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Bacterial exacerbations of chronic obstructive pulmonary disease. The role of lung function in aetiology of exacerbation

COPD is a major health problem in the world. It is the fourth leading cause of death for adults. COPD has been redefined in the GOLD (Global Initiative for Chronic Obstructive Lung Disease) guidelines as a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive. COPD patients frequently suffer from acute exacerbation of this condition. The most common causes of acute exacerbation are infections of the tracheobronchial tree and air pollution. About one third of exacerbations have no identifiable cause. The diagnosis of an acute exacerbation of COPD is primarily based on clinical symptoms like increased sputum production, shortness of breath, fever, symptoms of malaise, insomnia and depression. Chest x-ray can help rule out pneumonia, congestive heart failure, and pulmonary embolus (9).

The role of bacteria in precipitating exacerbation is to some extend controversial. There are various bacterial species present in the airways of 25–50% of patients with stable COPD. The fact that bacteria are more often recoverable during acute exacerbations compared with stable COPD support the role for bacterial infections (8). The major bacterial pathogens associated with exacerbations are *Haemophilus influenzae, Moraxella catarrhalis, Steptococcus pneumoniae*. During acute exacerbations in patients with FEV1 of 35% or less of predicted normal values (PV), Gram-negative bacteria, especially *Enterobacteriaceae* and *Pseudomonas aeruginosa* play a role in exacerbation (3). Patients with bacterial exacerbation of COPD are usually affected by worsening cough, shortness of breath, increasing sputum production and purulence of sputum (1). The effect of antibiotics also suggests an aetiological role for bacteria in exacerbations of COPD. The meta-analysis of nine studies showed a small overall benefit with antibiotics treatment for COPD exacerbations (10).

The study objectives were to investigate bacterial aetiology of acute exacerbations of COPD. Also, the effect of spirometric abnormalities on the occurrence of bacterial pathogens in sputum was assessed.

MATERIAL AND METHODS

The patients were enrolled in this study if they met the inclusion criteria: 1) patients exhibited evidence of COPD, defined as airflow obstruction, demonstrated by spirometry tests, defined as FEV1 <70% of PV; 2) patients had medical history of chronic bronchitis (productive

cough for >2 consecutive years and most days in a consecutive 3-month period; 3) patients met Anthonesin's type I criteria (increased dyspnea, increased sputum volume, and increased sputum purulence) 1; 4) patients had no other clear cause of health deterioration and had at least two of the following: fever >38°C, increased wheezing, increased cough, increased respiratory rate; 5) patients had a qualified (Gram stain) purulent sputum production (>25 WBC per field and <10 squamous epithelial cells at 100 x magnification.

Chest x-ray was made to exclude patients with other lung diseases like pneumonia, tuberculosis, lung abscess or tumour. The patients had not received any systemic antibiotic within two weeks prior to sputum investigation. Medical history was taken and included the following: signs and symptoms of this infections, whether subject received prior medical treatment for that infection, any underlying pulmonary or other clinically significant medical conditions, history of COPD, concurrent illnesses, history of tobacco use. The laboratory test including haemoglobin, haematocrit, White Blood Cell Count (WBC), Red Blood Cell Count (RBC), were performed. Spirometry was performed before sputum sample collection. Lung function measurements, including forced expiratory volume in one second (FEV1), forced vital capacity (FVC) were measured with pneumotachograph (ABC-Med, Cracow, Poland). Values are expressed as percentage of those predicted. FEV1/VC ratio was also calculated.

Sputum was obtained by spontaneous expectoration. Sputum smears were prepared and air-dried for Gram stain evaluation. Only Gram stain samples containing >25 leukocytes per field and <10 squamous epithelial cells at 100 x magnification were considered valid. Routine culture analysis was performed on all qualified sputum samples. Sputa were seeded in the following culture media: *Columbia blood agar, MacConkey, Chapman, Haemophilus agar*, and *Sabouraud's agar (bioMerieux)*. Identification of bacterial species was based on biochemical features using API Strep, API NH, API Staph, API E, API NE, API *Coryne (bioMèrieux)* kits.

Culture/susceptibility testing for *H. influenzae, S. pneumoniae, M. catarrhalis, S aureus, H. parainfluenzae* and other respiratory pathogens was performed. The laboratory specifically identified the pathogens as to genus and species. *In vitro* susceptibilities to a panel of antibiotics were determined by standardized methods. Determination of antibiotic sensitivity of isolated strains was made by means of the disc diffusion method (according to *Birby-Bauer*). Classifications as sensitive, intermediate and resistant were made.

Statistical analysis. The data were analysed in Statistica 5.0. Descriptive statistical analysis was made of the demographic data of the patients grouped according to the germs isolated. Mann-Whitney U test was applied for the statistical comparison of the results between different patients groups. The p-level value of 0.05 was treated as a "border-line acceptable" error level.

RESULTS

Twenty-eight patients with newly diagnosed exacerbation of COPD were treated in the Pulmonary Department between 09.2000-03.2001. The study population consisted of 20 male and eight female subjects, age 58.9±10.7 years (rank 42–74 years). Seven patients had never smoked, 21 persons were ex- or current-smokers. The number of pack-years of smoking was 27.95±12.1 (rank 51–10 pack-years). Sixteen patients suffered then from other pulmonary diseases.

Forty-nine microorganisms in 28 patients were isolated. Nine patients had more than one germ isolated. The following bacteria were isolated: *Haemophillus influenzae 7, Haemophillus parainfluenzae 13, Escherichia coli 5, Staphylococcus aureus 4, Pseudomonas aeruginosa 1, Pseudomonas putida 2, Moraxella atarrhalis 2, Sreptococcus pneumoniae 2, Klebsiella pneumoniae 1, Klebsiella oxytoca 2, Acinetobacter baumannii 2, Pantoea agglomerans 1, Enterobacter cloacae 1, Enterococcus faecalis 1, Serratia liquefaciens 1, Enterobacter intermedius 1, Lecreclia adecarboxylata 1, Citrobacter freundii complex 1, Serratia ficaria 1.*

The mean FEV1 value of the investigated patients was 48.5±16 (rank 14–70). The mean FVC value was 59.15±18 (rank 31–106). The percentage value of FEV1/FVC was 61.9±15.8 (rank 34–99). The WBC count was 11220±4320 (rank 5700-20300). Patients with *Haemophilus influenzae* in sputum had significantly lower FEV1 and VC values (p>0.05) compared to patients with *H. parainfluenze* in sputum (Tab. 1).

Table 1. Functional characteristic of patients basing on the main germs isolated from sputum

Characteristic	*H. influenzae* (n=7)	*H. parainfluenzae* (n=13)	Other Gram-negative bacteria
FEV1 % of PV	36.6 (24-54)	51.1 (30-67)	44.9 (14-70)
FVC % of PV	45.14 (31-61)	63 (39-106)	54.9 (31-85)
FEV1/FVC	58.96 (34.2-94.6)	59 (42-89)	60.9 (34-94)

The values are presented as the mean value (range)

Patients with *Haemophilus influenzae* in sputum did not differ in age and smoking habits from patients with *H. parainfluenze* and other Gram-negative bacteria in sputum. The group of patients with *Haemophilus influenzae* had the longest duration of COPD (Tab. 2).

Table 2. Characteristic of patients basing on the main germs isolated from sputum

Characteristic	*H. influenzae*	*H. parainfluenzae* (n=13)	Other Gram-negative bacteria
Age – yr.	56.3 (42-74)	56.23 (44-71)	64.56 (46-74)
Years of COPD	9.7 (3-16)	6.7 (3-11)	8.67 (2-25)
Smokers/never smokers	3/4	10/3	9/3
Pack-yr.	29.7 (25-34)	29.3 (10-51)	29.3 (10-40)
Concomitant disease (number of cases)	3/7	8/13	5/9
WBC	12700 (6500-19430)	10800 (5700-20350)	11550 (6280-15620)

The values are presented as the mean value (range)

Antibiotic sensitivity pattern of *H. influenzae* and *H. parainfluenzae* is shown in Table 3. Isolated germs were sensitive to Azithromycin, Amoxycylin, Ceftriaxon, Cefuroxym and Ciprofloxacin. In 10 patients with *Haemophilus parainfluenzae* in sputum, the species were the only germs present. Other microorganismss present in sputum of three *Haemofilus parainfluezae* patients were: *Moraxella catharalis* and *Steptococcus pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumani*, and *Haemophilus influenzae*. In patients with presence of *Haemohilus influezae* in sputum, there were other germs present (*Staphylococcus aureus*-3, *Moraxella catarrhalis*-1, *Pseudomonas aeruginosa*-1, *Escherichia coli*-1, *Streptococcus pneumoniae*-1, *Haemophilus parainfluenzae*-1). All germs of *Haemophilus influenzae* were B-lact (-).

Table 3. Antibiotic sensitivity patterns of the most common germs

	Clarithro-mycin			Azithro-mycin			Amoxy-cylin			Ampi-cylin			Ceftria-xon			Cefuro-xym			Ciproflo-xacin			Thrimeth /sulfam		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
H. influenzae	7	0	0	7	0	0	7	0	0	7	0	0	7	0	0	7	0	0	7	0	0	6	0	1
H. para-influenzae	5	5	3	13	0	0	13	0	0	13	0	0	13	0	0	13	0	0	13	0	0	9	0	4
S. aureus	4	0	0	4	0	0	4	0	0	0	0	4	4	0	0	4	0	0	4	0	0	4	0	0

DISCUSSION

H. parainfluenzae was present in 27% of our isolates. This germ was present in sputum from 46% of our patients with COPD exacerbation. In humans, *H. parainfluenzae* is present as a part of normal upper respiratory tract flora. *H. parainfluenzae* is frequently recovered from COPD adults sputum. DeAdate et al. found *H. parainfluenzae* in sputum of 32% of patients experiencing exacerbation of chronic bronchitis (2). Langan et al. performed bacteriological assessment of sputum in a large group consisting of 802 COPD exacerbated patients. 513 isolates were obtained from 400 culture positive patients. *H. parainfluenzae* was present in 27 % of isolates (5).

The presence of *H. parainfluenzae* in sputum of patients with COPD exacerbation does not establish firm aetiology of exacerbation. The possibility of contamination by saprophytic oropharyngeal flora is well known. Our study employed restrictive criteria of the patients' enrolment. All patients met Anthonisen type I criteria. Anthonisen developed a classification system to identify patients likely to be infected with bacterial pathogens based on presenting clinical symptoms. A type I exacerbation is defined as a patient with a combination of increased dyspnea, sputum volume and sputum purulence. A type II exacerbation refers to a patient with two of these symptoms, type III exacerbation occurs when patients have one symptom. Patients exhibiting type I or II exacerbation have a demonstrable benefit from antibiotic therapy, implying a bacterial aetiology of exacerbation, whereas patients with type III do not respond to antimicrobial therapy (1,11). Our study also used strict criteria to evaluate the quality of sputum samples. We think that with the implementation of these criteria sputum can be useful in analyzing bronchial infection.

In Fagon et al. study, *H. parainfluenzae* was found in 25% of specimens obtained by protected brush during bronchoscopy in patients with COPD exacerbation (4). Mitchel et al. investigated an immune response to *H. parainfluenzae* in patients with COPD exacerbation and showed that COPD patients had higher titres of antibodies to *H. parainfluezae* than healthy control group (7).

Bacteria are isolated from sputum in 50% patients with COPD exacerbation. The dominant bacterial pathogens isolated from sputum of acute exacerbation of chronic bronchitis are *Haemophilus influenzae, Moraxella catarrhalis, Steptococcus pneumoniae*. Less frequently isolated are: *Enerobacteriacae, Hemophillus parainfluenzae, Staphylococcus aureus, Pseudomonas aeruginosa*. The incidence frequency of these bacteria in sputum of patients with COPD exacerbation depends on patients' population and bacteriological technique (11). *H. parainfluenzae* was present in 14% of our isolates. It is the dominant bacterial pathogen isolated from sputum of acute exacerbation of chronic bronchitis. In our study, the patients with *Haemophilus influenzae* in sputum have significantly lower spirometric values compared to patients with *H. parainfluenze* and other Gram-negative bacteria in sputum.

A study of Miravitlles and Eller indicates that *H. influenzae* and *P. aeruginosa* occur more frequently in sputum of patients with exacerbation of COPD with large functional impairment of lungs (3,6). In their study, *H. influenzae* was isolated in sputum of 22% investigated patients and was isolated more frequently among patients with FEV1<50% than among those with FEV1>50%. Patients with the greatest degree of functional impairment presented a higher probability of having an isolation of *H. influenzae* or *P. aeruginosa* in sputum during COPD exacerbation. Zalacain et al. studied a group of severe COPD patients with FEV1<50% (13). They found that *H. influenzae* was the most common bacterium isolated. Some studies demonstrate increasing frequency of Gram-negative bacteria isolation not only from sputum but also from bronchoscopy material from patients with severe COPD exacerbation. Soler et al. found *Pseudomonas aeruginosa* and other Gram-negative bacteria in lower airway secretions in 44% and *H influenzae* in 33% of mechanically ventilated patients with COPD (12).

CONCLUSIONS

1. *H. parainfluenzae* may play an important role in COPD exacerbations. The recognition of *H. parainfluenzae* as a human lower respiratory pathogen needs more research.

2. The degree of respiratory impairment may have an influence on the presence of different bacteria in sputum of patients with COPD exacerbation.

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SUMMARY

The objective of the study was determination of the most frequent bacterial factors, including *Haemophillus parainfluenzae*, suspected of causing COPD exacerbation, of the relation between bacterial strains and respiratory system functional status as well as of antibiotic sensitivity of sputum isolated bacteria. The examined group comprised 28 patients treated in the Pulmonary Department of Medical University of Lublin. The subjects fulfilled the criteria of type I COPD bacterial exacerbation. Patient's chest x-ray and spirometry tests were performed. Forty-nine bacterial strains were isolated. In the case of nine patients, more than one strain was isolated. Subjects having *H. parainfluenzae* in sputum had significantly higher ($p<0.05$) FVC and FEV1 values comparing to patients with *H. influenzae* or other Gram-negative bacteria. *H. parainfluenzae* may be an important etiologic factor of COPD exacerbation. Aetiology of bacterial COPD exacerbation depends on the level of respiratory parameter limitation.

Bakteryjne zaostrzenia przewlekłej obturacyjnej choroby płuc (POCHP). Znaczenie badań czynnościowych w etiologii zaostrzeń

Celem pracy było określenie najczęstszych bakteryjnych czynników podejrzanych o przyczynę zaostrzenia POCHP z uwzględnieniem udziału *Haemophillus parainfluenzae*, ocena zależności występowania poszczególnych szczepów bakteryjnych od stanu czynnościowego układu oddechowego oraz ocena wrażliwości na antybiotyki izolowanych z plwociny szczepów. Grupa badana obejmowała 28 pacjentów Poradni Przyklinicznej Kliniki Chorób Płuc i Gruźlicy AM w Lublinie. Pacjenci spełniali kryteria bakteryjnego zaostrzenia POCHP typu I wg Anthonisen i wsp. Pacjenci mieli wykonywane zdjęcie radiologiczne klatki piersiowej oraz badanie spirometryczne. Wyizolowano łącznie 49 szczepów bakteryjnych. U dziewięciu pacjentów wyizolowano więcej niż jeden szczep. Pacjenci z obecnością *H. parainfluenzae* w plwocinie charakteryzowali się istotnie wyższymi ($p<0.05$) wartościami FVC i FEV1 w porównaniu z pacjentami z obecnością *H. influenzae* lub innych bakterii gram-ujemnych. *H. parainfluenzae* może być zatem istotnym czynnikiem etiologicznym zaostrzeń POCHP. Etiologia bakteryjnych zaostrzeń POCHP zależy od stopnia ograniczenia czynności oddechowej, ocenianej badaniem spirometrycznym.