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Anaerobic bacteria colonizing the lower airways in lung cancer patients

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Abstract: Anaerobes comprise most of the endogenous oropharyngeal microflora, and can cause infections of airways in lung cancer patients who are at high risk for respiratory tract infections. The aim of this study was to determine the frequency and species diversity of anaerobes in specimens from the lower airways of lung cancer patients. Sensitivity of the isolates to conventional antimicrobial agents used in anaerobe therapy was assessed. Respiratory secretions obtained by bronchoscopy from 30 lung cancer patients were cultured onto Wilkins-Chalgren agar in anaerobic conditions at 37°C for 72–96 hours. The isolates were identified using microtest Api 20A. The minimal inhibitory concentrations for penicillin G, amoxicillin/clavulanate, piperacillin/tazobactam, cefoxitin, imipenem, clindamycin, and metronidazole were determined by E-test. A total of 47 isolates of anaerobic bacteria were detected in 22 (73.3%) specimens. More than one species of anaerobe was found in 16 (53.3%) samples. The most frequently isolated were *Actinomyces* spp. and *Peptostreptococcus* spp., followed by *Eubacterium lentum*, *Veillonella parvula*, *Prevotella* spp., *Bacteroides* spp., *Lactobacillus jensenii*. Among antibiotics used in the study amoxicillin/clavulanate and imipenem were the most active *in vitro* (0% and 2% resistant strains, respectively). The highest resistance rate was found for penicillin G and metronidazole (36% and 38% resistant strains, respectively). The results obtained confirm the need to conduct analyses of anaerobic microflora colonizing the lower respiratory tract in patients with lung cancer to monitor potential etiologic factors of airways infections, as well as to propose efficient, empirical therapy. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 2, pp. 263–266*)

Key words: anaerobes, lower respiratory tract, antimicrobial agent susceptibility, lung cancer

Introduction

Anaerobes are the predominant components of the normal commensal oropharyngeal flora. In this part of the human body, they are present in amounts between 10^3–10^9/ml and outnumber aerobic and facultative microorganisms by 10:1. Anaerobic bacteria play a significant role in the prevention of oropharyngeal colonization by pathogens because some of them can interfere with the growth of potential pathogens on the competitive mechanism. However, under predisposing conditions such as smoking, alcohol abuse, bad oral hygiene, prolonged hospitalization, immunosuppression, chronic lung diseases, cancer, and cough reflex disorders in the early post-operative period, anaerobes can cause endogenous infections of the respiratory tract including bronchitis, lung abscess, thoracic empyema or necrotic lung inflammation. Furthermore, antibiotic-related selection pressure on the oropharyngeal flora is another known risk factor for anaerobic respiratory tract infections. Additionally, these organisms also present a significant role in escalating chronic obstructive pulmonary disease (COPD). The most frequent way of spreading these infections is the aspiration of oropharyngeal secretions in connection with defects in the clear-

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ance mechanisms of the respiratory tract. Although healthy adults can also experience the aspiration of oropharyngeal secretions, e.g. during sleep, due to efficient clearance mechanisms their lower respiratory tract is fortunately usually sterile [1–5].

Lung cancer patients are at high risk for respiratory tract infections because cancer therapy regimens, as well as the underlying disease, cause both transient neutropenia and disruption of the physiological barriers in airways. Although these infections are usually caused by aerobic or facultative bacteria, anaerobes comprising most of the endogenous oropharyngeal microbial flora can also cause infections of airways [6].

The objective of the present study was to determine the frequency as well as species diversity of anaerobes in the lower respiratory tract specimens of lung cancer patients. We assessed the sensitivity of isolated anaerobic bacteria strains to conventional antimicrobial agents used in the therapy of diseases caused by anaerobes.

Material and methods

Patients. A total of 30 patients suffering from lung cancer (29 men and 1 woman, mean age 46 years) were enrolled in the present study. Patients were qualified for the study after histopathological diagnosis. Histological type of lung cancer was determined before surgery on the grounds of examination of specimens obtained during bronchoscopy, sputum cytology, thin needle biopsy, supraclavicular lymph nodes biopsy and mediastinoscopy. In this group, 27 patients suffered from non-small cell lung cancer (NSCLC) and three from small cell lung cancer (SCLC). Among histological types of NSCLC of studied patients, squamos cell carcinoma predominated (50% of the patients), followed by large cell carcinoma, adenocarcinoma and mixed carcinoma. During the previous month, all patients qualified for the study had not: suffered from airways infections, taken antimicrobial agents, taken immunological system-influencing drugs, received a blood transfusion, or suffered from an allergic disease. The study was approved by the Ethical Committee of the Medical University of Lublin. Informed consent was obtained from all patients.

Microbiological assay. Respiratory secretions obtained by fiberoscopic bronchoscopy using a protected specimen brush (PSB) catheter from 30 lung cancer patients were cultured onto Wilkins-Chalgren Agar (Biocorp, Poland) with vancomycin and nalidixic acid and Wilkins-Chalgren Agar with colistin and nalidixic acid for selective isolation of Gram-negative and Gram-positive anaerobes, respectively. Plates were incubated in anaerobic conditions at 37°C for 72–96 hours. The isolates were identified using microtest Api 20A (BioMerieux). The minimal inhibitory concentrations (MICs) for penicillin G, amoxicillin/clavulanate, piperacillin/tazobactam, cefoxitin, imipenem, clindamycin, and metronidazole were determined by E-test (AB BIODISK) according to Clinical Laboratory Standard Institute (CLSI) recommendations. With each susceptibility test run, quality control was performed with *Bacteroides fragilis* ATCC 25285. Beta-lactamase production was detected using a nitrocephin test (Becton Dickinson).

Results

A total of 47 isolates of anaerobic bacteria were detected in the respiratory secretions of 22/30 (73.3%) lung cancer patients. The number of anaerobic species in a single specimen varied from one to four. More than one species of anaerobe was found in 16/30 (53.3%) samples: four, three and two different species of bacteria were identified in 2/30 (7%), 5/30 (17%) and 9/30 (30%) patients, respectively. The species of anaerobes colonizing the lower respiratory tract are listed in Table 1. Generally, from the examined samples, isolates of Gram-positive bacteria were cultured more frequently than those of Gram-negative bacteria, comprising 78.7% of all the isolates. Among Gram-positive anaerobes, the commonest was *Actinomyces* spp. (15 strains) and *Peptostreptococcus* spp. (14 strains), followed by *Eubacterium lentum* (seven strains); *Veillonella parvula* (six strains) was the commonest Gram-negative anaerobic bacteria.

The anaerobic bacteria obtained from respiratory secretions of lung cancer patients were characterized by high *in vitro* susceptibility to applied antibiotics and chemotherapeutics. The MIC values of antibacterial agents and number of resistant strains are listed in Table 2. Among the beta-lactam antibiotics used in the study,

Table 1. Anaerobic bacteria isolated from bronchial secretions in lung cancer patients

Species	Number (%) of isolates
Gram-positive	
Actinomyces israelii	6 (12.77)
Actinomyces viscosus	5 (10.64)
Acrinomyces naeslundii	4 (8.51)
Peptostreptococcus spp.	14 (29.79)
Eubacterium lentum	7 (14.89)
Lactobacillus jensenii	1 (2.13)
Gram-negative	
Veillonella parvula	6 (12.77)
Prevotella melaninogenica	1 (2.13)
Prevotella oralis	1 (2.13)
Prevotella spp.	1 (2.13)
Bacteroides spp.	1 (2.13)
Total	47 (100)

Table 2. Antimicrobial agent sensitivity of anaerobic bacteria isolated from bronchial secretions in lung cancer patients

Antibacterial agent	Range of MIC [μg/ml] for sensitive strains	Range of MIC [μg/ml] for resistant strains	Resistant strains (%)
Penicillin G	0.016–0.75	2–16	29
Amoxicillin/clavulanate	0.016–6	–	0
Piperacillin/tazobactam	0.016–16	256	16
Cefoxitin	0.016–16	256	9
Imipenem	0.016–0.38	64	2
Clindamycin	0.016–0.75	24–256	16
Metronidazole	0.016–3	256	38

the most *in vitro* active against these microorganisms were amoxicillin/clavulanate and imipenem (0% and 2% resistant strains, respectively), followed by cefoxitin (9% resistant strains), and piperacillin/tazobactam (16% resistant strains). 29% of isolates were resistant to penicillin G. Among antimicrobial agents not belonging to beta-lactams, the highest resistance rate was observed with metronidazole (38% resistant strains); 16% isolates were resistant in cases of clindamycin.

Among the tested anaerobes, 10/47 (21.28%) strains produced beta-lactamase as follows: *Actinomyces* spp. (4/47 strains; 8.51%), *Peptostreptococcus* spp. (3/47 strains; 6.38%), *V. parvula* (2/47 strains; 4.26%), *Bacteroides* spp. (1/47 strain; 2.13%).

Discussion

Pneumonia, including nosocomial pneumonia, is one of the main complications occurring in lung cancer patients due to defects in the clearance system in sites peripheral to the bronchial obstruction, or stenosis caused by cancer therapy as well as cancer itself and broad-spectrum antibiotics usage [7–14]. The role of anaerobes in the pathogenesis of pneumonia in hospitalized patients, especially in critically or chronically ill patients, is still controversial [4, 15, 16]. It is known that pneumonia is preceded by microbial colonization of the lower airways [17]. In our studies, heavy colonization of lower airways by anaerobic bacteria, mainly potentially pathogenic species, was found in the lung cancer group, comprising 73.3% of the patients. Fiberoscopic bronchoscopy using a protected specimen brush (PSB) catheter was applied as a highly sensitive and specific technique to obtain uncontaminated specimens for anaerobic cultures from the lower respiratory tract. Among the isolated anaerobes the clinically important Gram-positive bacteria were classified, including cocci belonging to *Peptostreptococcus* spp. and non-spore-forming bacilli, primarily *Actinomyces* spp.

Several classes of antimicrobial agents have good activity against anaerobic bacteria, including penicillins alone or in combination with beta-lactamase inhibitors, cephalosporins, carbapenems, chloramphenicol, clindamycin, metronidazole, glycopeptides, macrolides, tetracyclines or fluoroquinolones [2, 18, 19]. However, antimicrobial resistance of anaerobes has become an increasing problem over the past two decades; beta-lactamase production is one of the commonest resistance mechanisms [20, 21]. According to our data, the anaerobic bacteria isolated from bronchial secretions in lung cancer patients were susceptible to the majority of the tested antimicrobials. The strains producing beta-lactamase comprise only about 20% of the isolates. Amoxicillin/clavulanate was the only antimicrobial agent active against all the tested isolates, which may be important in the choice of empiric therapy not only for anaerobic but also for mixed infections. Moreover, among the tested anaerobes, representing mainly Gram-positive bacteria, the lowest resistance rates were for imipenem and cefoxitin, while the highest were for metronidazole and penicillin G. Consistent with the results of previous studies [18, 20, 22], the rates of resistance may show clinically important variations among geographic areas and between countries.

In conclusion, the results presented in this paper confirm the need to conduct analyses of microflora colonizing the lower respiratory tract in lung cancer patients, including anaerobic bacteria, to monitor potential etiologic agents of airways infections. Knowledge of the actual frequency of occurrence of pathogenic, as well as opportunistic, species in a given local population is necessary in order to propose efficient, empirical therapy for respiratory tract infections, taking into account the possible involvement of anaerobic bacteria.

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