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Original article

Bronchial Colonization in Patients with Non–Small Cell Lung Cancer

Ana Stojanović

Clinical Center Bežanijska Kosa , Pulmonology Department, Belgrade, Serbia

SUMMARY

Lower airways are sterile, but many researchers have reported the existence of bronchial colonization with pathogenic and non-pathogenic microorganisms in smokers and patients with chronic obstructive pulmonary disease (COPD) and lung cancer. The aim of this study was to determine the existence and type of bronchial colonization in patients with non–small cell lung cancer (NSCLC), as well as to determine whether colonization depends on patient's age, smoking habit, pulmonary function and body mass index (BMI). Another aim was to estimate whether colonization causes early postoperative complications. Fifty-five subjects with pathohistologically confirmed non–small cell lung cancer in the resectable stage and good performance status (ECOG 0 or 1) participated in this cross–sectional study. We assessed patient's degree of smoking, calculated their BMI and determined the existence and severity of chronic obstructive pulmonary disease if there was one. The patients underwent a flexible bronchoscopy when biopsy samples were taken with protected specimen brush (PSB) and bronchoalveolar lavage (BAL); the retrieved samples were inoculated. Colonization was marked by an increase in the number of bacteria, yielding >10^3CFU/L. In our study, bronchial colonization was found in 21 patients (38%). Potentially pathogenic microorganisms were isolated in 13 patients (*Streptococcus pneumonia* was found in five patients, *Streptococcus B haemoliticus* and *Pseudononans* in three patients each, *Haemophilus influencae* and *Enterobacter* in one patient each). Potentially non–pathogenic microorganisms were isolated in 8 patients (*Streptococcus viridans* was found in 7 patients and *Achromobacter xylosoxidans* in 1 patient). Patients' sex, age, smoking habit, body mass index and severity of chronic obstructive pulmonary disease were not statistically significant for developing bronchial colonization. Only one operated patient developed postoperative pneumonia, but he had not previously been diagnosed with bronchial colonization.

Key words: **bronchial colonization, lung cancer, brush biopsy**

Corresponding author:
Ana Stojanović
e-mail: dr.anastojanovic@yahoo.com

INTRODUCTION

Of all malignant diseases, lung cancer is the leading cause of death. As screening has not shown the expected results in this type of cancer, an early diagnostics plays a key role in decreasing death rate and increasing the overall survival rate (1, 2). The patients who can be surgically treated have the best chances for a five –year survival.

Early postoperative death rate in patients who have undergone lung cancer surgery is 2 – 7% and it additionally decreases their chances of healing. Postoperative complications can be caused by an infective agent (3), and potential bronchial colonization in these patients is a possible risk factor for developing an infection.

It is well–known that lower airways are sterile (4, 5), but many researchers have reported the existence of the so-called bronchial colonization with possibly pathogenic and non–pathogenic microorganisms (4-6). Colonization is diagnosed by means of quantitative and semi–quantitative methods, after inoculating samples retrieved from the lung parenchyma by bronchoscopy. Samples are retrieved by bronchoalveolar lavage and brush biopsy, which diminishes the possibility of sample contamination (7, 8). Bronchial colonization was examined and determined in smokers and some patients with chronic pulmonary diseases who have weaker immune system response. This group consists of patients with tracheostoma, chronic bronchitis, bronchiectasis and malignant pulmonary diseases (9-13).

The aim of this paper was to examine the existence and type of bronchial bacterial colonization in patients with non–microcellular lung cancer (NSCLC). Another aim was to determine whether colonization depends on patient's age, smoking habit, pulmonary function and body mass index (BMI).

MATERIAL AND METHODS

This cross–sectional study was conducted in the Clinical Canter ''Bežanijska Kosa'' in Belgrade and 55 patients participated in it. The participation criteria included histopathologically confirmed non–small cell lung cancer, resectable stage (I to IIIA according to TNM - classification), ECOG score 0 or 1, FEV larger than 2 l. The patients who had been treated with antibiotics in the previous month were excluded from the study. Patients' detailed medical history was taken, including collecting social epidemiological data by determining the pack/year index. We assessed patients' medical condition (ECOG score) through a detailed clinical examination and body mass index (BMI) was calculated. Patient's blood was taken for routine laboratory testing: complete blood count, sedimentation rate, fibrinogen, basic biochemical tests.

All the patients underwent spirometry and the quality of their pulmonary function was assessed according to forced expiratory volume in one second (FEV1) and the value of FEV1 ratio and forced vital capacity (FVC). According to these parameters, we determined the existence of chronic obstructive pulmonary disease and severity classification according to GOLD criteria. According to chest and abdomen HRCT result, we determined the stage of the disease consulting the current TNM classification.

Having performed a fibreoptic bronchoscopy, we obtained a biopsy sample with protected specimen brush, bronchoalveolar lavage and we also performed bronchoscopic biopsy in visualized tumorous growth (4). A bronchofiberscope Olympus BF–TE2 (with a working channel of 2 mm) was used in the study. For retrieving a brush biopsy sample, we used the bronchial brush (BC-202D- Olympus). The entire process was performed in sterile conditions and samples reached laboratory not later than ten minutes upon sampling. We did not administer a local anaesthetic through the working channel of the bronchoscope due to the anaesthetic's bacterial impact.

Brush biopsy was done on the location above the tumorous growth, and if the tumor was peripherally localized biopsy was done on the side of the lungs where tumor was localized. Upon taking a sample, the brush was cut into 1 mL of 0,9% saline. Bronchoalveolar lavage sample was taken in a repeated procedure and we examined the second sample due to possible contamination. In each procedure we would instil 50 mL of 0,9% saline and then collect the contents into a sterile test tube.

The retrieved samples were inoculated in the following agar media: blood agar, chocolate agar, MacConkey agar, Sabouraund agar and Lowenstein agar. The increase in the number of bacteria was monitored under aerobic and anaerobic conditions. Cultures were first evaluated for growth after 24h and we got final results 48h after inoculating the samples.

Colonization was marked by an increase in the number of bacteria larger than 10^3 CFU/L.

STATISTICAL EVALUATION

Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed in mean ± standard deviation of mean. Differences between the groups were assessed using parametric (paired T student test), or Mann–Whitney U test (for skewed data) for numeric variables, and the Chi-squared test for testing differences between proportions. A two-tailed p value < 0.05 was considered statistically significant.

RESULTS

Out of the total of 55 patients, bronchial colonization was found in 21 patients (38%) (Figure 1). There was no statistically significant difference in the age of patients with bronchial colonization and those without it (t=0.241; p>0.05).

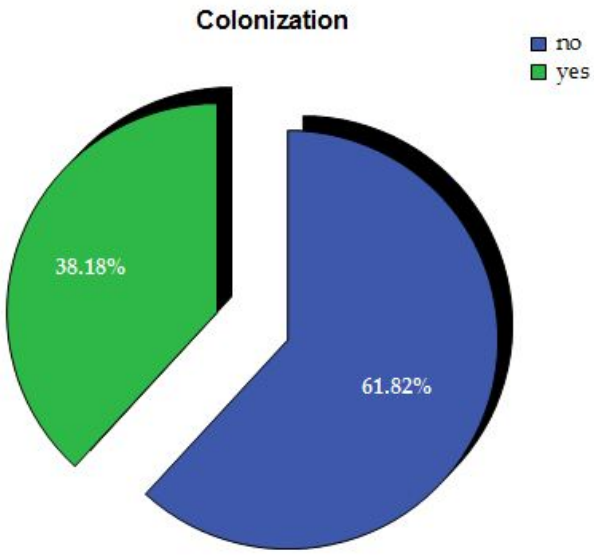

Figure 1: Presence of bronchial colonization in NSCLC patients

Also, there was no statistically significant difference in bronchial colonization in centrally localized tumors and peripherally localized ones (X^2=1.038; p>0.05) (Table 1).

Table 1: Colonization in central and peripheral lung cancer

			Colonization		Total
			No	Yes	
Type of tumor	Centrally localized	Number	10	9	19
		% Type of tumor	52.6%	47.4%	100.0%
	Peripherally localized	Number	24	12	36
		% Type of tumor	66.7%	33.3%	100.0%
Total		Number	34	21	55
		% Type of tumor	61.8%	38.2%	100.0%

The number of smokers and the number of non–smokers are approximately equal in groups of patients with or without bronchial colonization (X^2=0.048; p>0.05). There was no statistically significant difference in the existence of bronchial colonization according to the gravity of patients' smoking habit. (Z=0.249; p>0.05). Patients with and without bronchial colonization did not significantly differ according to the body mass index (BMI) (Figure 2).

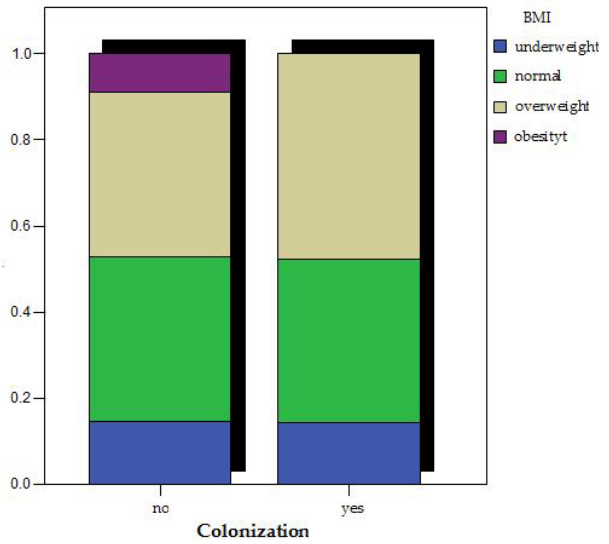

Figure 2: Body mass index – BMI in patients with and without bronchial colonization

Nine patients (43%) with bronchial colonization suffered from anaemia. Leukocytosis was found in 9 patients (43%) and all the patients had increased sedimentation rate (100%). High levels of fibrinogen were found in 20 patients (95%).

Bronchial colonization was by one third more common in patients with tumors localized in the right lung, but that difference was not statistically significant (X^2=0.008; p>0.05). In the group of COPD patients, bronchial colonization was found in 42,9% of patients. If patients with lung cancer also suffer from COPD, that does not mean they will be significantly more prone to bronchial colonization (X^2=1.583; p>0.05).

The largest number of patients with bronchial colonization were those suffering from adenocarcinoma (43%), planocellular carcinoma (24%) and adenosquamous carcinoma (24%) (Table 2).

Table 2: Prevalence of certain types of carcinoma in patients with bronchial colonization

			HP result					Total
			Adenocarcinoma	Planocellular carcinoma	Adenosquamous carcinoma	Carcinoid	Other	
Colonization	no	Number	20	10	3	0	1	34
		% of colonization	58.8%	29.4%	8.8%	.0%	2.9%	100.0%
	yes	Number	9	5	5	1	1	21
		% of colonization	42.9%	23.8%	23.8%	4.8%	4.8%	100.0%
Total		Number	29	15	8	1	2	55
		% of colonization	52.7%	27.3%	14.5%	1.8%	3.6%	100.0%

Most of the patients with bronchial colonization were those with IB stage of lung cancer, whereas those patients who were not diagnosed with bronchial colonization usually had IIA stage lung cancer. Patients with and without bronchial colonization did not statistically differ according to certain stages of the disease defined by TNM classification (Z=1.399; p>0.05).

Bronchial colonization was found in 21 patients with lung cancer (38%). Isolated bacteria were divided into two groups: potentially non-pathogenic microorganisms (nonPPMs) and potentially pathogenic microorganisms (PPMs). Isolated microorganisms belonged to the group of potentially non–pathogenic microorganisms in 8 cases (38%). *Streptococcus viridans* was isolated in 7 patients, whereas *Achromobacter xylosoxidans* was isolated in one patient. Potentially pathogenic microorganisms were isolated in 13 patients (62%). *Streptococcus pneumonia* was found in 5 patients, whereas *Streptococcus Bhaemoliticus* and *Pseudomonas* were isolated in 3 patients each. *Haemophilus influenzae* as

well as *Enterobacter* were isolated in 1 patient each.

All the patients underwent surgical treatment after they had been given preoperative prophylactic antibiotics. Most commonly administered antibiotics were third–generation cephalosporins. Pneumonectomy was performed in 23 patients (42%), lobectomy was performed in 27 patients (49%), segmental resection was done in 4 patients (7%) and 1 patient (2%) underwent exploratory thoracotomy.

During postoperative course, one patient (2%) developed postoperative pneumonia. A pathogenic cause of this complication was not isolated and the patient had not been previously diagnosed with bronchial colonization either.

DISCUSSION

Lower airways of healthy non–smokers are usually sterile, which has been revealed by several clinical studies (4-7). It is possible for them to remain sterile due to preserved immunological and non–immunological defence mechanisms of the intact bronchial mucosa. Cabello et al. (4) found only one healthy subject who yielded a pathogenic microorganism (*S. aureus)* in significant counts. Kirkpatrick and Bass (7), who examined 8 healthy subjects, got a similar result. There was one subject with potentially pathogenic microorganisms isolated from PSB and BAL cultures.

Some studies show that a significant number of patients suffering from certain chronic diseases that compromise their immune system and defence mechanisms also have bronchial colonization (9, 12, 13). Most commonly examined patients are those suffering from chronic obstructive pulmonary disease (4, 14), lung cancer (4, 10), bronchiectasis (4, 11) and those with tracheostoma (4).

It is well–known that lower airways of patients suffering from chronic obstructive pulmonary disease are colonized and similar results were obtained by examining bronchial colonization in patients with lung cancer (12, 13). Results obtained by examining the type of colonization in patients with lung cancer in the resectable stage can contribute to choosing an adequate preoperative antibiotic prophylaxis.

Our study has confirmed the existence of lower airways bacterial colonization in 38% of patients with resectable non–small cell lung cancer. This result is similar to the findings of other studies that have dealt with this problem (6, 12, 13). M. Ioanas et al. reported the existence of colonization in 41% of patients with resectable lung cancer (10) and Cabello at al. reported it in 42% of cases (4). In only one study lower airways colonization was 83% perioperatively. The reason for this significant difference probably lies in the fact that in this study samples were taken from both lungs. In our study and previously mentioned studies with similar colonization percentage, the sample taken from one lung was considered to be representative and if it was sterile it meant the entire bronchial tree was sterile, too.

The threshold for bronchial colonization has not been precisely defined yet, but in most of the studies $\geq 10^3$ CFU/L is considered to be positive (4, 10). In Cabell at al. frequently cited study (4) threshold for defining colonization is $\geq 10^2$ CFU/L. It is still not clear what causes bronchial colonization. Age and sex do not influence the development of bronchial colonization. In some studies (10) which examined the patients who had had cancer surgery, centrally localized tumors were more common in patients with bronchial colonization. Some researchers outline smoking as a risk factor for colonization. M Ioanas et al. (10) did not find any connection among the abovementioned factors in their study. The average age of colonized and non–colonized patients in our sample is identical and it is 60. In the group of patients with bronchial colonization there were twice as many men than there were women, but the difference was not statistically significant. The localization of tumor (central/peripheral) did not have an impact on the development of colonization. Our results show that there is no significant correlation between colonization and severity of smoking habit expressed through pack/year index.

Airway obstruction is another factor whose impact on bronchial colonization we examined. A large percent of patients with lung cancer also suffer from chronic obstructive pulmonary disease. In our sample, 43% of patients with bronchial colonization also had chronic obstructive pulmonary disease. There was no statistically significant difference between the patients with COPD and those without it. These data are in accordance with the findings of some other studies (10), but some authors explain that smoking and deteriorated pulmonary function are risk factors for developing bronchial colonization. The

pulmonary function of all the subjects in our study was good enough for surgical treatment, which means we had a very narrow variation range. That is a possible explanation why the degree of obstruction did not have an impact on the development of colonization.

Body mass index in our study did not have an influence on developing bronchial colonization unlike other studies that showed that obesity had an impact on colonization, but there were few obese subjects in the groups we examined (10).

In our sample, the patients with bronchial colonization had a marked positive inflammatory syndrome, but these values were not significantly different in patients with bronchial colonization and those without it. Anaemia of chronic disease was present in 43% of patients with colonization, which almost matches the percentage of patients with anaemia in total sample (44%). Anaemia of chronic disease and inflammatory parameters cannot indicate bronchial colonization development. These parameters were not examined in similar studies.

Bronchial colonization in the examined sample is by one third more common in the right lung than in the left lung. This finding can be explained by anatomy and the location of the right main bronchus which makes it easier for upper airway bacteria to reach it. More frequent colonization in patients with adenocarcinoma than in patients with planocellular carcinoma is in accordance with a higher percentage of patients suffering from adenocarcinoma in the total sample.

Bronchial colonization is influenced by centrally localized tumor and high body mass index (10), which was not confirmed by our study, possibly due to the sample size and a small number of obese subjects. In our study and in other studies (10), smoking, severity of smoking habit and airway obstruction degree do not have an impact on the development of bronchial colonization. Laboratory inflammatory parameters and the degree of anaemia cannot indicate the development of bronchial colonization.

PSB samples were more often positive than BAL cultures (15). PSB cultures were positive in 19 cases (90%), which matches the findings of other researchers (10, 14). This confirms the fact that colonization is a process which takes place in bronchi, not in alveoli (4).

In our study, potentially pathogenic microorganisms were isolated in greater percentage (62%) than non–pathogenic (38%) and it can be possibly explained by the existence of chronic obstructive pulmonary disease which is characterized by more frequent and recurrent infections.

Out of 55 patients from our sample who underwent lung cancer surgery, only one patient (2%) had a postoperative complication caused by an infective agent. This patient was diagnosed with postoperative pneumonia, but the cause of the infection was not isolated. The preoperative bronchial colonization was not confirmed in this patient. Many studies show that preoperative finding of bronchial colonization and the cause of postoperative bacterial complication do not match to a great degree and that patients are at risk even without colonization (17).

CONCLUSION

In our study, we proved the existence of lower airway bacterial colonization in 38% of patients with resectable non–small cell lung cancer, which is in accordance with other authors' findings. Potentially pathogenic microorganisms more frequently led to bronchial colonization than non–pathogenic ones. Age, body mass index and tumor localization did not influence bronchial colonization. The presence of chronic obstructive pulmonary disease did not have an impact on bronchial colonization either. In this study, we did not determine the link between the cause of previous colonization and the cause of postresectional infective complication.

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Kolonizacija bronha kod bolesnika sa nesitnoćelijskim karcinomom pluća

Ana Stojanović

Klinički centar Bežanijska kosa, Klinika za pulmologiju, Beograd, Srbija

SAŽETAK

Donji disajni putevi su sterilni, ali mnogi istraživači potvrđuju postojanje kolonizacije bronha patogenim i nepatogenim mikroorganizmima kod pušača i obolelih od hronične opstrukcijske bolesti pluća i karcinoma pluća. Cilj ove studije bio je da utvrdi postojanje i vrstu kolonizacije bronhijalnog stabla kod obolelih od nesitnoćelijskog karcinoma pluća (NSCLC). Utvrditi da li godine života, pušenje, disajna funkcija i indeks telesne mase (body mass index, BMI) obolelih utiču na postojanje kolonizacije. Takođe, proceniti da li kolonizacija utiče na pojavu ranih postoperativnih komplikacija lečenja. Ova studija preseka obuhvatila je 55 ispitanika sa patohistološki potvrđenim nesitnoćelijskim karcinom pluća u resektabilnoj fazi bolesti i dobrim performans statusom (ECOG 0 ili 1). Procenjivali smo težinu pušačke navike, izračunavali BMI i određivali postojanje i stepen težine eventualno pridružene hronične opstrukcijske bolesti pluća. Pri fleksibilnoj bronhoskopiji uziman je uzorak biopsije zaštićenom četkicom, kao i bronhoalveolarni lavat i materijal je zasejavan na hranljivim podlogama. Kolonizaciju je označavao porast bakterija veći od 10^3CFU/L. U našoj studiji, kolonizacija bronhalnog stabla bila je prisutna kod 21 bolesnika (38%). Potencijalno patogene mikroorganizme izolovali smo kod 13 bolesnika (Streptococcus pneumoniae kod 5, Streptococcus B haemoliticus kod tri i Pseudononas kod tri bolesnika, Haemophilus influencae kod jednog bolesnika i Enterobacter kod jednog bolesnika). Potencijalno nepatogene mikroorganizme izolovali smo kod 8 bolesnika (Streptococcus viridans kod 7, Achromobacter xylosoxidans kod jednog bolesnika). Pol, starost, pušenje, indeks telesne mase, kao ni težina ispoljene hronične opstrukcijske bolesti pluća, nisu statistički značajno uticali na pojavu kolonizacije bronhijalnog stabla. Samo je jedan operisani imao postoperativno pneumoniju, ali kod njega prethodno nije utvrđena kolonizacija bronhijalnog stabla.

Ključne reči: **kolonizacija bronha, karcinom pluća, braš biopsija**