	n.s. (0.9)		
Septic shock (n)	19 (19)	17 (52)	2 (3)	14 (4–37)	<0.0001	7.2 (2–19)	<0.001
ARDS (n)	7 (7)	2 (6)	5 (7.5)	0.7 (0.55–2.7)	n.s. (0.7)		
$\triangle PL$ (cmH_2O)	23.7 (14.2)	24 (12.4)	23.6 (15.1)	0.85 (0.67–4.9)	n.s. (0.85)		
$\triangle Paw$ (cmH_2O)	9 (6.5)	10.7 (7.7)	8 (4.9)	1.6 (0.67–2.6)	n.s. (0.7)		
TF < 20%	17 (17)	13 (39.4)	4 (6)	3.17 (1.9–4.9)	<0.0001	3.7 (1.1–6.9)	0.003
C reactive protein (mg/dl)	14 (13.4)	9.7 (8.5)	18.5 (14.6)	0.4 (0.1–1.7)	n.s. (0.08)		
Procalcitonin (ng/L)	8.6 (5.5)	14.4 (4.2)	6.9 (6.2)	1.8 (0.67–5.9)	n.s. (0.25)		
Glucose (mg/dL)	155 (45)	156 (37)	154.7 (49)	1 (0.80–1.3)	n.s. (0.9)		
Albumin (mg/dL)	3 (1.7)	2.9 (1.5)	3.1 (1.9)	1.7 (0.78–2.56)	n.s. (0.15)		
Creatinine (mg/l)	1.29 (0.8)	1.3 (0.76)	1.27 (0.9)	1.1 (0.77–1.9)	n.s. (0.44)		
Lactate (mg/dL)	17 (10)	22.34 (12)	14.2 (9)	3.5 (0.8–9.2)	n.s. (0.07)		
pH	7.33 (0.1)	7.29 (0.12)	7.36 (0.1)	2.9 (0.91–6.7)	n.s. (0.06)		
P/F ratio	189 (55)	206 (61)	165 (52)	1.4 (0.7–2-3)	n.s. (0.32)		
Arterial pCO_2	60 (11)	66.6 (14)	56 (9)	3.4 (0.81–6.7)	n.s. (0.09)		
Recent hospitalization[a] (n)	29 (29)	15 (45)	14 (21)	1.8 (1–3.6)	n.s. (0.06)		
Multidrug resistant (MDR) micro-organism colonization	26 (26)	13 (39)	13 (20)	1.7 (0.9–3.2)	n.s. (0.14)		
MDR infection	12 (12)	9 (27)	3 (4.5)	4.7 (1.6–13)	0.009	4.7 (1.3–9)	0.021
Second infection during RICU stay	33 (33)	17 (52)	16 (24)	3.17 (1.1–3.1)	0.031	1.9 (1.1–4.5)	0.027
CRP worsening (n)	33 (33)	17 (52)	16 (24)	3.1 (2–8.1)	0.006	2.1 (1.4–6.1)	0.036

Data are presented as number and percentage for dichotomous values or mean value and standard deviation for continuous values.

[a] Within 6 months.

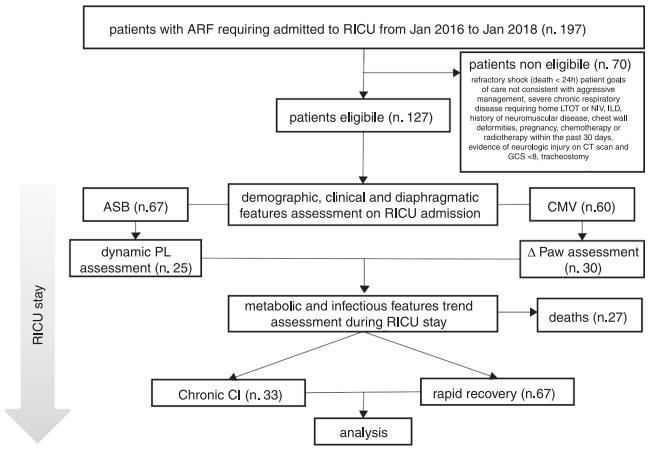

Figure 1 Description of the study population.
ARF = acute respiratory failure, MV = mechanical ventilation, RICU = Respiratory Intensive Care Unit, LTOT = Long Term Oxygen Therapy, NIV = Non Invasive Mechanical Ventilation, ILD = Interstitial Lung Disease, CT = Computed Tomography, GCS = Glasgow Coma Scale, ASB = Assisted Spontaneous Breathing, PL = Transpulmonary Pressure, CMV = Controlled Mechanical Ventilation, Paw = Driving Pressure, Chronic CI = Chronic Critical Illness

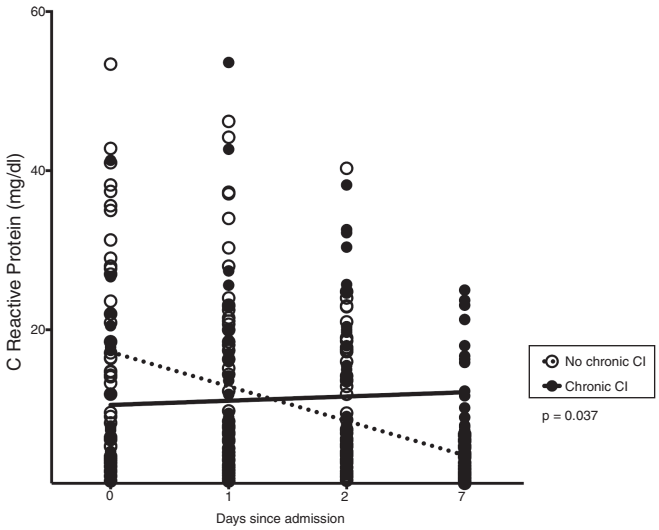

Figure 2 Time course of CRP serum level in patients developing chronic CI or recovering.

ond infection during stay, and a trend of CRP to increase at day 7 after admission were independently associated with chronic CI development; ROC analysis indicated that septic shock was the strongest predictor (AUC = 0.92, p < 0.0001) of chronic CI onset (see Fig. 3).

Discussion

With this cohort study, we have observed a 33% prevalence of chronic CI in patients admitted to RICU due to *de novo* ARF. We have also shown that a worse clinical severity and diaphragmatic dysfunction at admission, early worsening of systemic inflammation, type of infection, and septic

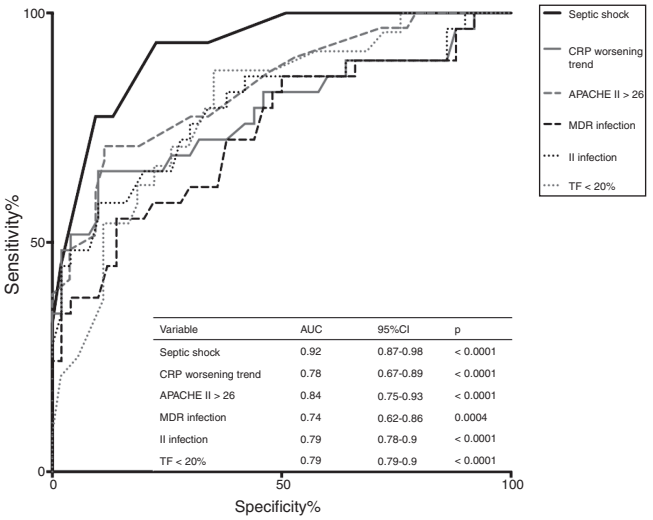

Figure 3 ROC analysis comparing predictors of chronic CI.
AUC = Area under the curve.

shock in particular, are factors independently associated with chronic CI development.

Prevalence of chronic CI in the ICU

Given the lack of a univocal definition of chronic CI, the exact prevalence of this condition remains unknown. As a whole in patients admitted to ICUs, the prevalence of chronic CI defined as the need to prolong MV is around 10%.[1] Moreover, chronic CI represents a predominant clinical feature in up to 50% of patients who survive an episode of sepsis.[10] In our study performed in a medical specialized ICU in patients with *de novo* ARF, onset of chronic CI was lower than that reported in a surgical ICU.[10] However, in the latter study[10] the definition of chronic CI referred to the length of stay in ICU for $\geq$14 days with persistent organ dysfunction as assessed by SOFA score. In our study, chronic CI was defined as the need for respiratory intensive care for $\geq$8 days associated with the decision to perform tracheostomy and to prolong MV. Prolonged mechanical ventilation and tracheostomy are factors that increase per se the risk of bed-lying, thus requiring combined strategies to avoid complications and speed-up recovery in specialized intensive care settings.[22]

Inflammation and chronic CI

During stay in ICU, persistence of inflammatory state is the pathophysiological key which may favor chronic CI development.[23] The term ''*persistent inflammation, immunosuppression and catabolism syndrome*'' (PICS) has been coined to describe a phenotype of chronic CI in surgical ICU patients who suffered from major pro-inflammatory event (blunt traumatic injury/sepsis).[24,25] A self-perpetuating cycle of inflammation constitutes the pathobiological hypothesis underlying PICS development.[26] Patients who develop chronic CI after sepsis exhibit persistent elevations of systemic inflammatory cytokines (IL-6 and IL-8) even at 28 days following onset of ARF,[21] which is

the case of the inflammatory response during ARDS.[27] Persistence of elevated levels of inflammatory cytokines and chemokines in circulating fluids and bronchoalveolar lavage, could lead patients with ARF to chronic CI.[28] In patients with ARDS, longitudinal sampling has proved that local and systemic inflammation may persist for several weeks, even after the resolution of the respiratory syndrome.[29] Recently, both hyperinflammatory and hypoinflammatory subphenotypes were identified analyzing biomarkers plasma levels from two trials in patients with ARDS.[30] The hyperinflammatory subphenotype is characterized by fewer days free from ventilatory and/or organ failure, conditions which constitute a risk factor for chronic CI. In our study, the measurement during the first week after admission of CRP as a marker of inflammation was able to distinguish the group of patients who would develop chronic CI from those who did not and would recover (Fig. 2).

Infection and chronic CI

To study the underlying mechanisms that drive chronic CI progression over the course of sepsis, different animal models and clinical studies in survivors have been conducted.[30–32] Data show that immunological dysfunction and repeated infections have a role in maintaining inflammation and, finally, trigger chronic CI.[33,34] In line with these findings, our study confirms that patients with ARF presenting a second infection during stay in RICU or having a MDR bacterial infection are more likely to develop chronic CI. Overall, this suggests that immunological dysregulation and susceptibility to infection constitute a mechanism favoring the development of chronic CI in these individuals. Interestingly, septic shock, a condition characterized by profound circulatory, cellular and metabolic alterations, was the strongest predictor of chronic CI development in our study (Fig. 3).

DD and chronic CI

In patients suffering from acute critical illness of various etiologies, respiratory muscle dysfunction is quite frequent and may develop early. Demoule et al. have shown that 64% of patients admitted to ICU have DD at the acute onset, sepsis being the major independent risk factor.[35] The mechanisms underlying sepsis-induced DD (SIDD) are complex.[36] At the molecular level, there is evidence that cytokines (TNF-alpha, IL-1alpha, IL-1beta and IL-6) and inflammatory signaling (NF-kappaB pathways) are involved,[37] and may promote mitochondrial dysfunction, reactive oxygen species (ROS) production, and breakdown of sarcomere proteins during infection.[38] In the present study, we have reported that DD (i.e. TF < 20%) by noninvasive assessment early at the time of admission is a risk factor in the specific population of difficult-to-wean patients. To the best of our knowledge, this result is new and opens further research perspectives.

Ventilatory induced lung injury and chronic CI

There is evidence in literature that indicates that MV plays a role in determining ventilator-induced lung injury.[39] In particular, non-physiological stress/strain could act as a trigger and amplifier for local and systemic inflammation.[40] In our study, we measured $\triangle$Paw as a surrogate marker for cyclic lung strain in patients receiving ventilator support[41] and $\triangle$PL as a marker of lung stress in patients with ASB[16]; however, we failed to find any correlation with chronic CI development. Therefore, even though lung mechanics have been used with a small group of patients, data suggest that mechanical stress/strain on lung parenchyma might play only a minor role in the physiopathology behind the onset of chronic CI.

Limitations

Despite intriguing findings our observational study presents some major problems which limit generalizability. First, the evidence of persistent inflammation as a potential risk for chronic CI development lacks measurement of specific inflammatory cytokines. Second, immunological assessment has not been investigated in our population. Third, the definition of chronic CI as applied here excludes those patients with persistent signs of organ dysfunction (i.e. renal insufficiency), or without any need for tracheostomy and prolonged MV.

Conclusions

Patients admitted to RICU with *de novo* ARF frequently progress to chronic CI, infection-related factors being crucial in the pathogenesis. Data here presented warrant further research to clarify all the potential physiopathological factors predisposing patients to chronic CI development.

Ethics approval and consent to participate

Approval from the local ethics committee of Modena was obtained (registered protocol number protocol 839/C.E. and 266/16 C.E). Written informed consent to participate was obtained by all patients enrolled.

Consent for publication

Consent for publication was obtained by all patients enrolled.

Availability of data and materials

Data are available at the Respiratory Disease Unit of the University Hospital of Modena, Italy.

Declarations of interests

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

We would like to thank Colin Woodham at Alpha Science Editors for language editing.

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Pulmonol. 2020;**26(3)**:159–165

PULMONOLOGY

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REVIEW

Physiological rationale of commonly used clinical exercise tests

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Received 16 October 2019; accepted 17 October 2019

KEYWORDS
Exercise testing;
Oxygen uptake kinetics;
Carbon dioxide output kinetics;
Endurance tests;
Six-Minute walk test

Abstract In order to measure cardiopulmonary performance for clinical and investigation purposes we need standardized tests which allow the comparison with standard values, between people, or individuals with themselves over time. The quest for the ideal exercise test has led to the development of several formats, the so called laboratory and field tests. Incremental exercise tests allow measurement of maximal exercise capacity and a host of submaximal variables of great interest. The physiological rationale of the tests and of the detection of interesting submaximal variables can be explained from the oxygen uptake and carbon dioxide output kinetic response to constant power exercise. When the muscles have to produce very high energy, the exercise is physiologically limited to relatively short duration. The minimum power at which an exercise can no longer be sustained for long periods of time is called critical power. Above critical power the time-power function shows a hyperbolic shape. This shape provides the rationale for understanding the properties, limitations and responsiveness to interventions of endurance tests such as constant power test on a cycle-ergometer or treadmill, endurance shuttle walk test and six-minute walk test.

Introduction

Performance during standardized exercise tests (i.e. laboratory and field tests) and their associated physiological or pathophysiological responses are recognized biomarkers of considerable importance in the multidimensional evaluation of cardiac and respiratory diseases.[1–4] From an evidence-based perspective, exercise testing is fundamental to accurately quantify cardiorespiratory fitness,[1–4] it may uncover the pathophysiologic mechanisms underlying exercise intolerance and it is independently related to major outcomes such as survival and hospital admissions.[1–5] Therefore, exercise capacity assessment is a valuable tool to evaluate the severity of impairment, as it provides meaningful clues for tailoring individualized rehabilitative interventions and considerably improves prognostic stratification.

In order to measure cardiopulmonary performance for clinical and investigation purposes we need standard-

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https://doi.org/10.1016/j.pulmoe.2019.10.004

ized tests which enable comparison with standard values, between different people, or individuals with themselves over time. These tests are broadly divided into laboratory-based exercise tests and field tests. Laboratory tests are usually conducted on either a cycle-ergometer or a motorized treadmill. A comprehensive array of physiological system response can be measured, which provide for accurate definition of responses both at the limit of tolerance and throughout the course of the test. On the other hand we have the so called field tests that consist of asking the persons being tested to walk at a specific rate or the fastest pace possible during a specific time. In these tests the physiological information attainable is usually limited.

In this review we will start by describing the responses of the oxygen and carbon dioxide transport and utilization/production systems to a single step increase of power, since they are the cornerstone to understanding the physiological rationale and limitations of all exercise tests.

Single-step moderate constant power exercise

In repose to the onset of constant exercise in the upright position, oxygen uptake ($V'O_2$) initially rises abruptly (phase 1 component). Phase 1 is the gas-exchange expression of the immediate increase in venous blood flow through the lungs as a consequence of both the compression of the veins by contracting muscles and the activation of the sympathetic nervous system leading to increased cardiac chrono- and inotropy and to venous vasoconstriction.[5] Subsequently a more dominant exponential phase 2 component develops, determined by the hemodynamic response (increase in cardiac output and venous extraction in the working muscles). In moderate intensity of exercise (i.e. exercise at which no sustained increase in blood lactate takes place) phase 2I response follows a single-exponential growing function. As phase I is very difficult to identify in single breath-by-breath traces, for the interest of this review, the initial increase in VO_2 can also be modelled by a single exponential growing curve encompassing phase 1 and 2, which time constant is called mean response time (MRT) and is typically between of 30–45 sec in healthy people.[5–8] MRT is the amount of time that it takes to increase VO by a factor of 1-1/e (because 1/e is approximately 0.368, MRT is the amount of time that takes VO_2 to increase to approximately 63.2% of what it is needed to reach the steady state situation (i.e. oxygen supply and demand are matched) corresponding to a given moderate power. Eventually (after a time span of 4× mean response time $\tilde{2}$ −3 min) such a steady state (phase 3 component) is achieved (Fig. 1).[5,9] The steady state VO_2 attained in phase 3 s at any exercise intensity of the moderate domain keeps a remarkably constant relationship with power (VO_2 /Δ power) is $\tilde{1}0\,ml \cdot min^{-1} \cdot W^{-1}$ with slight variations depending on the relative proportions of carbohydrate and fatty acids being catabolized and the involved muscle-fibers.[5]

Carbon dioxide output ($V'CO_2$) follows the same pattern of response, but because at the beginning of exercise the body is able to accumulate a fair amount of the carbon dioxide produced by the working muscles MRT for VCO_2 is longer (50–60 s) and it usually takes $V'CO_2$ around 4 min to reach its steady state [5–7]. Ventilation (V'_E) follows $V'CO_2$ with a slight delay[5–7,10] and heart rate is faster than $V'O_2$[5–8,11,12] (Fig. 1).

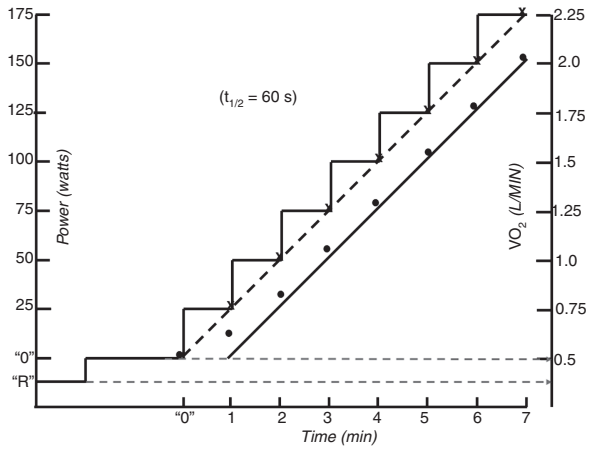

Fig. 1 Schematic of the oxygen uptake (VO_2) response to ramp incremental exercise. It can be seen how in both one-minute step and ramp (solid) increase (dashed) there is a lag between power and oxygen uptake.

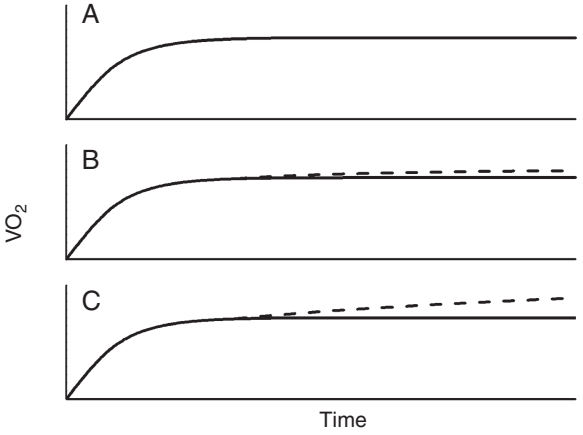

Fig. 2 Schematic representation of the oxygen uptake (VO_2) response to moderate exercise (panel A), Heavy exercise (panel B) and very heavy (above the critical power) exercise (panel 3).

Single-step intense constant power exercise

In contrast to moderate-intensity exercise, which duration will depend on factors such as availability of fuels (mainly glucose, but also certain proportion of fatty acids (especially in trained subjects), dehydration or in real life locomotor or feet injuries, intense exercise is limited by factors related with the oxygen flow to the muscles and deteriorating homeostasis when it does not match the demands. It is beyond the scope of this work to discuss whether there is a bioenergetics threshold at the cellular level, however what it is clear and has been repeatedly demonstrated, is that above a certain intensity exercise, $V'O_2$ kinetics becomes unquestionably different from moderate exercise (Fig. 2).[13–15] Thus, above certain repeatable threshold, the VO_2 keeps on rising after the third minute, resulting in a greater VO_2 proportion with respect to the power than with moderate exercise.[5,13–15] While the physiology of this extra VO_2 (usually called the second component) is not completely understood, it has been modelled as an additional

slow and delayed (by several minutes) VO_2 component superimposed on the phase 2 response.[5] The region of intense exercise can be subdivided in two domains regarding lactic acid accumulation and VO_2 rise (Fig. 2). Below certain exercise intensity, called critical power (CP),[16,17] the increase peripheral oxygen extraction by ensuing acidemia and the metabolic transformation of lactate back to pyruvate by the liver and less active muscles[18,19] can match the lactic acid produced by the exercising muscles and an equilibrium is achieved, discernible because VO_2 and lactic acid rising ends and a new steady state is reached.[5,13–15] Above the CP, though, a steady state is not achievable and VO_2 rises either to its maximum, presumable because the upper limit for O_2 conductance and utilization is achieved,[1,5,15] or to the tolerable limit, because the symptoms associated with approaching to the physiological limits compel the individual to quit.[1,5]

It is not clear whether the ''second component'' of $V'O_2$ derives into increased $V'CO_2$,[7] nonetheless, in the domain of intense exercise new sources of carbon dioxide are added to the metabolic $V'CO_2$. In first place the additional carbon dioxide released by the bicarbonate buffering of lactic acid becomes noticeable soon after lactic acidosis develops and muscle and blood bicarbonate are utilized.[5] $V'CO_2$ consequently becomes higher than $V'O_2$ (since working muscles use mainly glucose as fuel, their respiratory quotient is close to 1 and so does the respiratory exchange ratio (RER) when the muscles are the main metabolic source of carbon dioxide—as it happens in exercise— therefore RER becomes higher than 1 when bicarbonate buffering of lactic acid takes place. As bicarbonate is consumed, this source of carbon dioxide fades away and a different source of carbon dioxide takes over i.e. the carbon dioxide washed out from the body to offset the ongoing lactic acidemia, what it is manifested as hypocapnia.[5,6,10,14,20] The intense exercise $V'CO_2$ kinetic response frequently appears as monoexponential, but this resemblance is deceptive since it is the result of the coincidence of several physiological mechanisms with different temporal characteristics.[7,21]

As with moderate intensity exercise, V_E follows $V'CO_2$ until acidosis develops, afterwards it is driven by the falling pH and hyperventilation, and it is V_E that starts driving the extra $V'CO_2$ release from body carbon dioxide stores (hyperventilation).[5,6,10,14,20] In heavy exercise as in moderate exercise heart rate kinetics is faster than $V'O_2$, nonetheless, heart rate does not reach a steady state, rather it keeps increasing parallel to $V'O_2$.[5,19]

Laboratory tests

Incremental tests

Incremental exercise tests are tests in which the power increase is a linear function of time. These tests are aimed at maximally stressing the O'_2 transport and utilization systems. They are routinely used in the clinical setting because they measure maximal aerobic capacity and provide information about all the physiological domains of exercise (i.e. moderate, heavy and very heavy) as well, in a compact format. These tests consist of either 1-min increases in power or the so called ramp tests with continuous (every 2–5 sec) escalations of power. Imposing linear increase profiles for treadmill exercise can be problematic because most speed/grade increments incorporated into clinical exercise testing do not result in linear increases in power.[22]

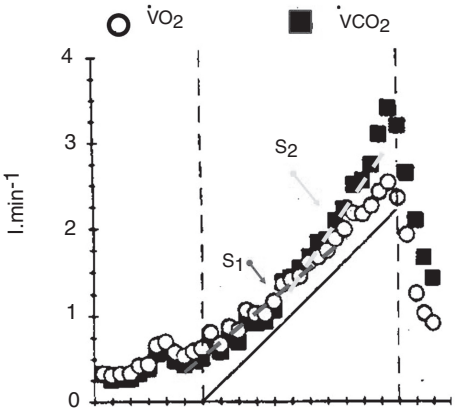

Fig. 3 Showing the profiles of VO_2 and VCO_2 along an incremental exercise test and the S_1 and S_2 slopes.

The cycle-ergometer is used more often than the treadmill because it is less expensive, occupies little space, is less prone to movement artefacts, makes it easier to take additional measurements, requires relatively little practice and unlike the treadmill, the external power output is accurately known.[1,4,5,23] On the other hand, walking on treadmill is more familiar for the patient, and has been proposed that it may better reflect an exercise modality encountered during daily living.[1,4,5,23] Physiological responses to cycle ergometer and treadmill tests differ and so can the physiological factors limiting exercise tolerance. For example, in patients with pulmonary disease, cycle-ergometry results in a greater likelihood for exercise intolerance to result from leg fatigue rather than dyspnoea.[1,4,5,23–26] However, desaturation occurs more frequently walking than cycling in respiratory patients.[27]

The normal $V'O_2$ response to incremental test is the direct consequence of the response to moderate constant power exercise. The expected $V'O_2$ increase approximately $10\,ml \cdot min^{-1} \cdot W^{-1}$ (see before).[5] As the incremental test can be considered to be the continuous sequential summation of constant power stimulus, then the expected $V'O_2$ accumulated response will be also the sequential summation of each step response. Hence, the linear phase of $V'O_2$ lags behind the steady state $V'O_2$ response by the MRT.[8,28] (Fig. 1). Because steady states are never achieved in incremental protocols,[29] the actual $V'O_2$ at any instant of the moderate intensity region will always be inferior to what it is expected for the isochronal power (i.e. $\tilde{1}0\,ml \cdot min^{-1} \cdot W^{-1}$ plus unload pedaling $V'O_2$. This linearity is no longer possible at powers over the LAT due to the superimposition of the $V'O_2$ ''slow component'' to the anticipated value.[5,13–15] It would be expected that above latacte thershold the slope of $V'O_2$ would become higher, however as the slow component is very sluggish, it has been empirically shown that it becomes less than expected at very rapid power increments for the fitness of the person (due to a high anaerobic metabolism), about the same for intermediate power rises and greater, as expected, for slow power increments.[30] Thus, while the

speed of the test does not usually affect the peak $V'O_2$ it changes the $V'O_2$ - WR relationship, making peak power a poor surrogate of peak $V'O_2$.

The $V'CO_2$ response to incremental exercise tests relative to $V'O_2$ in the sub-LAT region is displaced further from the VCO_2 steady state to power relationship because of its slower kinetics.[5,7] This is the reflection of the part of the metabolic carbon dioxide retained in the body's stores.[5,7] Thus, in this domain of exercise, following an initial period of transient carbon dioxide stores accumulation, which may last up to 3 min, the $V'CO_2$ ramp response also becomes relatively linear (S_1 slope) with respect to power. For this reason, in the moderate exercise region RER at the lung will slightly underestimate the tissue respiratory quotient.[31]

At powers above the lactate threshold, the addition of carbon dioxide coming from bicarbonate buffering of lactic acid, drives $V'CO_2$ to an steeper slope with respect to the power increase, this region has been called isocapnic buffering region or ''S_2''[7] (Fig. 3), This change in slope is the physiological rationale for the non-invasive detection of the lactate threshold.[5,7] When the blood bicarbonate becomes insufficient to buffer the lactic acid produced, a new source of carbon dioxide takes over i.e. the hyperventilation phase, characterized by the decrement in end-tidal arterial carbon dioxide pressure and a third change in slope (Fig. 3). The point at which this second change in slope is discernible is called the respiratory compensation point.

The faster the increases in power, the greater the lactic acid production, and therefore the greater the S_2 slope and therefore the RER at maximum exercise may increase at levels higher than 1.2 with rapid tests, but only 1.05 in slower test.[5,7,31] This limits the usefulness of RER as marker of good effort and underlines one of the drawbacks of this format, the standardization of the speed of the power increments, particularly in sick individuals. It is beyond the scope of this review to describe the proposed methods to standardize the speed of the test. The reader is referred to further texts.[4,5,32]

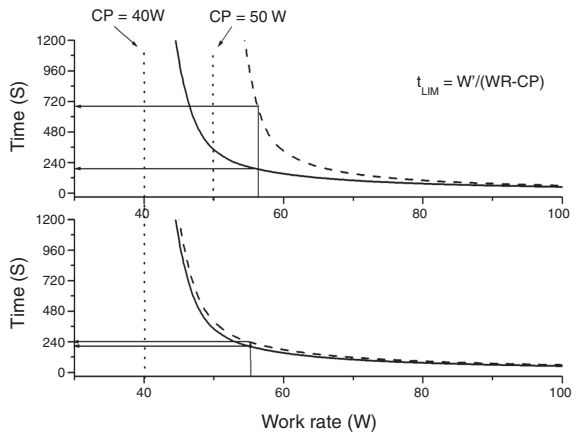

Fig. 4 Power-duration curves before and after interventions endurance time of high intensity exercise.

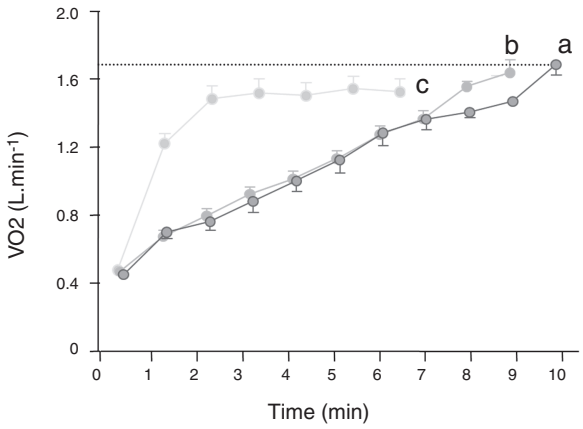

Fig. 5 VO_2 profile in 3 different exercise tests in 8 COPD patients: a) incremental cycle-ergometer exercise test b) incremental shuttle walking test (ISWT) and; c) 6-minute walking test.

Constant power (endurance) tests

The relationship between power and endurance time (t_{LIM}) for constant-work-rate exercise is hyperbolic [33,34], (Fig. 4):

$$t_{LIM} = W'/(P-CP) \quad (1)$$

where P is the imposed power and W' is the curvature constant (having units of work). CP, as we said before, represents the highest power that can be maintained without VO_2 continuing to increase with time towards VO_2max rather than attaining a submaximal steady state.

From the hyperbolic shape of the power-duration relationship (Fig. 4), it can be inferred that both the duration of the test and the magnitude of its response to an intervention will depend on the position of the selected power on the power-duration relationship.[35] The practical expedient of selecting a fixed percentage of the peak power obtained on a prior incremental exercise test takes no account of such issues, since CP does not occur at a fixed percentage of the peak $V'O_2$ (or peak power). This has two major consequences: firstly, the inter-subject variability will be greater than if the intensity for the constant test were normalized to CP[35,36] and secondly, for interventions that increase CP, improvements in t_{LIM} are highly dependent on the difference between selected power for the endurance test and CP.[35,36] Thus, the use of t_{LIM} as a robust measure of efficacy of interventions—as well as in other non-time-limited endurance tests such as the endurance shuttle walking test (ESWT)—for which improvement depends on the magnitude of effect of interventions on CP - requires that the pre-intervention intensity be normalized for the fitness (i.e. CP) of the tested person. However, estimating CP is cumbersome, requiring the performance of several constant tests above the LAT. A practical approach to normalizing constant power test intensity when CP is not known has been proposed.[4]

Field tests

The incremental shuttle walking test

The incremental shuttle walking test (ISWT) is an externally-paced incremental walking test.[37] Subjects are required to walk around two markers 9 m apart (10 m course). Single

audio cues (beeps) signal the time at which the subject is expected to turn at the marker. Walking speed is increased each minute. The ISWT has 12 levels (walking speeds) and therefore lasts a maximum of 12 min. No encouragements are given during the test: the only verbal cues provided refer to an impending increase in walking speed.[37] While the profile of the test is not exactly linear, it is very close[38] (Fig. 5) and available data suggests that ISWT distance correlates well (r = 0.66-0.88) with measured peak $V'O_2$ in incremental tests.[4,39] ISWT performance is usually defined as the distance achieved.

Endurance shuttle walking test

The ESWT[40] is derived from the ISWT, much like the laboratory constant-power test derives from the incremental tests. Like the ISWT, it is externally paced and its intensity is tailored to the exercise tolerance of the individual patient. The ESWT uses the same course and auditory signal method as the ISWT, however, a constant walking cadence is maintained throughout the test. The ESWT starts with a 100 s ''warm-up'' at a slow pace,[40] followed by the ''exercise'' phase at the prescribed speed (typically 80% of ISWT peak) calculated from a previous ISWT.[40] Results are expressed in seconds or in metres.

As the considerations with respect to the power-duration curve for the ESWT are essentially identical to those of the constant-work-rate tests, inter-individual variability of test duration is expected to be high, unless the duration is purposely standardized. There is little information on the mechanisms determining baseline ESWT duration. It is likely that the physiological determinants are analogous to those of constant power test although some evidence suggests that ventilatory limitation may be more prominent in walking than in cycle-ergometry.[41] It is possible that some patients may fail because of a ceiling (see below) effect.[42]

Six-minute walking test

During the six-minute walk test the patient is encouraged to walk at the maximum possible speed compatible with covering as much distance as possible in 6 min. Individuals acquainted with the test and able to complete it in one bout, tend to select a roughly constant pace throughout the test[38] (Fig. 5). Therefore, although the test is not intended to be a constant test, the power (i.e. the speed at which patients carry their own weight during walking) is usually fairly constant,[38] as illustrated by the attainment of a plateau in the oxygen uptake and heart rate responses during the test.[38,43] Moreover, it has been reported that, in individuals familiar with the test and with encouragement, the selected walking speed is comparable to the critical speed (the equivalent of critical power for walking).[38,43] Thus six-minute walk test comes to be a kind of constant power test[38,43] (Fig. 5). However, two particular features make it different, and potentially less sensitive, to interventions than proper endurance tests.[43] On the one hand several studies have shown that respiratory patients tend to select a relatively constant speed of walking[38,43] and, unless walking itself is specifically trained,[45] they may not adopt a faster walking pace after interventions improving their pulmonary function.[43,44] On the other hand six-minute walk has been shown to have a ceiling effect, as the linear relationship between peak VO_2 and six-minute walk test is lost in less impaired subjects.[43,46]

Conclusions

Performance during standardized exercise tests (i.e. laboratory and field tests) and their associated physiological or pathophysiological responses are recognized biomarkers of considerable importance in the multidimensional evaluation of cardiac and respiratory diseases.

The oxygen uptake ($V'O_2$) response when the skeletal muscles have to generate a moderate intensity constant power can be described by a monoexponetial function with an amplitude of 10 ml $\cdot$ $min^{-1} \cdot W^{-1}$ and a MRT usually between 30 and 60 s. At this steady state the oxygen supply matches the oxygen demand of the working muscles. For exercise of higher intensity—coincident with the accumulation of lactate—the $V'O_2$ continues to rise above the steady-state amplitude reached at moderate exercise until a new steady state is attained—this time because lactate metabolism by the liver and less active muscles are able to metabolize the lactate produce by the working muscles—or, at higher intensities until the maximum VO_2 is reached or closely approached. The minimum power at which this second steady state is no longer possible is called critical power (CP). The time that exercises above CP can be sustained is limited and can be modelled as a hyperbolic function of power. From these physiological concepts, on the one hand the amplitude and MRT and on the other the hyperbolic shape of the power- duration relationship, can be explained the profile of VO_2 in incremental test and the duration and responsiveness to interventions of constant work rate tests respectively.

VCO_2 profile is also exponential, but slower because part of the carbon dioxide produced by the active muscles is dissolved in the body water, however its kinetic response is the result of the coincidence of several physiological mechanisms with different temporal characteristics. Indeed VCO_2 comes mainly from three different sources: metabolism, buffering of lactate by bicarbonate and hyperventilation. The different kinetics of these three sources provides the rationale for the detection of the LAT and for the standardization of the speed of the test.

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Pulmonol. 2020;**26(3)**:166–168

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CASE REPORT

Novel complications of the tunnelled indwelling pleural catheter

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Received 2 June 2019; accepted 21 August 2019
Available online 19 November 2019

KEYWORDS
TIPC;
Polyester plug;
Tumour clot

Abstract Tunnelled indwelling pleural catheters (TIPC) are a modality of treatment for malignant pleural effusions. Though relatively easy, safe and efficacious, they are associated with a small risk of complications. We describe newer complications of the TIPC including the retention of the polyester plug and the blockage of the catheter with thick organised material consisting of malignant cells taking the shape of the catheter.

Introduction

Tunnelled indwelling pleural catheters (TIPC) have gained a significant role in the ambulatory management of patients with malignant pleural effusions. The modern day TIPC consists of a multi-fenestrated chest drain of flexible silicone, with a small polyester cuff enveloping the medial portion of the tube. The latter part is tunnelled through subcutaneous tissue before the distal portion enters the pleural space, with the cuff acting as a focal point for fibrous growth to allow the drain to remain in place. At the proximal (external) end is a one-way access valve designed to be attached to proprietary vacuum drainage bottles.[1] TIPC use is safe and efficacious but certain complications have been described.[2] We describe two new and previously unreported complications of the TIPC.

Case summary

Case 1. A 75 year old man was a known case of carotid vasculopathy on clopidogrel therapy, COPD with cor-pulmonale on oxygen therapy and hypertension. In 2017 he was evaluated and proved to have adenocarcinoma of lung (cT4 N3 M0-Stage IIIB), given symptoms of cough, dyspnoea and the chest radiograph and computed tomography (CT) of thorax suggestive of right lower hilar mass encasing the right main bronchus on bronchoscopy guided endobronchial biopsy. The patient was referred for oncology assessment and treated with chemotherapy, radiotherapy and later immunotherapy.

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https://doi.org/10.1016/j.pulmoe.2019.08.008

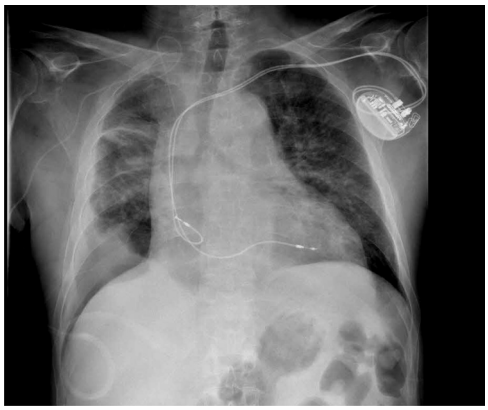

Figure 1 Chest radiograph of case 1 showing right pleural thickening with TIPC in situ.

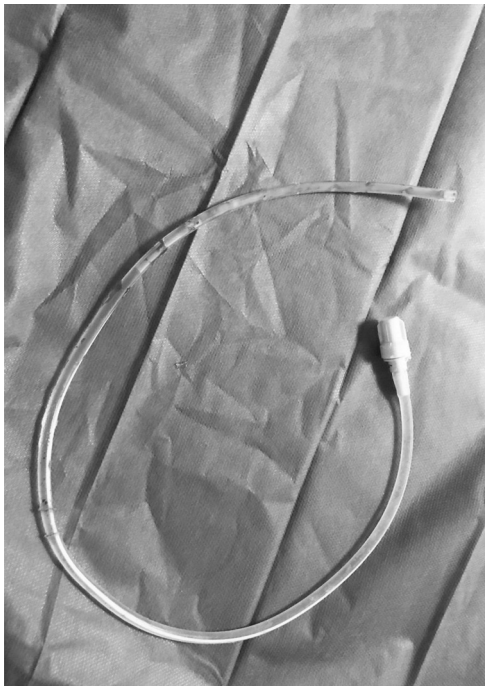

Figure 2 Image of the TIPC of case 1 with the white polyester plug missing.

In 2019, the patient presented with progression of the lung malignancy and a right recurrent pleural effusion which was managed with insertion of the TIPC. After two months of drainage the patient was referred back by the nurse due to nil drainage. In view of spontaneous pleurodesis and chest X-ray (CXR) showing right pleural thickening (Fig. 1), the catheter was removed. Post removal it was observed that polyester plug on the TIPC was missing (Fig. 2). An ultrasound of the local area showed a hypoechogenic spot in the medial tract of the previous tunnelling extending from the subcutaneous tissue down due to the acoustic shadow from the retained plug in the subcutaneous space (Fig. 3). As the patient was on antiplatelet therapy and material being inert; skin and subcutaneous tract dissection was not done. The patient and relatives were informed about the situation and he was sent home with the retained polyester plug.

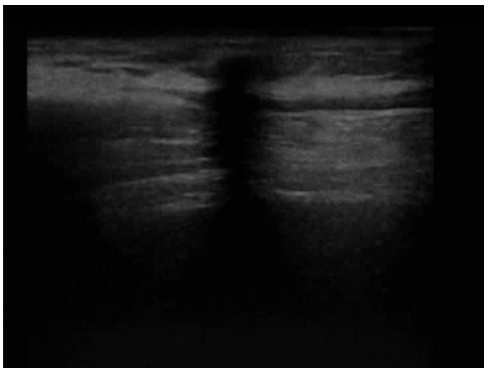

Figure 3 Ultrasound image of the local area showing a hypoechogenic spot in the medial tract of the previous tunnelling extending from the subcutaneous tissue down due to the acoustic shadow from the retained plug in the subcutaneous space.

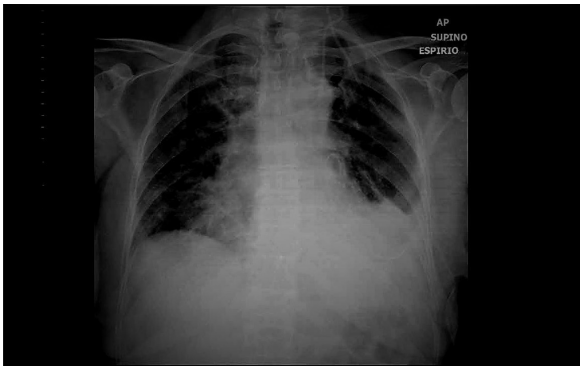

Figure 4 Chest radiograph of case 2 showing left pleural thickening with TIPC in situ.

Case 2. A 77 year old lady, a known case of breast cancer was managed with surgery, chemotherapy and radiotherapy in 1989, then with chemotherapy for chest wall metastasis in 2013. Subsequently she had lung nodules and a small right pleural effusion which was managed with further chemotherapy. In January 2019, she presented with dyspnoea and large metastatic left pleural effusion which was treated with TIPC. In view of non-functionality and CXR suggestive of residual small pleural effusion the IPC was removed after two months (Fig. 4). After removal and dissection of the old IPC, it was observed to be filled with a long, thick, haemorrhagic organised material, completely intact, taking the shape of the IPC and appearing like the plant ''corallo rosso'' (Fig. 5). The material on histopathological examination was positive for metastatic adenocarcinoma cells.

Discussion

Treatment of malignant pleural effusion is intended to be palliative, to relieve symptoms and improve quality of life. The two main approaches involve drainage followed by pleurodesis versus continuous drainage with TIPC. The former is preferred for patients with a longer life expectancy. But the latter is associated with fewer complications and shorter hospital stay.[3] A TIPC is indicated in cases of malignant pleural effusion with symptoms, recurrent pleural

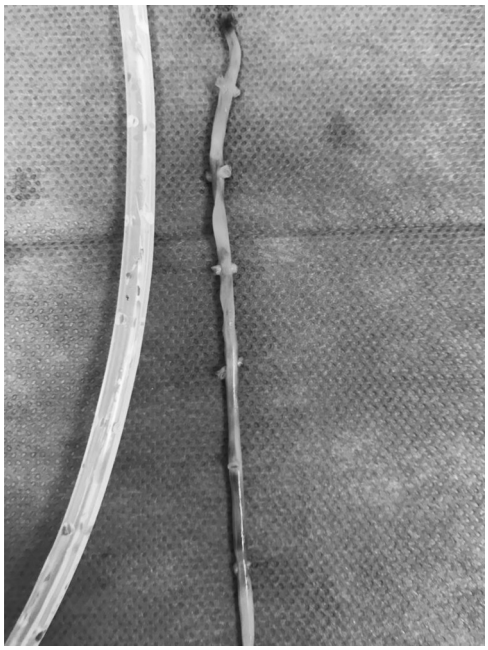

Figure 5 Cut open IPC filled with a long, thick, haemorrhagic, intact organised material taking the shape of the IPC and appearing like the plant ''corallorosso''.

effusions, where the patients have short to intermediate life expectancy or trapped lung or previous unsuccessful pleurodesis.[4,5]

TIPC has been reviewed as being safe and efficacious for management of malignant pleural effusions.[6,7] Lui et al review in detail the complications of TIPC such as pleural infections, catheter tract metastasis, symptomatic loculations, nutrition and cell loss, fractures of catheter on removal, catheter blockage, chest pain and the costs of TIPC management. Catheter related pleural effusions were further sub divided into cellulitis, empyema and tunnel infections.[9] Fysh et al reported complications in 9.8% cases of TIPC removal leading to fracture or iatrogenic severing and four patients having catheter fragments retained within the pleural space without any complications.[10] Grosu et al describe the various reasons for fractures of TIPC such as, greater than 1 cm placement of the catheter cuff within the tunnel, longer than 5 cm subcutaneous tract, catheter tract metastasis, mesothelioma and changes in the manufacturing process of the catheters.[2]

Management of TIPC complications has been reviewed in literature. TIPC related pleural infections can be avoided by continual assessment of patient related, TIPC related and clinician related risk factors, including need for drainage and prompt removal when further drainage was not indicated. Patients with catheter tract metastasis have been treated with analgesia and external beam radiotherapy. Symptomatic loculations have been addressed with pleural aspiration or second catheter placement. General dietary care can cover the nutritional losses due to TIPC drainage. Catheter fractures can be avoided by careful removal, using less traction force. Retention of fractured fragments was not associated with any complications. Saline flush and manipulation along the catheter have been documented in literature to prevent catheter blockage. Chest pain required appropriate analgesia and withdrawal of negative suction if used.[8]

Polyester plug retention in the subcutaneous tissue was a novel complication associated with the TIPC and has never been reported before. The TIPC of our patient was removed intact without any signs of fractures or retained fragments which have been more commonly described. The polyester plug must have mostly organised due to dense fibrosis in the adjacent area and hence was retained on removal. While catheter blockage has been described in literature, our patient had the same thing due to a thick organised material consisting of blood and malignant cells which took a very fascinating shape like that of the plant ''corallo rosso''.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Pulmonol. 2020;**26(3)**:169–180

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LETTER TO THE EDITOR

Balance impairment in chronic respiratory patients

Dear editor:

Balance integrity is critical for an individual's functional independence. Disruption of balance can cause falls with negative consequences for older adults including loss of autonomy and increased morbidity and mortality.[1] Successful maintenance of balance and postural control is a complex skill that requires the integration and coordination of musculoskeletal systems (ie, biomechanics, range of motion, flexibility) and neural systems (ie, motor, sensory, and higher-level pre-motor processes), which must be continuously adapted to suit an array of situations in daily life.[2,3]

Chronic respiratory diseases stand out as leading causes of morbidity and mortality worldwide, with chronic obstructive pulmonary disease (COPD) expected to become the 3rd leading cause of death by 2020.[4] Chronic respiratory patients (CRP) share risk factors that have been associated with an increased propensity for balance impairment, such as muscle weakness and consumption of multiple medications.[5] Among patients with COPD, peripheral muscle impairment, particularly of the lower limbs, is a persistent finding that usually results from or is aggravated by peripheral muscle deconditioning resulting from a reduction in physical activity which is frequently observed in COPD patients.[6] Since lower limb muscle strength plays an essential role in balance maintenance it is not surprising that COPD patients frequently report impairments in balance, coordination and mobility.[6] Complementary to this explanation, another possible cause for balance impairment in CRP is diaphragm weakness which relates to a core deficit that can disrupt the balance.[7]

Structured Pulmonary Rehabilitation Programs (PRP) that include exercise training are currently recognised as a core component of the management of CRP. The ever-growing body of evidence among PRP places it indisputably among the most effective therapeutic strategies to improve shortness of breath, health status and exercise tolerance among patients with COPD.[8] Nevertheless, balance impairment and specific balance training programs still lack robust evidence, and for the time being are not routinely addressed by most Pulmonary Rehabilitation settings.

Given this we conducted a prospective study among the unselected (real-world) CRP patients referred to our PRP, in order to evaluate balance integrity and to unveil potential demographic, functional and clinical factors associated with balance impairment.

We included all CRP referred to our PRP from September 2017 to December 2018. At baseline all patients were evaluated by a dedicated pulmonologist, responsible for the PRP and aside from patients with known associated neuromuscular diseases and those with gait impairment resulting from osteoarthropathies (in which case, balance impairment could not be undissociated from conditions other than the respiratory disease), all patients were offered the possibility of participating upon written informed consent previously approved by our institution's Ethics Committee.

We collected demographic data, primary diagnosis, number of comorbidities and medication, baseline body mass index, baseline results of pulmonary function tests, distance walked in the six-minute walk test (6MWT), modified Medical Research Council (mMRC) dyspnea scale, St. George Respiratory Questionnaire (SGRQ) and Hospital Anxiety and Depression (HADS) scores. Balance assessment was performed through the fulfilment of three tests: Timed Up and Go (TUG) test, Tinetti test (TT) and Activities-Specific Balance Confidence (ABC) scale. The TUG was used to provide a timed measure of balance and functional mobility in our patients. Briefly, in this test, the patient rises from a standard chair, walks 3 m at a normal pace, walks back to the chair, and finally sits down. Two attempts are made, and the best is recorded. A test duration scored below the upper limit of the 95 % confidence intervals normalised for age can be identified as having a lower performance (impaired balance) (9). The TT consists of 19 items divided into two sections: balance (9 items) and gait (10 items). Individuals scoring less than 26 points are considered to have an increased risk of falling.[10] Finally, the ABC scale requires the patient to indicate the degree of confidence, measured in percentage (0–100 %), in performing 16 activities without losing balance or becoming unstable. This test has been previously validated for use within the Portuguese population[11] and it is especially beneficial for addressing the efficacy of specific intervention.

Statistical analysis was performed with the software SPSS version 24. Results are presented as medians and range for non-normally distributed continuous variables and as percentage of total for categorical data. Inferential analysis was performed with U-Mann-Whitney test and Pearson chi-square for continuous and categorical variables, respectively, considering a significance level of 5 %.

We enrolled 28 patients, mainly male (89.3 %) aged 66 (35–78) years. The most frequent diagnosis was COPD, 60.7 % of patients, followed by interstitial lung diseases (ILD),

Table 1 Baseline characteristics of COPD *versus* ILD patients.

	COPD	ILD	p-value
Patients	17	11	
Age (years)	67 (35–78)	65 (51–77)	0.279[a]
Males	15 (88.2)	10 (90.9)	0.664[b]
BMI (kg/m^2)	25.4 (17.8–35.8)	25.4 (17.1–32.2)	0.527[a]
Nº comorbidities	2 (1–7)	2 (1–4)	0.939[a]
Nº medications	4.5 (2–11)	4 (1–10)	0.550[a]
FEV1 (%)*	**37.1 (19.4–56.4)**	**74 (40.1–121.9)**	**0.001**[a]
6MWT (meters)	413 (267–522)	400 (324–440)	0.266[a]
mMRC	2 (0–4)	3 (1–4)	0.195[a]
SGRQ (%)	51.9 (30.1–80.5)	47.4 (37.9–66.7)	0.537[a]
HADS Anxiety	7.5 (2–14)	8 (2–12)	0.816[a]
HADS Depression	8.5 (2–10)	6.5 (0–11)	0.938[a]

Data are presented as median (minimum-maximum) for non-parametric continuous variables and number (percentage) for categorical variables.

Definition of abbreviations: COPD: chronic obstructive pulmonary disease; ILD: Interstitial lung disease; BMI: Body mass index; FEV1: Forced expiratory volume at the 1° second; 6MWT: 6-minute walk test; mMRC: Modified medical research council dyspnea scale; SGRQ: St. George Respiratory Questionnaire; HADS: Hospital anxiety and depression scale;

[a] U-Mann-Whitney test for continuous variables.

[b] Pearson chi-square for categorical variables.

[*] p-value < 0.05.

39.3 % of patients. The median number of comorbidities was 2 (1-7), and medication was 4 (1-11) per patient. Baseline FEV1 was 47.6 % (19.4–121.9), and the distance walked at the 6MWT was 409 (267–522) meters. The mMRC was equal or superior to 2 in 71.2 % of patients. Median SGRQ was 49.5 % (30.1–80.5), HADS in anxiety 7.5 (2–19) and in depression 7.5 (0–11). Aside from the somewhat expected difference in FEV1, patients with COPD and ILD scored similar results in all of the above measures at baseline (Table 1). The results of the balance tests revealed a TUG of 6.36 (5.15–9.22) seconds, a TT of 26 (22–28) and an ABC scale of 70.63 % (39–94). Of the total, 67.8 % of patients had at least one abnormal balance test.

When comparing patients with and without balance impairment, we found no differences in relation to age, gender, body mass index and overall disease severity and functional capacity (assessed by FEV1 and distance walked in the 6MWT). The initial assessment of symptoms and quality of life scores was also similar in both groups. Nevertheless, patients with a higher intake of medications ($p = 0.035$) were more prone to present balance impairment. Co-morbidities also seemed to influence negatively the presence of balance impairment, but the p-value, though borderline, did not reach statistical significance ($p = 0.066$). Detailed results are presented in Table 2.

When exploring the results per group of pathology, we found that both COPD and ILD patients scored similar results in the balance tests performed at baseline. Due to the low sample size of each individual group, statistical inference could not be performed to unveil individual factors associated with balance impairment per group of pathology.

Our results are in agreement with those of more extensive studies addressing this issue and confirm balance impairment as a frequent finding within CRP.[3,6,12] When unveiling potential factors associated with this impairment our results were only positive for the total number of medications in use, regardless of the type of medication. Previous publications on this subject have described a positive association between psychotropic medication use and balance impairment in older and middle-aged adults[12–14]; which from a conceptual point of view is easier to understand since most of these drugs can have direct effects on balance control. In our sample, we postulate that both the cumulative effect of multiple medications (raising a higher possibility of drug-drug interactions) as well as the overall higher number of comorbidities (which is perhaps the basis of the higher number of medications) can offer an explanation for the higher prevalence of balance impairment in this group of patients, but of course further studies and larger samples are required to confirm this hypothesis.

Due to the limited sample size we could not evaluate the presence of factors associated with balance impairment in each individual pathology group, which would be desirable since ILD and COPD patients have different pathophysiological pathways. Nevertheless we could observe that neither FEV1 nor the distance walked in the 6MWT (two commonly used physiological markers of disease severity) were associated with balance impairment in our sample. On the other hand side, COPD and ILD patients were seemingly comparable in relation to number of medications, the only variable that proved to be associated with increased balance impairment in our analysis.

We acknowledge that our study has some limitations regarding sample size and also the operational characteristics of some of the balance tests utilised. This is particularly important in the specific case of the TUG for which the cut-off used by age can vary significantly from population to population and larger validation studies in our specific population would be desirable.[9] To overcome this limitation, we chose to utilise all three balance tests as previously

Table 2 Comparison of patients with and without balance impairment.

	Total	Normal Balance	Impaired Balance	p-value
Patients	28	9	19	
Age (years)	65.5 (35–78)	67 (35–77)	64 (51–78)	0.699[a]
Males	25 (89.3)	8 (88.9)	17 (89.5)	0.963[b]
BMI (kg/m^2)	25.4 (17.1–35.8)	25.7 (17.8–34.0)	25.0 (17.1–35.8)	0.829[a]
Nº comorbidities	2 (1–7)	2 (1–3)	3 (1–7)	0.066[a]
Nº medications*	**4 (1–11)**	**1 (1–5)**	**5.5 (1–11)**	**0.035**[a]
FEV1 (%)	47.6 (19.4–121.9)	50.4 (32.4–86.6)	43.5 (19.4–121.9)	0.537[a]
6MWT (meters)	409 (267–522)	409 (292–451)	409 (267–522)	0.554[a]
mMRC	2 (0–4)	3 (1–4)	2 (0–4)	0.285[a]
SGRQ (%)	49.5 (30.1–80.5)	48.7 (30.1–80.5)	49.8 (40.8–69.3)	0.721[a]
HADS Anxiety	7.5 (2–14)	10.5 (2–12)	7 (2–14)	0.589[a]
HADS Depression	7.5 (0–11)	7 (0–11)	7.5 (2–10)	1.000[a]

Data are presented as median (minimum-maximum) for non-parametric continuous variables and number (percentage) for categorical variables.

Definition of abbreviations: BMI: Body mass index; FEV1: Forced expiratory volume at the 1° second; 6MWT: 6-minute walk test; mMRC: Modified medical research council dyspnea scale; SGRQ: St. George Respiratory Questionnaire; HADS: Hospital anxiety and depression scale;

[a] U-Mann-Whitney test for continuous variables.

[b] Pearson chi-square for categorical variables.

[*] p-value < 0.05.

described, but we are aware that there is still a possible slightly biased estimation of balance impairment within our sample.

We are currently preparing to conduct a second phase of this study where we aim to both enlarge the sample size and evaluate the cut-off validation for TUG. In this second phase of the project, we also expect to ascertain the benefits of both standard hospital based PRP as well as specific balance training (as an add-on to standard PRP) in balance-impaired patients.

Nevertheless, we consider that our findings so far, impart an essential message to clinicians dealing with CRP: the careful review of the individual patientś drug chart should represent an indispensable step of the baseline evaluation, and awareness of overuse of medication should be emphasised as it can contribute to balance impairment.

Conflicts of interest

The authors have no conflicts of interest to declare.

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8 April 2019
Available online 12 December 2019

https://doi.org/10.1016/j.pulmoe.2019.11.009
2531-0437/

Lung transplant complicated with unexpected neoplasm in explanted lungs: A difficult challenge to manage

Introduction

Lung transplantation is an established treatment for end-stage lung diseases such as fibrotic interstitial lung diseases (ILD) and chronic obstructive pulmonary disease which are both the most common indications for lung transplant.[1,2]

Despite extensive pre-transplant evaluation, neoplasm can be found in explanted lungs with an incidence ranging between 0.8% and 2.2%,[3] for several reasons. The differential diagnosis between imaging features associated with pulmonary fibrosis such as fibrotic nodules, ground-glass opacities or consolidations may be difficult to distinguish from neoplastic nodules and invasive diagnostic approaches may not be recommended due to associated risks such as pneumothorax and worsening respiratory failure.[1,3] The incidental finding of neoplastic diseases raises difficult issues in management due to the need for immunosuppressive therapy with unpredictable effects on disease progression, interactions with chemotherapy and other oncology treatments and with increased risk of infection and other complications.

We describe a clinical case in which the unexpected finding of adenocarcinoma in the explanted lungs poses delicate management problems.

Clinical case

We present a case of a 44-year-old woman, former smoker for 10 years (21 pack-years) with previous contact with birds during six years. The patient was referred to a Diffuse Lung Diseases clinic due to progressive fatigue, worsening in the previous two years, with exertional dyspnoea (modified Medical Research Council [mMRC] — 2); no other respiratory symptoms were noticed. A thoracic computed tomography (CT) showed a diffuse pulmonary cystic disease suggesting advanced Langerhans cell histiocytosis (Fig. 1). Blood tests were unremarkable. Pulmonary functional tests revealed a mild pulmonary restriction (FEV_1 68% of predicted, FVC 68% of predicted, FEV_1/FVC 86%, TLC 64% of predicted) and a marked decrease in DL_{CO} (16% of predicted, increasing to 20% when corrected to the alveolar volume). The 6-minute walk test showed desaturation to 62% and a walking distance of 260 meters. The histology from surgical lung biopsy confirmed hypersensitivity pneumonitis (HP) and pulmonary Langerhans cell histiocytosis (PLCH).

In spite of immunosuppressive treatment with prednisolone (10 mg/day) and mycophenolate mofetil (MMF), she presented clinical, functional and radiology worsening and was referred for lung transplant two years after the initial diagnosis.

A year and a half later, she underwent a bilateral lung transplant under extracorporeal membrane oxygenation (ECMO). Histopathology analysis of explanted lungs identified advanced pulmonary fibrosis and multi-focal and bilateral nodules of adenocarcinoma with solid and acinar pattern, positive for TTF-1 and CK7 with focal invasion of the visceral pleural. Post-transplant staging through 18-flourdeoxyglucose positron emission tomography (18-FDG-PET) did not suggest evidence of distant metastasis. Two-months later, due to severe back pain, a hip-CT showed a lytic lesion in the sacrum with joint bone destruction and cortical disruption. The 18-FDG-PET revealed a hyper-metabolic focus (maximum SUV 11.6) in the same location. The tumor was then classified according the American Joint Committee on Cancer (AJCC) 8th edition as pT4N0M1b (stage IV). Molecular characterization showed 10% of tumoral cells expressing the PD-L1 receptor and only the not targetable mutation [c.34 G > T (p.Gly12Cys)] in the KRAS gene (exon 2) was detected by Next-Generation-Sequencing (NGS).

The patient never met clinical conditions for systemic neoplastic treatment due to recurrent respiratory infections, which were further complicated by progressive stenosis of the right bronchial anastomosis despite endoscopic dilation attempts (Fig. 2A). She underwent stereotactic radiotherapy on the bone lesion for palliative pain control and was referred to the palliative care unit. Despite all efforts, she had an unfavorable course with documented progression of the oncological disease with progressive enlargement and metabolic increase of bone involvement (SUV 21.9) and with emergence of voluminous infra-carinal adenopathy of high metabolism (SUV 15.7) (Fig. 2B,C).

She died 12 months after lung transplant due to septic shock and respiratory failure.

Discussion

The current case shows that distinguishing fibrotic interstitial lung diseases (ILD) from lung cancer can be a diagnostic challenge. The prevalence of lung cancer in interstitial pul-

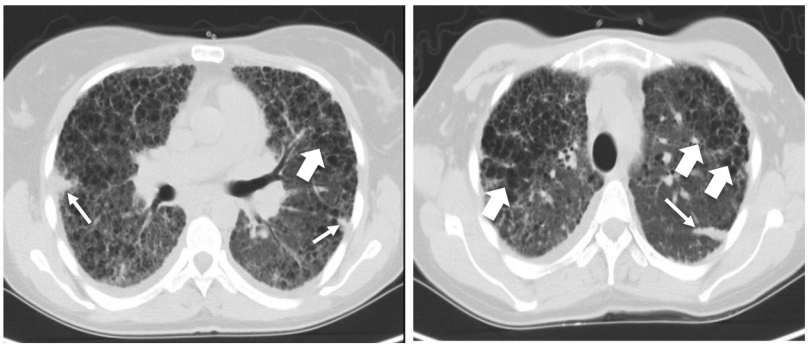

Figure 1 Pre-transplant computed tomography of the chest in lung window showing a diffuse change in the pulmonary architecture with multiples cists, some of them confluent with thin walls (large arrows) in relation with Langerhans cell histiocytosis. It is also described a septal thickening due to confluence of fibrotic areas with formation of some nodules (thin arrow).

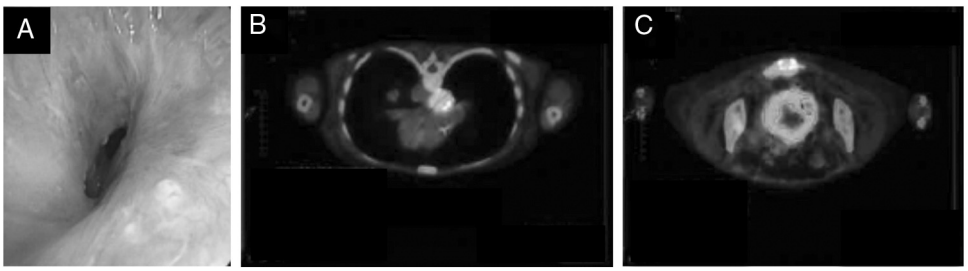

Figure 2 A) Bronchoscopy showing the right bronchial anastomosis stenosis. B) Positron emission tomography (PET-CT) showing the emergence of voluminous infra-carinal adenopathy of high metabolism (SUV 15.7) and C) worsening of metabolic (SUV 21.9) and dimensional size of the bone lesion.

monary fibrosis (IPF), the most common ILD, ranges from 2.7% to 48%,[4] significantly higher than in general population. It is recognized that both entities share multiple common genetic, molecular and cellular processes. Some studies also describe a preferential distribution of tumors in lower lobes suggesting a relationship between fibrotic areas and cancer development, a phenomenon called as ''scarcinoma'', although it is premature to prove a direct association with this finding.[5]

PLCH is known as an uncommon interstitial lung disease in young adults with an unpredictable course that may be associated with an increased susceptibility to the development of malignant neoplasms.[6] Lung cancer is the most common solid organ malignancy occurring in approximately 5% of PLCH patients.[7] An interesting feature related to the pathogenesis of PLCH is the presence of a recurrent $BRAF^{V600E}$ mutation present in almost 50% of LCH lesions.[8] This mutation is also found in other different tumors[9] supporting the association between PLCH and neoplastic diseases. Smoking habits are another recognized potential risk factor for both PLCH and lung cancer. It is estimated that more than a half of PLCH patients have previous or current smoking habits.[9]

Since an active neoplasm is an absolute contra-indication for lung transplant, its diagnosis during pre-transplant evaluation is crucial.[10] Patients with fibrotic ILD and solid pulmonary nodules are challenging to approach. There are no protocols to guide the surveillance of such nodules: CT screening is recommended when the nodules are inferior to 8 mm, and PET-CT should be performed when the nodules are over 8 mm with a low or moderate pretest probability of malignancy.[5] Endobronchial ultrasonography with transbronchial needle aspiration is one of the diagnostic techniques to be considered for histological sampling in patients with high pretest probability of malignancy.[2] In our particular case, during the time on waiting list for transplant, annual thoracic CT showed progression of disease to pulmonary fibrosis with pulmonary nodules that remained stable over time. Evaluating retrospectively, some of those nodules could have been of neoplastic origin, however the invasive diagnostic approaches available at that time were extremely risky for the patient due to the possible fatal complications, namely respiratory failure.

An interesting aspect of this case is the disagreement between the initial and post-transplant histological diagnosis. As far as we know, there are no published papers analyzing the histological transformation that can occur after lung transplant, despite descriptions of some ILDs evolving to pulmonary fibrosis over time. An unpublished retrospective study demonstrated that in the majority (80.5%) of patients submitted to lung transplant due to ILD their explanted lungs showed a histologic pattern of usual interstitial pneumonia (UIP), although only one third of them had previous diagnosis of IPF.[11] This finding suggests a fibrotic evolution between initial diagnosis and lung transplant in non-IPF patients, which is consistent with the finding of pulmonary fibrosis on the resected lungs from our patient.

Another increased risk factor for neoplastic disease is the immunossupression that was started some years before the lung transplant. MMF is inclusively described as being

related to tumor progression, possibly due to various mechanisms, including disruption of apoptosis and DNA repair.[3] After lung transplant, managing immunosuppressive therapy was the most challenging approach. It was decided to discontinue MMF and remain with tacrolimus and prednisone at the lowest limit of the desired range.

This case offers some noteworthy learning points. The increased risk of primary lung cancer in lung transplant candidates must be acknowledged. Certain imaging features, including pulmonary nodules or masses, should be followed or biopsied, depending on how advanced the ILD is and the urgency for transplant. Patients with ILD should undergo bronchoscopy lavage for cytological samples. It is important to have a high level of suspicion for neoplastic disease since the clinical scenario does not always point to this differential diagnosis. This is reflected in the present case, as the patient had only a remote smoking history and had no on-going weight loss or other signs that would suggest a concurrent malignant disease. This case also reinforces the need for research for noninvasive blood tests that could identify neoplasms. These tests would be extremely useful in such challenging clinical situations, once the invasive alternative diagnostic approaches they have are highly risky.

Conflicts of interest

The authors have no conflicts of interest to declare.

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13 May 2019

https://doi.org/10.1016/j.pulmoe.2019.10.002
2531-0437/

Risky diving

Dear Editor,

The mediastinum is an anatomic compartment in the thoracic cavity located between both pleural cavities.[1] Pneumomediastinum is a rare clinical condition in which air leaks into the mediastinum[2,3] resulting from physical trauma or other situations that lead to air escaping from the lungs, airways or bowel into chest cavity.

It has an incidence of 1: 44,500 patients admitted to the emergency room[3] and it may also be spontaneous[4] or secondary to other clinical situations (iatrogenic or non-traumatic).[2,3] The majority of patients are males accounting for 76% of reported cases. Many authors believe that it is an underdiagnosed condition as symptoms may be easily attributed to other causes.[3] One of the possible etiologies is an abnormal increase of intra-mediastinal pressures,[3] which forces the air into the intra-thoracic tissues to balance pressures.[5,6]

Diving associated pneumomediastinum has been progressively increasing and occurs mainly during the decompression phase.[7] Patients may have cervical pain or swelling,[2] dyspnea, cough, thoracalgia[2,6] and less frequently anxiety, dysphagia, sialorrhea and fever.[2]

Although the diagnosis is usually confirmed by thoracic radiography,[3] this exam may be normal in about 30% of the patients[2] and for these, a computed scan tomography (CT) is mandatory.[3] Laboratory findings are frequently inconclu-

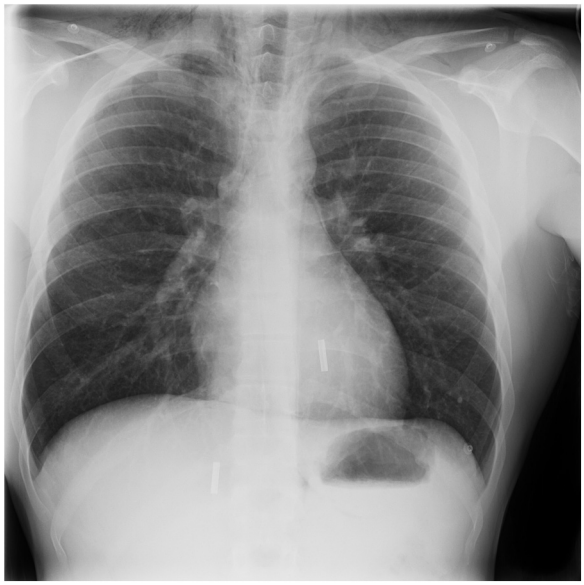

Figure 1 Chest radiography with evidence of pneumomediastinum.

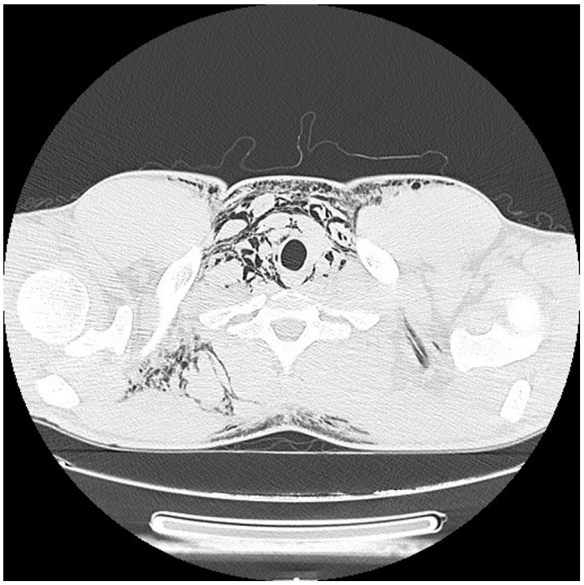

Figure 2 CT scan of the patient.

sive but some may reveal minor elevation of inflammatory parameters.[3]

The authors present the case of a 21-year-old male professional fisherman, who, after 90 min of surface diving, increased dive depth to seven meters with compressed air bottle. At this point he emerged rapidly after feeling an unusual thoracic discomfort, which become worse as he ascended. Immediately after the emersion he developed complaints of cervical swelling and dysphonia, and was admitted to our hospital emergency department. Physical examination revealed a subcutaneous cervical and supraclavicular emphysema in chest radiography (Fig. 1), which later extended to the abdominal region. The patient remained hemodynamically stable, with peripheral oximetry between 98 and 100% breathing room air.

Besides a smoking habit (seven smoking pack year) his previous medical history was unremarkable. No relevant alterations in peripheral blood analysis were observed but thoracic CT confirmed the presence of pneumomediastinum with subcutaneous emphysema (Fig. 2). The Hyperbaric Medicine Service decided that the patient did not need be exposed to hyperbaric treatment as there were no neurological symptoms. Nasal canula oxygen treatment was initiated to increase gas reabsorption and he was closely monitored for potential esophagic or tracheal rupture in the following 24 h.

After that period, patient was discharged as there was total subcutaneous and mediastinal emphysema reabsorption without evident sequelae. The subsequent follow-up appointment showed no clinical or imagiological evidence of relapses. He followed a respiratory functional study as an outpatient which was normal.

Pneumomediastinum is usually a benign medical situation[3] and although there is no consensus regarding treatment, most studies support a conservative approach with rest and analgesia.[6] Oxygen administration can increase gas reabsorption up to six-times and should be considered as an alternative treatment.[3] Relapses are rare, so a short-term medical surveillance is recommended.[3] This condition may be responsible for a high incidence of morbimortality such as facial or cervical lesions and esophagic or tracheal rupture,[2] which justifies a complementary study carried out after the acute onset. Although spirometry is not recommended in the acute setting, however, it must be performed to exclude pulmonary fragility that may worsen the overall pneumomediastinum prognosis.

With this case, the authors aim to alert to a rare and otherwise underdiagnosed situation which, although benign, requires a prompt diagnosis and acknowledgement of the risks that may be associated.

Funding

The authors declare that no funding was received for this paper.

Conflicts of interest

The authors have no conflicts of interest to declare.

Author contributions

- Catarina Cascais-Costa wrote the paper.
- Gilberto Teixeira contributed to data collection.
- Gilberto Teixeira and Lília Andrade contributed to the revision of the manuscript.
- All the authors read and approved the final manuscript.

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1 June 2019

https://doi.org/10.1016/j.pulmoe.2019.09.010
2531-0437/

Community-acquired *Klebsiella pneumoniae* liver abscess: a case complicated by metastatic lung abscesses

To the Editor,

A 74-year-old caucasian male, presented with a two-week history of fever, shivering, right scapular pain and dry cough. His medical history recorded high blood pressure, smoking and alcohol drinking. He had no recent history of antibiotic use, hospitalization or travelling to foreign countries. On admission he was sweaty and febrile, without any other significant alterations on physical examination. Laboratory analyses detected hypocapnia, elevated white blood count, C-reactive protein level as well as elevated liver enzymes. Chest X-ray revealed faded round densities on both lungs while abdominal ultrasound showed an hyperreflective liver with an oval, hypoechoic and heterogenic lesion (7.3×3.9 cm). Patient was hospitalized and started on doxicicline. Abominal MRI confirmed the nodular lesion in the the IV/VIII segments of the liver, with fluid and internal septae (Fig. 1). Simultaneously, the patient was diagnosed with diabetes mellitus and Kp resistant only to ampicillin was isolated in 2 blood cultures. The antibiotic was changed to amoxicillin-clavulanic acid plus metronidazole and the patient became afebrile and without pain. Percutaneous drainage was not executed due to high risk related to subphrenic location. Subsequent contrast CT-scan also revealed multiple nodules on both lungs, mostly peripheral, the bigger ones being cavitated and were considered as septic pulmonary emboli (Fig. 2). The patient was discharged after 2 weeks, antibiotics were continued until 8 weeks and no recurrence has been reported after 2 years .

We believe that this patient had a distinctive form of community acquired *Klebsiella pneumoniae* (Kp) infection causing liver abscess and complicated with septic metastatic pulmonary emboli, forming abscesses.

Liver abscess due to *Klebsiella pneumoniae* (KLA) is a distinct syndrome characterized by monomicrobial liver abscess, almost exclusively acquired in the community and in the absence of hepatobiliary disease. It is strongly associated with diabetes mellitus and Asian ethnicity and has a higher probability of complicating with metastatic infection sites than liver abscesses of other etiologies.[2] Some particularly virulent strains expressing hypermucoviscous phenotype are responsible for this invasive syndrome, despite not being naturally resistant to antibiotics.[3] It was geographically confined to Southeast Asia until the past decade, when other reported cases indicate the emergence of this syndrome worldwide.[3] In up to 11–12% of cases, KLA can be complicated with other septic metastatic lesions.[3]

Our patient was Caucasian and a 74-year-old male, consistent with published demographic data.[3] He fulfilled the requested diagnosis criteria for KLA[2,5,6]; 1. Clinical symptoms and laboratory findings of liver abscess: fever, chills, referred scapular pain, elevation of white-blood cell count and C-reactive protein, abnormal liver function tests; 2. Compatible imaging: as in this case, KLA has distinctive imaging features, being more often single, solid in appearance and septated, comprising multiple non-communicating locules; 3. Isolation of Kp in blood culture/abscess aspiration culture: although serotyping was not conducted, antimicrobial susceptibility of Kp isolated in blood culture meets the characteristic pattern of virulent KLA, described to be resistant to ampicillin and ticarcillin/carbenicillin but susceptible to all other antibiotics.[2,3,5]

That this patient had no underlying hepatobiliary disease, no previous hospitalizations or antibiotic use having acquired Kp in the community, also favored this diagnosis. Furthermore, he was simultaneously diagnosed with diabetes mellitus, the most common host risk factor for KLA.[3] Metastatic complications are more frequent in KLA than liver abscesses of other etiologies[2]; They can occur in up

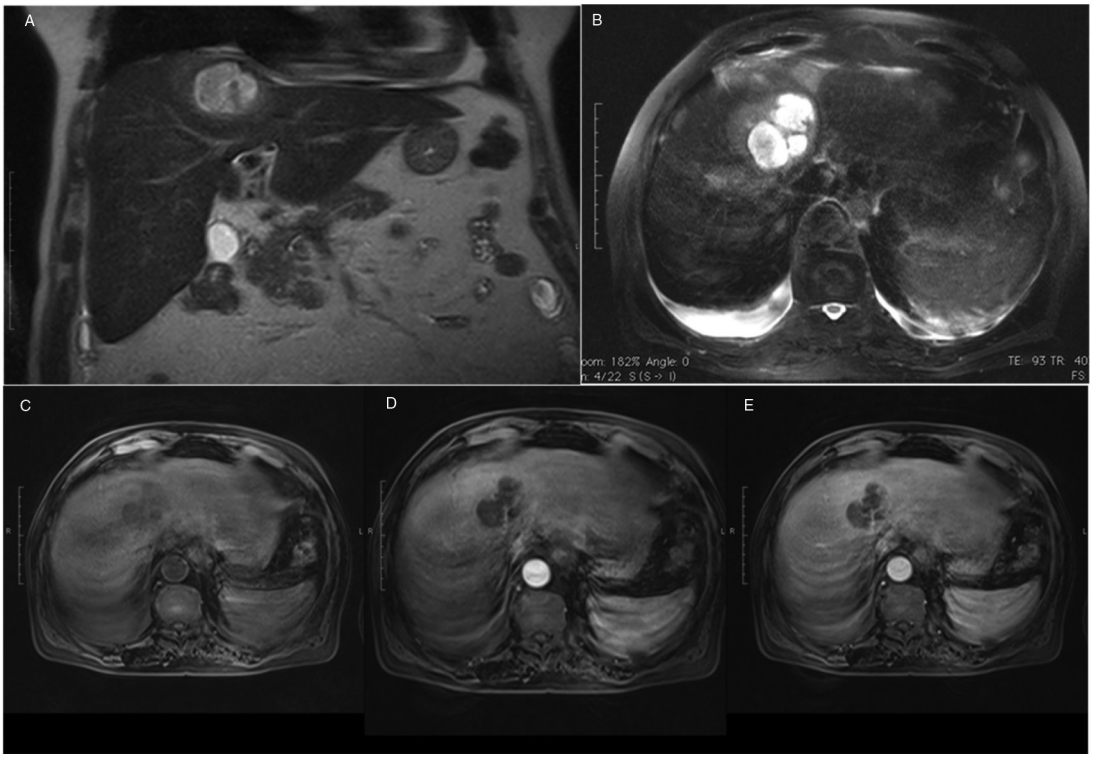

Fig. 1 Abdominal MRI (A) Coronal plan, T2 HASTE sequences – nodular lesion in the IV/VIII segments of the liver (B) Axial plan, T2 FS weighted sequences – hypersignal with fluid and internal septae (C) before gadolinium injection (non-enhancement phase) (D) after gadolinium injection, arterial phase – without contrast enhancement (E) after gadolinium injection, portal phase- peripheral contrast enhancement of the lesion.

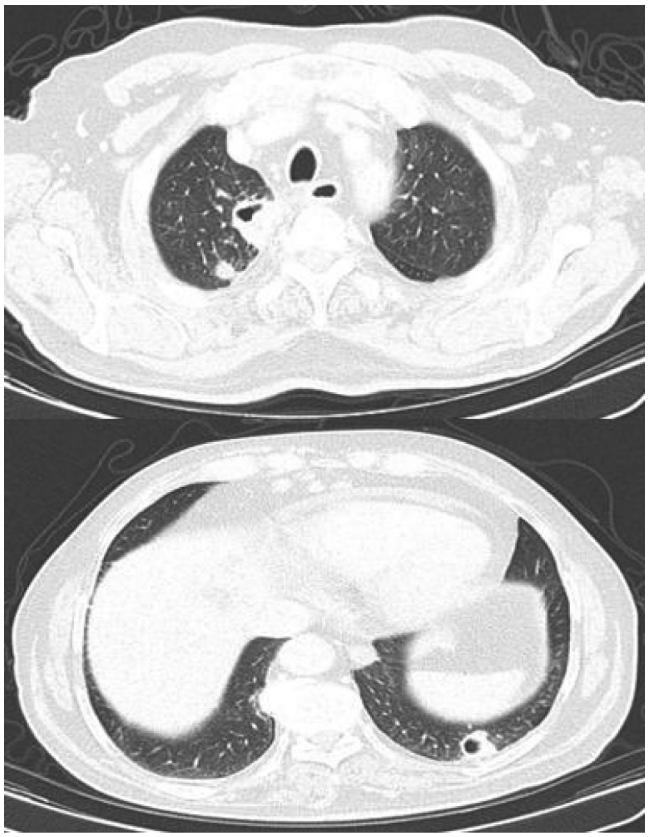

Fig. 2 Thoracic CT scan – axial plan – multiple peripheral nodules of both lungs, the bigger ones, cavitated.

to 11–12%, most commonly as endoftalmitis and meningites but SPE is a rare complication and it is generally present at hospital admission.[3–6] Our patient met SPE diagnostic criteria: had dry cough and hypocapnia, lung infiltrates, KLA as the embolic source, other potential explanations for lung infiltrates excluded and lung infiltrates resolved after appropriate antimicrobial therapy.[5,6] A broad spectrum of CT-scan findings can be present but less commonly compatible with lung abscesses.[6]

In conclusion, we describe a case of KLA with high clinical importance since it is the second case reported in Portugal [1], and the only one with the exceptionally rare complication of lung abscesses.[3,4,7] This case is an additional proof of the emergence of this syndrome worldwide. In cases of multiple lung abscesses with an acute presentation with dry cough in diabetic patients Kp should be considered, since it may be the first manifestation of serious underlying infection.

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24 June 2019

https://doi.org/10.1016/j.pulmoe.2019.08.006
2531-0437/

rs1573858 GATA-2 homozygote variant associated with pulmonary alveolar proteinosis, cytopenia and neurologic dysfunction

Dear editor,

Pulmonary alveolar proteinosis (PAP) is a syndrome characterized by the accumulation of alveolar surfactant. Although auto-immune PAP is responsible for 90% of all cases, association with genetic abnormalities has also been established.[1,2]

The authors present the case of a 43 years old non-smoker female. She has a previous medical history of idiopathic CD4 lymphocytopenia, idiopathic segmental dystonia, lymphedema and septicaemia due to parvovirus B19. Two years ago, she was treated for *Pneumocystis jiroveci* pneumonia with cotrimoxazol with clinical resolution.

Ten months ago, she was admitted to Hospital for dyspnea with acute respiratory failure. She was assumed to have had acute cardiac failure with good response after diuretic treatment. After discharge, the echocardiogram was normal but in this context she performed a thoracic computed tomography scan which showed diffuse pulmonary ground-glass opacities and slight thickening of the interlobular septa — ''*crazy-paving*'' pattern (Fig. 1). At this time, she was referred to our Department of Thoracic Diseases due to these imaging findings and chronic respiratory insufficiency. She performed bronchoalveolar lavage that evidenced rare alveolar macrophages in an amorphous granular background with proteinaceous material; the cytopathological study was negative for malignant tumour cells and microbiological study showed no pathogenic microorganisms. Routine blood tests reported: hemoglobin 9.0 g/dL, platelets 59,000/μL, leukocytes 3100/μL, neutrophils 1181/μL, lymphocytes 1160/μL and monocytes 40/μL. Results were positive (1:320) for antinuclear antibodies (ANA), and negative for anti-neutrophil cytoplasmic antibodies (ANCA) and anti-extractable nuclear antigens (ENA) antibodies. Anti-granulocyte-macrophage colony-stimulating factor (anti-GM-CSF) antibodies were negative. A required genetic test for GATA-2 gene mutation identified a rs1573858 homozygote, benign variant. Although this mutation is considered not pathogenic, the whole clinical profile, except segmental dystonia, is typical of GATA-2 deficiency. Therefore, she was diagnosed with GATA-2 deficiency related PAP and whole lung lavage (WLL) was proposed. Right WLL was initially performed. A total of eight litres of warmed saline solution was instilled in the right lung and the fluid was then consecutively collected by gravity after opening an outflow tube. The procedure was aided by mechanical chest percussion. After 20 days, left WLL was performed with eight litres of saline solution (Fig. 2). There were no procedure complications and currently the patient does not require oxygen therapy.

Macrophage homeostasis is highly relevant to lung hygiene. Conditions reducing either the number or functions of alveolar macrophages would be expected to reduce their capacity to clear surfactant from the lung surface, promoting secondary PAP.[1,3]

GATA-2 belongs to a family of transcription factors that are critical regulators of gene expression in hematopoietic cells. Therefore, GATA-2 gene mutations may lead to haploinsufficiency associated with profound cytopenias. It remains unclear why but dendritic cells, monocytes, B and NK lymphoid cells are the ones mainly affected.[3,4] Given this involvement, an association between GATA-2 insufficiency and PAP is to be expected. Given the usual abundance of alveolar macrophages in bronchoalveolar lavage fluid of these patients, PAP in GATA-2 deficiency must reflect more an alveolar macrophage dysfunction than a quantitative deficit, presumably by direct effects on alveolar macrophage phagocytosis.[4] GATA-2 also interacts with different signaling cascades through modulating the expression of key receptors or transducing proteins, such as M-CSF receptor or phospholipase C.[3] *Collin et al.*[3] reported the presence of 18% of PAP and 50% of abnormal pulmonary function cases in GATA-2 deficiency while *Vinh* et al.[5] found it in 33% of patients. As expected, our patient had no

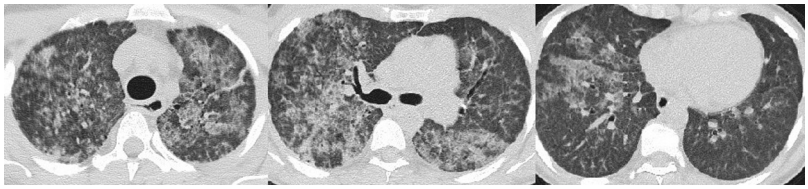

Figure 1 ''Pulmonary alveolar proteinosis – imaging. Chest CT showing diffuse ground-glass areas with juxtaposition of healthy zones and thickening of the interlobular septa — *crazy-paving*''.

Figure 2 ''Left whole lung lavage – procedure and fluid collected. (A) Patient is intubated with a double-lumen endotracheal tube in a right lateral decubitus position; 1 L of warmed saline is being instilled and subsequently collected by gravity after opening the outflow tube. Mechanical chest percussion is improving drainage. (B) From left to the right: from the 1 st to the 8th litre to be instilled and collected; the fluid becomes less opaque''.

detectable anti-GM-CSF antibodies. In our case, the mutation identified is considered a benign variant. However the clinical profile (except the segmental dystonia) is typically observed in GATA-2 deficiency and therefore another unidentified genetic hit may be hypothesized as a promoter factor.

The literature has highlighted the association between PAP and secondary opportunistic infections. However some studies have suggested that these microorganisms may even contribute to a secondary PAP.[2,6] In our report, the patient presented a previous *Pneumocystis jiroveci* pneumonia. The authors question whether the potential role of *Pneumocystis jiroveci* is another contributing factor.

In recent years, some clinical syndromes have been associated with GATA-2 deficiency. *Vinh* et al. described in 2010 the *Dendritic cell, Monocyte, B and NK Lymphoid Human Deficiency* (DCML) *Syndrome*. This syndrome results from a progressive absence of multi-lymphoid or lymphoid-primed multipotent progenitors and a severe depletion of CD38+ granulocytic monocytic progenitors.[5] In our report, the patient has a previous medical history of idiopathic CD4 lymphocytopenia. Considering these data, GATA-2 deficiency proved to be the explanation for the patient idiopathic CD4 lymphocytopenia. At the time of PAP diagnosis the patient also presented with a severe monocytopenia and a mild neutropenia. These findings are suggestive of DCML syndrome. However, a more detailed haematological study must be carried out to confirm this hypothesis. *Ferreira et al.* also described a patient with PAP that fulfilled the diagnostic criteria for DCML syndrome but until that date they had not confirmed the GATA-2 mutation.[7]

WLL is the current standard of care in auto-immune PAP.[2] However, some secondary PAP cases have poor response to WLL and no reported series have specifically evaluated GATA-2 deficiency related PAP. In our report the patient presented an optimal clinical response. It will be important to note the clinical evolution in the coming months.

To sum up, our report shows a very rare cause of PAP suggesting that a benign variant of GATA-2 mutation may contribute to the onset of a complex syndrome. This emphasizes the need to search for secondary causes as well as the current available treatments.

Author contributions

Venerino Poletti and Stefano Maitan conceived the idea and made the diagnosis. Nuno China and Venerino Poletti collected the data and wrote the manuscript. Venerino Poletti and Carlo Gurioli were responsible for cytopathologic analysis. All the authors have read and approved the final version.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pulmoe.2019.09.008.

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23 June 2019

https://doi.org/10.1016/j.pulmoe.2019.09.008
2531-0437/