The role of respiratory microbiota in the response to systemic treatment of lung cancer

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Introduction

There is growing interest and awareness concerning the human microbiome and its role. The average human body hosts $3.8 \cdot 10^{13}$ bacteria, which is about 0,2 kg (1). The concept of a sterile lung is now decisively outdated. Next Generation Sequencing (NGS) of 16S rRNA allowed us to conduct thorough research on the composition of the human microbiome. Commensal flora turned out to impact a vast number of diseases, including obesity, diabetes mellitus (2), asthma (3), cystic fibrosis (4), cardiovascular diseases (5), or exacerbations of COPD (4). This topic also occurred to be significant in oncology and during the studies concerning the role of the microbiome in cancer, emerged a new term: oncobiom. Lung cancer is still the most common cancer in men and women combined and the leading cause of cancer deaths (6). Recently we acquired new methods of treatment in advanced stages of lung cancer, one of them being immune checkpoint inhibitors (ICIs). Since commensal flora has an impact on lung cancer pathogenesis, there is a question of whether it can also influence the effectiveness and adverse effects of the treatment. There is multiple evidence that aspiration of microorganisms from the oral cavity is essential to formation of individual respiratory microbiomes and may participate in development of a proinflammatory phenotype that promotes cancerogenesis (7-9). This phenomenon explains the decision to use buccal swabs in our study to collect microbiological samples for NGS of 16S rRNA.

Aim and research hypothesis

In our study, we want to assess the composition of the respiratory microbiome in patients undergoing systemic treatment of lung cancer and its role in the response to systemic treatment.
We established the following hypotheses:

There is a difference in the composition of the microbiome between lung cancer patients and the control group.

Composition of microbiome changes during systemic treatment.

Changes in the microbiome differ depending on the type of administrated treatment.

The specific composition of the microbiome can be responsible for better course and adverse effects of administrated treatment.

Materials and Methods

The study was approved by the Bioethics Committee of the Medical University of Lodz and is covered by mandatory insurance for medical experiments conducted as part of the Medical University of Lodz. It is conducted since September 2022 at the Clinical Department of General and Oncological Pulmonology of Military Medical Academy Memorial Teaching Hospital of the Medical University of Lodz and the Biobank Lab of the University of Lodz. It is a non-interventional, observational, longitudinal, single-center study.
Lung cancer diagnosis, staging, and type of treatment are defined according to current guidelines according to Good Clinical Practice principles. Patients are qualified for immunotherapy according to the B6 drug program. Participants are recruited to the study before the administration of systemic treatment and undergo procedures listed in Table 1 at the starting point and every 12 weeks, which is a checkpoint visit for patients treated with immunotherapy. Patients are enrolled after obtaining informed, written consent.

Table 1

Performed procedures

Buccal swab
Blood sample analysis
Analysis of body composition with electrical bioimpedance

Buccal swabs are done twice and are collected with Isohelix DNA/RNA Buccal Swabs, stabilized and stored in a freezer in 4°C until isolation of DNA. DNA is isolated from the collected material. Isolation is done with Dneazy PowerSoil Pro Kit. Then the material is frozen in 20°C until further assays in Biobank. Microbiom is analysed by 16S rRNA sequencing. It is a method that focuses on bacteria among microorganisms and estimates their abundance ratio. The gene is amplified and decoded. 16S gene is different for each bacterial species, so we can assess what type of bacteria are present in the sample, and in what proportion they are represented (10). To identify taxa abundant in lung cancer patients Analysis of Composition of Microbes (ANCOM) will be performed.

Blood is collected from patients into test tubes without coagulant. After collection, it is centrifuged, and the supernatant is frozen at -80 ° C until further tests are done.
Electrical bioimpedance is performed on every assessment point. Each procedure contains two measurements to ensure its repeatability. Data with normal distribution is analyzed with Student's t-test for paired samples. Data deviating from the normal distribution is analyzed with Mann-Whitney U test.

Results

We retrospectively analyzed the demographical and clinical features of patients undergoing immunotherapy in our clinic since 2020 (Table 2) and the type of their treatment (Table 3). We also acquired archival data from bacterial cultures from bronchoscopy to characterize which bacteria were present in their samples of bronchoaspirate (Figure 1).

Table 3

Type of received treatment among patients undergoing immunotherapy from 2020 to 2023.

Type of treatment, n (%)	
Immunotherapy in monotherapy	29 (47%)
Immunochemotherapy	33 (53%)
Line of treatment, n (%)	
I line of treatment	41 (66%)
II line of treatment	15 (24%)
Adjuvant treatment	6 (10%)

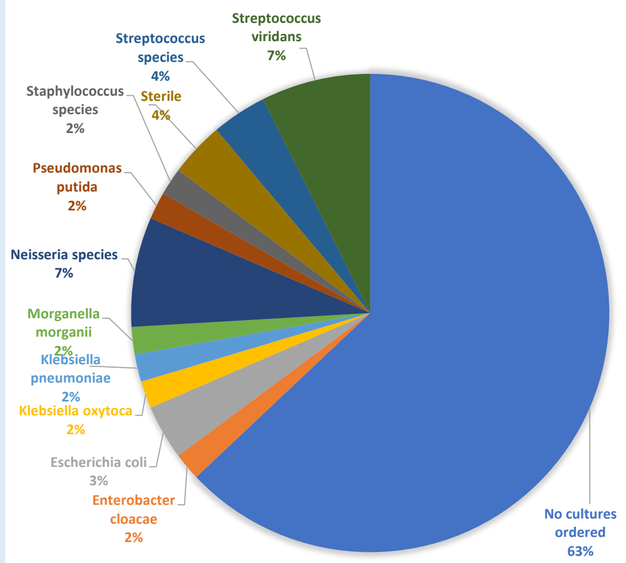
Table 2

Baseline characteristics of the patients undergoing immunotherapy from 2020 to 2023.

Sample size	62
Age, mean (SD)	68 (7)
Sex, n (%)	
Men	40 (65%)
Women	22 (35%)
Histopathological type, n (%)	
Adenocarcinoma	31 (50%)
Squamous cell lung cancer	23 (37%)
Large cell lung carcinoma	1 (2%)
Neuroendocrine lung cancer	1 (2%)
Small cell lung cancer	6 (10%)
PD-L1 expression, n (%)	
> 50%	14 (23%)
1-49%	10 (16%)
< 1%	13 (21%)
not determined	25 (40%)

Figure 1

Results of cultures of bronchospirate of the patients undergoing immunotherapy from 2020 to 2023

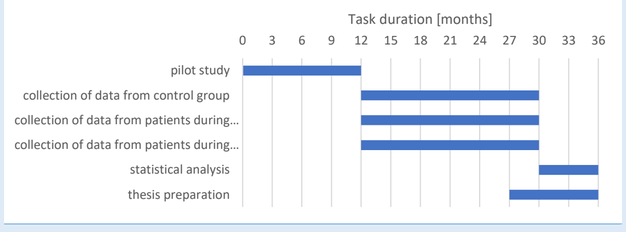


Conclusion

The study limitations are bound to a relatively small test group. At this point, we are in the process of a pilot study (Fig. 2) to validate our methods and assess the needs of the test group since oncological patients require targeted conduct.

Figure 2

Gantt chart depicting duration and distribution of Research Tasks



Even though there is evidence of a correlation between development and progression of lung cancer and the composition of respiratory microbiome, the mechanism is still unclear with inconsistencies between current studies. A lot of promising research on the role of microbiota in systemic treatment was done on animal models and requires further studies. Since lung cancer is still the leading cause of cancer deaths, despite the advances made in systemic treatment, we have to look for all the factors that can improve the outcome of the treatment. Reports on how lung microbiome changes during immunotherapy are contraindicatory.

Acknowledgement

The authors express gratitude to prof. dr hab. n. med. Paweł Majak for valuable assistance.

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