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MICROBIOLOGICAL MONITORING OF NASAL LAVAGE FLUID AS A METHOD FOR EARLY DETECTION AND PREVENTION OF BACTERIAL LUNG COMPLICATIONS IN A PATIENT WITH CYSTIC FIBROSIS

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Abstract. The severity of complications in cystic fibrosis are determined by microorganisms colonizing the lower airways. Paranasal sinuses can be a reservoir of aggressive pathogens. We have developed a method for collection and primary inoculation of nasal lavage fluid from cystic fibrosis patients for microbiological investigation. As a clinical case illustrating the feasibility of this technique, we describe the dynamics of the microflora composition in a patient with cystic fibrosis. The patient had a clinical and microbiological picture of *P. aeruginosa* eradication from the lung tissue, owing to which the antibacterial therapy was stopped. Six months later, the microflora in the nasal lavage fluid and sputum were assessed in parallel. The growth of *P. aeruginosa* (10^2 CFU/mL) but not *P. aeruginosa* in sputum was detected. To determine origin of this strain, the degree of genetic relationship between 5 strains obtained from the patient from 2008 to 2016 was assessed based on bacterial protein profiling. A typical strain of *P. aeruginosa* ATTS 27853 was used as a control. Strains isolated from the patient in 2009 and 2016 were identical suggesting that the antibacterial therapy led to eradication of *P. aeruginosa* in the lungs, but not in the upper airways. Four months later, the growth of *P. aeruginosa* was found in sputum. The patient was prescribed to use antibacterial drugs inhaled into paranasal sinuses. Repeated test performed 3 months later resulted in growth of *P. aeruginosa* 10^1 CFU/mL from nasal lavage fluid, but not from sputum. The patient was referred to a risk group on airway colonization by pathogen strains derived from the upper airway tract. The clinical example illustrates relevance of conducting a regular microbiological study of nasal lavage fluid in order to early identify clinically significant pathogens to prevent their spread to the lower airway tract.

Key words: *cystic fibrosis, nasal lavage, lower airways, paranasal sinuses, sputum, MALDI-ToF mass spectrometry.*

ОПЫТ МИКРОБИОЛОГИЧЕСКОГО МОНИТОРИНГА ЖИДКОСТИ НАЗАЛЬНОГО ЛАВАЖА ДЛЯ РАННЕГО ВЫЯВЛЕНИЯ И ПРОФИЛАКТИКИ БАКТЕРИАЛЬНЫХ ОСЛОЖНЕНИЙ ЛЕГКИХ У ПАЦИЕНТА С МУКОВИСЦИДОЗОМ

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Резюме. Тяжесть осложнений при муковисцидозе определятся микроорганизмами, колонизирующими нижние дыхательные пути. Однако параназальные синусы также способны быть резервуаром агрессивных

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Кондратенко О.В., Лямин А.В., Ерещенко А.А., Антипов В.А. Опыт микробиологического мониторинга жидкости назального лаважа для раннего выявления и профилактики бактериальных осложнений легких у пациента с муковисцидозом // Инфекция и иммунитет. 2023. Т. 13, № 1. С. 167–170. doi: 10.15789/2220-7619-MMO-2055

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патогенов. Нами был разработан способ сбора и первичного посева жидкости назального лаважа от пациентов с муковисцидозом для микробиологического исследования. В качестве клинического примера, иллюстрирующего возможности применения данной методики, приводится описание динамики микрофлоры пациента с муковисцидозом. У пациента отмечалась клиническая и микробиологическая картина эрадикации *P. aeruginosa* из легочной ткани, в связи с чем было остановлено проведение антибактериальной терапии. Спустя 6 месяцев было проведено исследование микрофлоры жидкости назального лаважа пациента с параллельным посевом мокроты. Получен рост культуры *P. aeruginosa* 10^2 КОЕ/мл, в мокроте роста культуры *P. aeruginosa* получено не было. Для решения вопроса о происхождении данного штамма была проведена оценка степени генетического родства между 5 штаммами, полученными от пациента в период с 2008 по 2016 гг. на основании их белкового профилирования. В качестве контрольного штамма был использован типовой штамм *P. aeruginosa* АТТС 27853. Установлено, что штаммы, выделенные от пациента в 2009 и 2016 гг., являются идентичными. Это обстоятельство свидетельствует о том, что проведенная антибактериальная терапия привела к эрадикации возбудителя в ткани легких, но при этом не воздействовала на него в верхних дыхательных путях. Спустя 3 месяца вновь был получен рост культуры *P. aeruginosa* в мокроте. Пациенту назначена антибактериальная терапия, включающая введение ингаляционных антибактериальных препаратов в параназальные синусы. При повторном исследовании жидкости назального лаважа с параллельным посевом мокроты пациента спустя 3 месяца получен рост культуры *P. aeruginosa* 10^1 КОЕ/мл из жидкости назального лаважа, то есть отмечена тенденция к снижению титра возбудителя в верхних дыхательных путях. В мокроте роста культуры *P. aeruginosa* получено не было. Пациент отнесен к группе риска по колонизации нижних дыхательных путей штаммами из верхних дыхательных путей. Клинический пример иллюстрирует необходимость и актуальность проведения регулярного микробиологического исследования жидкости назального лаважа с целью раннего выявления клинически значимых возбудителей и проведения профилактических мероприятий по недопущению их распространения в нижние дыхательные пути.

Ключевые слова: *муковисцидоз, назальный лаваж, нижние дыхательные пути, параназальные синусы, мокрота, MALDI-Tof масс-спектрометрия.*

Introduction

Cystic fibrosis is the most common genetic pathology. The prognosis of the disease, in most cases, will be determined by bacterial pathogens colonizing the lower airways (LA) [1, 2, 3]. The works of foreign authors show that paranasal sinuses are able to be a reservoir for infection and a zone for adapting aggressive clones. Such pathogens are *Pseudomonas aeruginosa, Burkholderia cepacia* complex, MRSA and others, which become sources of infection with LA [5, 6]. A unified algorithm for the study of nasal sinus microflora in patients with cystic fibrosis has not been developed in the Russian Federation at the moment. We have developed a method for collecting and primary inoculation of nasal lavage fluid from cystic fibrosis patients for microbiological examination (Patent for Invention No. 2659155) [4].

As a clinical example illustrating the possibility of using this method in the practical work of doctors of centers for the treatment of cystic fibrosis, we describe the dynamics of the microflora of patient R. Patient is 16 years old and is being monitored at the center for the treatment of cystic fibrosis with a diagnosis: Cystic fibrosis, mixed form, severe course. delF508/N1303K mutations. The diagnosis was made at the age of two, in 2004. From 2004 to 2014, the sputum showed an increase in *P. aeruginosa*. During antibacterial therapy from 2014 to 2016, a clinical and microbiological picture of eradication of the pathogen from pulmonary tissue was noted (in accordance with the requirements of the European Consensus: Early therapy and prevention of lung damage in cystic fibrosis (2004) — presence of at least three times negative crops within six months)) [7]. Considering the clinical improvement and the results of the microbiological study in 2016, antibacterial therapy for *P. aeruginosa* was discontinued. In November 2016, a parallel research of the microflora of nasal lavage fluid and sputum was conducted. The study resulted in growth of *P. aeruginosa* 10^2 CFU/mL culture in nasal lavage fluid with no growth of *P. aeruginosa* culture in sputum.

For the period from 2008 to 2016, we preserved 5 strains of *P. aeruginosa* isolated from this patient, including a strain obtained from nasal lavage fluid. Considering the previous clinical and microbiological picture of eradication from LA, it was unclear whether this was colonization of paranasal sinuses with a new strain of *P. aeruginosa*, or whether the strain was eroded from LA, but was able to persist in the sinuses. In order to understand these epidemiological aspects, we assessed the degree of genetic relationship between strains obtained from the patient at different times based on their protein profiling. For this purpose, protein spectrums of strains were obtained

using the formic acid extraction method. The results were then cluster analyzed using the MALDI-ToF mass spectrometry method.

The strains obtained from the patient were numbered from 1 to 5, while strain 1 was obtained in July 2008 (sputum), strain 2 — in March 2009 (sputum), strain 3 — in May 2009 (sputum), strain 4 — in December 2008 (sputum), strain 5 — in November 2016 (nasal lavage liquid). Typical strain *P. aeruginosa* ATTS 27853 (strain 6) was used as a control strain for dendrogram construction. The obtained data were visualized using a cluster dendrogram (Fig., see color plate, p. I).

The dendrogram shows that strains from 1 to 5 have signs of genetic relationship. At the same time strain 6 is significantly distanced from them. Strains 1 and 3 as well as 4 and 5 are found to be descendants of the same clone. Strains 1 and 3, as well as 4 and 5 have a minimum distance level, which allows them to be considered genetically identical. The presented dendrogram clearly demonstrates that the strains isolated from the patient in 2009 and 2016 are identical. This circumstance indicates that the conducted antibacterial therapy led to the eradication of the pathogen in the lung tissue, but did not affect it in the upper airways (UA). In March 2017, the patient's sputum was retested. The recommended sinus debridement was not carried out due to the low compliance of the patient, taking into account his age characteristics. In sputum, growth of the *P. aeruginosa* culture was obtained again. Analyzing the results of the microbiological research, the patient was re-prescribed antibacterial therapy for this pathogen. It included not only nebulizer therapy with LA, but also the introduction of inhaled antibacterial drugs into paranasal sinuses. In June 2018, a retest of the nasal lavage fluid with parallel culture of the patient's sputum was performed. As a result of testing of the nasal lavage fluid, growth of the culture of *P. aeruginosa* 10^1 CFU/ml was obtained. Thus, there was a tendency towards a decrease in the titer of the pathogen in UA. No growth of *P. aeruginosa* culture was obtained in sputum after antibacterial therapy. However, the persistence of the strain in UA suggests the possibility of its appearance in sputum in the coming months, if the corresponding therapy with UA is not continued. This patient is considered by us as being at risk for airway colonization of strains from UA.

This clinical example clearly illustrates the necessity and relevance of conducting a regular microbiological study of nasal lavage fluid in order to early identify clinically significant pathogens and carry out preventive measures to prevent their spread in the LA.

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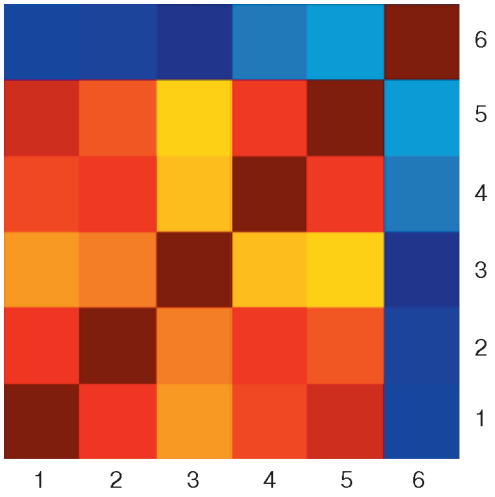

Figure. Cluster dendrogram constructed by using 5 strains isolated from patient R. (1–5) and a typical strain of *P. aeruginosa* (6)
Note. Mass spectrums of bacterial strains are located along the Y-axis (vertical line) from bottom to top of the dendrogram, along the X-axis (horizontal lines) from left to right of the dendrogram. The color of the cell reflects the degree of affinity of the corresponding strain pair. The range of cell colors corresponds to the thermal imaging scale from dark blue (with absolute difference in strains) to dark red (with complete coincidence).