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ASSESSMENT OF INFECTIOUS ETIOLOGY IN SEVERE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE; RELATIONSHIP WITH RESPIRATORY FAILURE

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Abstract

Acute exacerbation of chronic obstructive pulmonary disease (COPD) is a negative event in disease evolution which leads to higher morbidity and mortality of patients. Infectious agents are the main cause of exacerbations and they can be easily obtainedusing new molecular methods.

Our objective was to detect respiratory pathogens in patients hospitalized for severe acute exacerbation of COPD and to analyze their relationship with respiratory failure. We examined sputum from 49 patients (male, n=32) older than 40 years, using multiplex PCR microarray for 34 targets of which 18 bacteria, 9 viruses and 7 markers of antibiotic resistance. Blood gas analyses and other clinical and laboratory measures were provided.

Infectious etiology was found in 51% of acute exacerbations in hospitalized patients. Of all sputum samples, influenza A was the most frequently detected respiratory pathogen (n = 9, 18.4%) followed by *Haemophilus influenzae* (n = 7, 14.3 %). We found asignificant negative correlation between the presence of the type of detected pathogens and the level of pCO2 in the blood ($r = -0.437$; $p = 0.029$); thus, the higher the level of pCO2, the greater likelihood that it is a bacterial infection.

Detection of sputum bacteria in patients with severe acute exacerbation of COPD can be an independent risk factor for acute hypercapnic respiratory failure.

Keywords: acute exacerbation, COPD, respiratory pathogens, PCR, blood gas analyses

Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease which is characterized with airflow limitation and progressive impairment of pulmonary function over time, mainly caused by inhalation of noxious particles and gaseslike cigarette smoke, with higher prevalence in older age and in males [1-2].

Acute exacerbations of COPD are the main cause of mortality as well as increased morbidity and low health status in these patients [3].

They are often called catastrophic eventsin clinical course of this prevalent disease. Worsening of symptoms like increased sputum production and purulence, cough, dyspnea, wheezing and respiratory rate are main features [4,5]. Onset of acute exacerbation requires modification in management, thus Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines exacerbations as acute worsening of respiratory symptoms which requires an additional therapy [6].

Depending on appropriate treatment they are classified as mild, moderate and severe. Severe exacerbations require hospitalization and are usually associated with acute respiratory failure upon chronic carbon dioxide retention. Their frequency is a main prognostic factor for further exacerbation and worse prognosis of COPD [7].

The main etiological cause of acute exacerbation are respiratory infections found in approximately 60-70% of cases; other causes are inhalations of harmful particles like PM 2,5 matter usually from polluted air and changes of ambient temperature [8].

The most commonly found respiratory pathogens are viruses with a higher prevalence of Rhinovirus. Bacterial infections are also very important feature of exacerbations of COPD with *Haemophilus influenzae* as the most common isolated bacteria. These data differ according to different methods of examinations - using microbiological culture or new molecular and PCR-based methods as

well as using different specimens. Bacteria are easily isolated by using classical microbiological culture and until recently, they have been considered as a main causative respiratory pathogen. Detection of viruses is now routinely donewith new molecular methods which have shown their importance in acute exacerbations of COPD [9-15].

These respiratory infections lead to immune-inflammatory respond in lungs and increased mucus, edema and bronchospasm with local and systemic inflammation. There are many studies which have attempted to determine the specific inflammatory pattern in acute exacerbations, but no specific biomarkers have been found. CRP is the only one that is increased in all infections but there is data that suggest that CRP and procalcitonin cannot distinguish bacterial from viral etiology [16-18].

Some studies have evaluated therelationship between the type of respiratory pathogens and some of the clinical characteristics of COPD. They found lower values of FEV1 associated with the presence of *Pseudomonas aeruginosa* and with combined exacerbations, viral and bacterial. In addition, patients with severe exacerbations have more compromised lung function and worse outcomes accompanied with specific spectrum of respiratory microorganisms. In these patients acute hypercapnic respiratory failure is mostly seen, if respiratory acidosis is present and it requires more aggressive treatments like NIV (Non-Invasive ventilation) or intubation and mechanical ventilation [19-22].

By using new multiplex PCR microarray we can easily obtain presence of microorganisms in acute exacerbations of COPD as well as in stabile COPD. We can determine infectious etiology of acute exacerbation in short time and apply appropriate treatment [23].

In this context, the objective of this study was to detect various types of respiratory pathogens as causative agents of acute exacerbation of COPD in hospitalized patients and to analyze their relationship with respiratory failure.

Methods

This was an observational, prospective, monocentric study, which included 49 patients older than 40 years. All patients had severe acute exacerbation of COPD and were hospitalized at the University Clinic for Pulmonology and Allergology in Skopje. Patients had been previously diagnosed with chronic obstructive pulmonary disease according to actual GOLD guidelines [1].

Exclusion criteria for participation in the study were presence of other clinically significant respiratory disorder as asthma, cystic fibrosis, lung carcinoma, interstitial lung disease, tuberculosis, pneumonia as well as other clinically manifested infection. Patients who were on long-term corticosteroid and antibiotic treatment and patients whocould not expectorate sputum despitethe applied methods for forced expectoration, were not included in the study.

Physical examination and assessment of symptoms were made in all patients. On assessment day, we measuredbody temperature, body mass index (BMI), blood pressure, heart rate, chest radiography and made a standard 12-channel electrocardiogram. Laboratory blood tests were performed with measuring of complete blood count, sedimentation, lipid and protein profile, C-reactive protein (CRP), eosinophil and neutrophil count,AST,ALT and fibrinogen. Lung function was assessed with spirometry using the Power Cube spirometer (Ganshorn, Niederlaner, Germany) according to current recommendations of the European Respiratory Society (ERS) and the American Thoracic Society. All patients completed COPD assessment test (CAT) and mMRC (modified Medical Research Council (UK)) dyspnea scale questionnaires.

Blood gas analyses were performed by taking arterialized blood from the earlobe and processing it with a gas analyzer: "Prime-star profile", nova biomedical, according to a standard protocol. They were obtained from all patientsby measuring of pH, PaO2 (kPa), PaCO2 (kPa), SaO2 (%), HCO3- (moll / L) [24].

Sputum examinations

All patients who could produce sputum (n=49) were included in the study. Expectorated sputum was analyzed with the rapid, nested, multiplex PCR microarray using “Pneumonia plus” panel. This method in one sample can simultaneously detect 34 respiratory pathogens: 18 bacteria (11 gram-negative, 4 gram-positive and 3 atypical), 9 viruses and 7 markers of antibiotic resistance. It consists of a closed

system with reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR) and detection in order to isolate, amplify and detect nucleic acids from multiple respiratory pathogens.

All targets are qualitatively determined and marked as detected or notdetected, except typical bacteria which are determined not only qualitatively but also quantitativelyusing a number of genomic copies per milliliter. The result is considered positive if a value of 10ˆ4 copies / mL and above is obtained.

For statistical data analysis we used SPSS, version 26.0 (IBM SPSS, Inc., Chicago, Illinois) with a p-value ≤ 0.05 considered significant.

Results

We examined 49 patients with a mean age of 63. 51 ± 9.10. Basiccharacteristics of patients, lung function and smoking status are shown in Table 1.

Table 1. Basic characteristic of patients

Features	**Values (n=49)**
Age (years)	63.51 ± 9.10
Max-min	82-43
Gender (n/%)	
Male	32/65.3
Female	17/34.7
BMI (kg/m2)	31.52 ± 7.15
Max-min	53.33-19.00
Body temperature (C^0)	36.8 ± 0.6
Max-min	39.0- 36.2
SyBP(mmHg)	126.5 ± 15.2
Max-min	180-90
DiaBP (mmHg)	78.3 ± 7.6
Max-min	100-60
HR	90.3 ± 16.1
Max-min	129-59
Smoking status	
Pack/years of smoking	60.24 ± 31.12
Max-min	140-20
Current smoker (n/%)	18/36.7

Ex-smoker (n/%)	24/51.0
Non- smoker (n/%)	8/16.3
Lung function (postbronchodilatatory testing)	
FEV1 (L)	1.08 ± 0.40
FEV1 (%)	40.69 ± 13.13
FVC (L)	1.90 ± 0.63
FVC (%)	57.08 ± 14.20
FEV/FVC (%)	56.67 ± 9.65
Blood gas analyses	
pO2 (kPa)	7.93 ± 1.67
pCO2 (kPa)	6.76 ± 2.32
pH	7.41 ± 0.04
HCO3 (mmol/L)	33.35 ± 11.49
SO2 (%)	87.77 ± 8.42

Note: BMI: Body mass index; SyBP: Systolic blood pressure; DiaBP: Diastolic blood pressure; HR: heart rate; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity. pO2: partial pressure of oxygen in blood. pCO2: partial pressure of carbon dioxide in blood

Male were predominant (32/65, 3%) over female (17/34, 7) patients; most of the patients were ex-smokers (n=24) with mean standard deviation (SD) of pack/years of smoking of 60.24 ± 31.12.

All patients had a severe acute exacerbation of COPD and treated according to a standard protocol.

The majority of patients belonged to GOLD II-GOLD IV stage of COPD with mean post broncho dilatatory FEV1 values of 1.08 ± 0.40 and 40.69 ± 13.13% of the predicted.

Respiratory microorganisms were detected in 25 patients, 7 were bacteria, 11 viruses and in the remaining7 patients we found a combination of viruses and bacteria in sputum.

According to these results, patients were divided in two groups of pathogen detected and pathogen notdetected group with further division according to type of the pathogen to viral, bacterial and combined subgroups. (Table 2)

Table 2. Presence and types of respiratory pathogens detected with PCR method

Features	**Value (n=49)**
Detected pathogens by PCR method (n /%)	
Present	25/51
Absent	24/49
Type of pathogen detected (n /%)	
Bacteria	7/28
Viruses	11/44
Combined (bacteria + viruses)	7/28

From all sputum samples we found that inluenza A was most frequently detected (n = 9, 18.4%) followed by *Haemophilis influenzae* (n = 7, 14.3%), *Pseudomonas aeruginosa* (n = 6, 12.2%), *Streptococcus pneumoniae* (n = 5, 10.2%) and human rhinovirus /enterovirus (n = 5, 10.2%), (Table 3).

Blood gas analyses showed mean values of partial pressure of oxygen in capillary blood of 7.93± 1.67 and pCO2 of 6.76 ± 2.32 (Table1).

TheMann-Whitney nonparametric test showed no significant difference in blood gas analyses betweengroups of patients with detected respiratory pathogens and the one with not detected pathogens (Table 4).

No significant difference in values of blood gas analyses was found between groups with bacterial, viral and combined types of pathogens using the ANOVA comparison test (Table 5).

A negative significant correlation was found between the presence of the type of detected pathogens and the level of pCO2 in the blood ($r = -0.437$; $p = 0.029$).

Thus, the higher pCO2, the greater likelihood that it is a bacterial infection (Fig.1).

Table 3. Pathogens detected on panel

Features	**Values (n=49)**
Acinetobacter calcoaceticus-baumannii complex (n/%)	1/2.0
10^4 (n/%)	1/100%
Enterobacter cloacae complex (n/%)	2/4.1
10^4 (n/%)	2/100%
Escherichia coli (n/%)	1/2.0
10^4 (n/%)	1/100%
Haemophilis influenzae (n/%)	7/14.3
10^4 (n/%)	1/14.3
10^5 (n/%)	1/14.3
10^6 (n/%)	2/28.6
≥ 10^7 (n/%)	3/42.9
Moraxella catarrhalis (n/%)	3/6.1
10^4 (n/%)	1/33.3
≥ 10^7 (n/%)	2/66.7
Pseudomonas aeruginosa (n/%)	6/12.2
10^4 (n/%)	3/50%
10^6 (n/%)	1/16.7
≥ 10^7 (n/%)	2/33.3

Table 4. Comparison of blood gas analyses in patients with detected and not detected respiratory pathogens

Features	**Pathogen detected (n=25)**	**Pathogen not detected (n=24)**	**p**
pO2 (kPa)	8.13 ± 1.57	7.72 ± 1.79	0.395
pCO2 (kPa)	6.33 ± 1.83	7.20 ± 2.7	0.453
pH	7.41 ± 0.41	7.42 ± 0.45	0.276
HCO3 (mmol/L)	30.78 ± 8.83	36.03 ± 13.39	0.193
SO2 (%)	89.22 ± 6.89	86.25 ± 9.69	0.548

Table 5. Comparison of blood gas analyses in patients divided by type of pathogen detected

Features	**Bacteria (n=7)**	**Viruses (n=11)**	**Combined (n=7)**	**p**
pO2 (kPa)	7.88 ± 1.65	7.85 ± 1.78	8.80 ± 1.03	0.424
pCO2 (kPa)	6.98 ± 1.63	6.62 ± 2.21	5.23 ± 0.76	0.160
pH	7.40 ± 0.02	7.41 ± 0.05	7.40 ± 0.03	0.959
HCO3 (mmol/L)	33.58 ± 8.39	32.06 ± 10.66	25.97 ± 3.64	0.229
SO2 (%)	87.84 ± 7.78	87.82 ± 7.84	92.80 ± 6.89	0.281

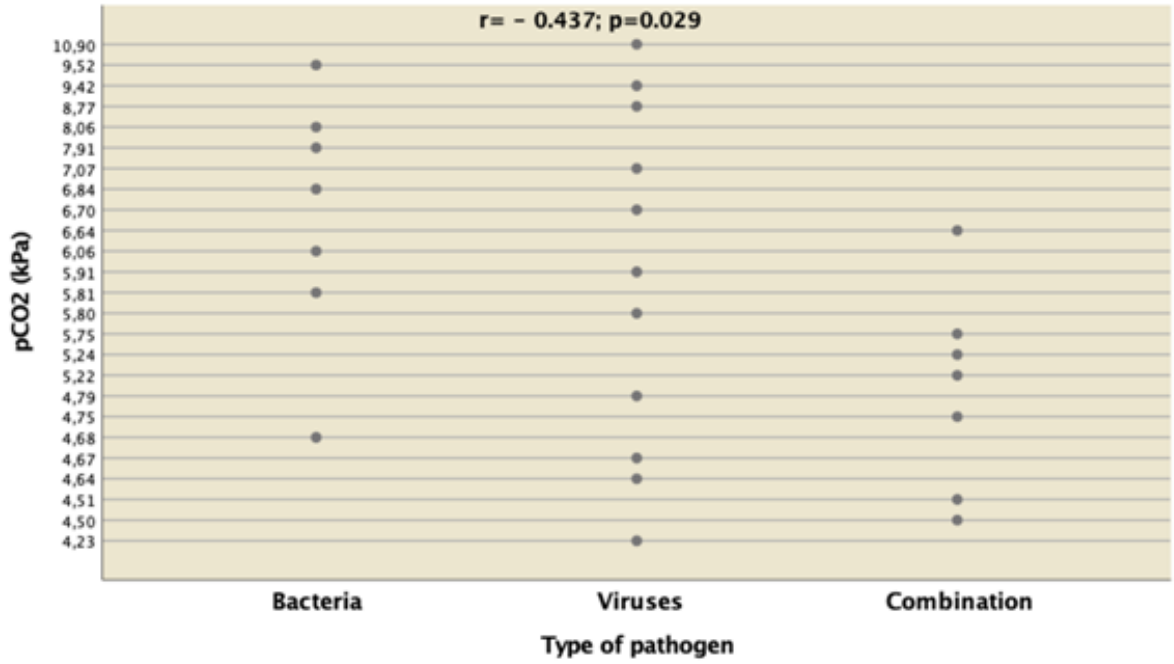

Figure 1. Correlation between type of pathogen found in sputum using PCR method and the level of pCO2 (kPa)

Discussion

This is the first study in our country that prospectively assessed etiology of acute exacerbation of COPD in hospitalized patients. By using molecular methods which analyzed nucleic acids from a large number of microorganisms (multiplexing) in sputum specimen, we managed to detect the real causative agent ofrespiratory infection in acute exacerbation and to initiate antibiotic treatment.

The strength of our study is that we used clinically approved pneumonia respiratory panel, which can detect 34 targets simultaneously in one sputum sample for one hour. This enables very quick diagnosis and appropriate treatment.

There are many studies which have examinedthe profile ofrespiratory pathogens in COPD patients, but they have usually used various methods, tests and samples like microbiological culture for typical bacteria, nasopharyngeal swabs for virusesand urine for atypical bacteria [25].

Other studies that have used multiplex PCR-based methods usually take longer time for results [26]. Data in current literature show that infectious agents are responsible for approximately 70% of acute exacerbations of COPD [8], which did not correspond with our results; we found that 51% of exacerbations were pathogen positive and had infectious etiology. This suggests that other reasons like worsening of comorbidities may be responsible for this percentage of pathogen negative group. Heart failure, atrial fibrillation, pulmonary thromboembolism, gastroesophageal reflux and other should be considered [27, 28].

Our results regarding prevalence of viruses and types of respiratory pathogens were similar to other studies. The only difference was in findinginfluenza A asthe most common detected virus in our patients' sputum. These results differ from West European studies, which found rhinovirus to be the most common viral agent in acute exacerbation of COPD, but they are similar to Asian author's findings [11,29].

Respiratory failure is a condition in which the respiratory system fails in one or both of its gas exchange functions, i.e., oxygenation of and/or elimination of carbon dioxide from mixed venous blood. It is defined by an arterial oxygen pressure (Pa, O2) of 8.0 kPa (60 mmHg), an arterial carbon dioxide pressure (Pa, CO2) of 6.0 kPa (45 mmHg) or both. Patients with COPD usually develop chronic hypercapnic respiratory failure. In acute exacerbation of COPD, we found an overall worsening of patient health status and respiratory failure, which is known as acute or chronic respiratory failure. This condition is more often seen in severe COPD patients [7].

Our results have shown that hypercapnia is associated with the presence of bacteria as an etiological factor for acute exacerbation. A negative significant correlation was found between the presence of bacteria and the level of pCO2 in the blood ($r = -0.437$; $p = 0.029$). This shows that more severe COPD is likely to have bacterial exacerbations.

Conclusion

The rapid detection of respiratory pathogens with the PCR method in our study found infectious etiology in 51% of acute exacerbations of COPD in hospitalized patients, with prevalence of viruses. Influenza A was the most common detected pathogen overall (18.4%), *Haemophilus influenza* (n=7) and *Pseudomonas aeruginosa* (n=6) were the most common detected bacteria.

We also found that the higher pCO2, the greater likelihood that it is a bacterial infection. Hypercapnic failure in patients with severe acute exacerbation of COPD is most likely associated with bacterialetiology. We can conclude that detection of sputum bacteria can be an independent risk factor for acute or chronic, hypercapnic respiratory failure in patients with severe acute exacerbation of COPD.

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