The case of diagnostics of invasive pulmonary aspergillosis by in vivo probe-based confocal laser endomicroscopy of central and distal airways

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1. Introduction

Aspergillus spp. are the cause of a variety of pulmonary abnormalities that range in severity from airways colonization to invasion of the lung and its blood vessels, leading to sepsis and death [1]. There are approximately 300 species of the genus Aspergillus. They are saprophytic ubiquitous fungus and its spores can be easily inhaled everywhere [2]. But only approximately 8 species are responsible for the vast majority of human disease [3]. Aspergillus fumigatus is the most common pathogen, followed by A niger, with A nidulans, A terreus, A clavatus, A flavus, A niveus, and A ustus [4].

These fungi get their name from a ritual liturgical implement, called aspergillum, used in Roman Catholic ceremonies, which resembles the fruiting body of Aspergillus spp [5]. It is necessary to stress that fruiting bodies develop from mycelia in areas of high oxygen tension, such as lung/or sinus cavities, and do not develop in tissues [5].

The manifestations of pulmonary aspergillosis are determined by the host’s immune status, and have been classified into 4 main groups: saprophytic aspergillosis (aspergillosina), allergic bronchopulmonary aspergillosis (ABPA), semi-invasive aspergillosis (chronic necrotizing pulmonary aspergillosis, CNPA), and invasive pulmonary aspergillosis (IPA) [6,7].

IPA is the most severe type of the disease with a significant cause of morbidity and mortality in immunocompromised patients [8].

Extensive work has been done in the field of diagnostic imaging of IPA. On chest CT invasive pulmonary aspergillosis usually manifests as dense, well-circumscribed lesions with or without a halo sign, an air crescent sign and cavity [9].

On microscopy, the hyphae of Aspergillus spp. range in diameter from 2.5 to 4.5 μm and exhibit frequent septation. And there is one differential characteristic of Aspergillus spp. hyphae: they tend to branch dichotomously, progressively, and primarily at acute angles of approximately 45°, mimicking an arborizing tree branch [10].

But the diagnosis is still difficult to ascertain, that lead to late treatment of IPA resulting in a high mortality.

A relatively new technology of pulmonary imaging, probe-based confocal laser endoscopy (pCLE), which is also called alveoscopy, allows for real-time non-invasive visualization of intraacinar structures in vivo. The term “optical biopsy” is becoming more and more popular in respect of in vivo pCLE and reflects the ability of the technique to substitute to some extent real biopsy, what is the most essential in critically ill
immunocompromised patients with contraindications to lung biopsy. The role of in vivo pCLE in diagnostics of IPA has not been yet assessed in humans. The only study of Morisse H. and colleagues describes the use of fibered confocal fluorescence microscopy for in vivo and in situ imaging of experimental invasive pulmonary aspergillosis in immunosuppressed rats [11].

Here, we report a case of the first in vivo pCLE imaging of IPA. The study was approved by the Ethical Committee of Federal Research Clinical Center FMBA of Russia. We obtained the patient informed consent before the procedure. The pCLE technique is being explored in a research setting and that this gave the opportunity to perform this procedure in the patient.

2. Case

A 41-year-old male was admitted to our clinic (day “0”) in a severe condition with a three-week history of productive cough, dyspnea, high fever and general weakness appeared after an episode of hypothermia. First he was treated in one of the Moscow hospitals with the diagnosis of right-sided upper lobe multisegmental necrotizing pneumonia. Antibacterial and empirical treatment for tuberculosis had been ineffective and patient was transferred to the Federal Research Clinical Center for the specialized treatment.

From the personal history of the patient it is known that 3 months before he underwent colectomy for familial adenomatosis polyposis, that was complicated by multiple perforations of small bowel in the postoperative period with the following development of sepsis and massive adhesion in the abdominal cavity. Two relaparotomies were performed with the bacteriological analysis that showed Candida albicans, Enterococcus faecalis, Klebsiella pneumoniae. Therapy with meropenem and vancomycin was applied. The patient was sent to the outpatient treatment with the open granulating wound.

Laboratory test performed on day 0 revealed abnormal changes in WBC up to 39.1 × 10^9 L⁻¹, with a differential of 85% neutrophils and C-reactive protein (CRP) 272.5 mg/L. The clotting parameters were saved. All liver markers showed expressed dysfunction, including albumin 28.9 g/L, alanine aminotransferase 132 U/L, creatinine 266 μmol/L. Tests for human immunodeficiency virus were negative.

Arterial blood gas analyses were: PaCO₂ 31.2 mmHg, PaO₂ 31.2 mmHg, pH 7.34. High resolution computed tomography (HRCT) on day 1 showed big thick-walled ring-shaped zone of consolidation with polymorphous cavitory destructive changes in the right upper lobe (Fig. 1A and B). The features of bilateral multisegmental pneumonia were observed both sides (Fig. 1C).

Bronchoscopy on day 2 revealed that the back of the throat, mucosa of larynx, trachea and bronchial tree was inflamed and covered by dim-gray color membrane presented in a fixed thick layer all over the listed parts. The only focuses of free mucosa were observed in the lower lobe bronchi both sides. Differential diagnostics for mycotic lesion, tuberculosis and neoplastic process was needed. Pulmonary biopsy was impossible because of the patient’s condition. The membrane was collected by brush biopsy for pathogen culture.

The Ziehl-Neelsen stain of acid-fast bacilli in the material on day 2 was negative, but Aspergillus niger was isolated from the culture.

The diagnosis of IPA was made on day 2 and based on the culture-positive A. niger, the imaging findings and the absence of the response to empirical treatment for tuberculosis and pneumonia.

pCLE was performed on day 4, after the diagnosis was developed with the purpose to estimate IPA endomicroscopic features. pCLE was provided with the commercial Cellvizio system (Mauna Kea Technologies, Paris, France). We used a 1.4-mm diameter miniprobe (Alveoflex; Mauna Kea Technologies). This probe uses a laser (wavelength 488 nm) directed into the alveolar space, generating real time moving images with an optical area of 600 μm at a video frame rate of 12 images per second and a focus depth of 50 μm. The probe was introduced through the working channel of the bronchoscope and gently pushed down first to bronchial wall and then to the alveolar ducts and sacs. We did not use any exogenous fluorophore. The investigation was made in lungs segments with and without (Fig. 2) changes according to HRCT data.

![In vivo alveolar pCLE imaging at 488 nm in patient with IPA. Normal alveoli in left lower lobe.](image)

**Fig. 1.** Extensive area of the destructive changes, surrounded by halo-shaped consolidation with the expressed perifocal reaction, in which center different shape and size air cavities are visualized on the background of infiltration (A, B). Confluent infiltrates in segments of middle and lower right lobes and separate focuses in lingular lobes of the left lung reveals the signs of bacterial pneumonia (C).
In the zone with the most expressed changes in the right upper lobe pCLE imaging demonstrated the absence of the differentiation of alveolar walls resulting in their complete destruction with well distinguishable fibrillar branching fluorescent structures and no cells (Fig. 3). The same image with fibrillar fluorescence was got from the bronchial wall covered by the dim-gray color membrane.

In other alveolar zones where on HRCT there were pneumonia signs on the background of the preserved alveolar elastin network (Fig. 4A) we found great amount of highly fluorescent cells appears to be macrophages (Fig. 4B), but considerable amount of them were much bigger than typical macrophage (Fig. 4C). In some alveoli we observed irregular complexes most probably being big highly fluorescent cells sticking together (Fig. 4D).

The therapy with voriconazole injection (first day 400 mg × 2, 200 mg × 2) was immediately started additionally to the antibiotic therapy and immunotherapy, on the day 2.

Unfortunately, in spite of the treatment on day 9 patient died from advanced pulmonary sepsis, septic shock and multiple organ failure.

Autopsy on day 10 showed the lung parenchyma with dichotomy branching mycelia and partially septated hyphae with extensive invasive growth (Fig. 5). Culture of the autopsy tissue was positive for A. niger.

3. Discussion

Patients with immunosupression have much higher risks of the development of IPA than those with the safe immune status. Various diseases and disorders can become risk factors for IPA,
such as corticosteroid therapy, prolonged neutropenia, etc. [11]. In our case study the immune system of the patients can be compromised by the mutilating surgery and the following interventions due to the complications developed in postoperative period.

Clinical signs of IPA have no specific features therefore lack of suspicion of this disease along with low activity of diagnostic actions often lead to postmortem diagnosis or to the critical condition of patients by the time of the beginning of the target therapy, as far as the success of the treatment increases at early application of antymycotic drugs [12].

Treatment with voriconazole, which efficiency is confirmed with a number of randomized researches [13], in this case was short and did not lead to positive result.

The time spent on inadequate therapy, appeared inefficient due to the wrong diagnosis of tuberculosis, as the most frequent disease in a differential diagnostics at destructive pneumonia, played the crucial role in a lethal outcome for our patient. IPA is really not a frequent event, but nevertheless taking into account the severity of the disease course and risks for the patient, any methods that can increase the efficiency of diagnostics of IPA are useful even if it is a question of expensive technologies such as pCLE.

pCLE is a rather new method of visualization in pulmonary diagnostics, having a unique opportunity to investigate distal airways, including respiratory compartment. Today foreign researchers have already accumulated a certain material, studying endomicroscopic features at the central [14] and peripheral [15] diseases of inflammatory and neoplastic character.

We did not find any cases of the application of pCLE in patients with IPA in the current available literature.

Having the experience of pCLE examining patients with various pulmonary pathology [16] we believe that IPA (at least among such diseases as bacterial pneumonia, sarcoidosis, emphysema, COPD, cancer, interstitial pneumonia, alveolar proteinosis, etc.) is the only disease at which fluorescent fibrillar structures with the dichotomizing branching, corresponding to hyphae of Aspergillus spp. are visualized. This finding is also confirmed by the researches of Morisse H. and colleagues [10] who described the similar fibrillar structures at pCLE in situ after thoracotomy with the histologically confirmed diagnosis of invasive aspergillosis in the experiment on rats.

The images on the Fig. 4 most likely characterize the process of incomplete phagocytosis with the formation of giant macrophages for the activation of the elimination of structures of Aspergillus spp., however these pictures are not specific only for IPA, and also can be seen at alveolar proteinosis and probably at other diseases when there are foreign substances or products of the metabolism disorders in alveoli lumen that need to be utilized.

D. Denning and coauthors [17] have shown that fungal hyphae cross the elastic fibers of pulmonary artery without any evidence of damage to the latter. What concerns the elastin destruction in our study, which is observed both on pCLE and histologic images, we believe that neutrophil granulocytes are responsible for this process due to the proteases release, rather than elastase production by Aspergillus.

We cannot say that pCLE is superior to HRCT for diagnosis of IPA, but it gives us valuable additional information, that can save the time for making correct diagnosis. Limitation of using pCLE is connected with that fact that only definite structures such as elastin, collagen type IV, proteins, lipids and some others are well distinguished during the examination. One more limitation is that the miniprobe tip is uncontrollable, moving ahead to distal airways based on the path of least resistance, so we can not accurately see the same alveoli during the second study. And the last one limitation is that this technology cannot be specifically used to diagnose IPA since we know the dichotomous branching is not unique for Aspergillus. Several other filamentous fungi can exhibit similar structures, such as hyalohyphomycetes: Acremonium spp., Fusarium spp., S. apiospermum, S. prolificans, Peacilomyces lilacinus, etc., so it is impossible to differentiate them histologically.

We can conclude that pCLE is a perspective method of in vivo express diagnostics of IPA, especially in critically ill patients. Its application can allow us to improve the efficacy of the treatment and to lower the lethality at this pathology.

Conflict of interest statement

There are none.

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