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Detection and identification of mucormycosis agents in native and formalin-fixed paraffin-embedded tissue samples of patients with mycosis using multiplex real time PCR

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Purpose

The aim of the study was to test a multiplex real time PCR with High Resolution Melt analysis (mHRM-RT-PCR) on clinical samples for detection and identification of *Mucormycetes* spp.in tissue samples.

Methods

The study included 8 native and 22 formalin-fixed paraffin-embedded tissue samples from 25 patients with mucormycosis in Saint-Petersburg between 2013 and 2021 yy. As controls, 21 tissue samples were collected from patients without mycoses. We investigated the native tissue samples by direct microscopy method with calcofluor white. Fungal cultures were obtained by sample inoculation on Sabouraud glucose agar. Histological sections of tissue samples were stained by PAS and Grocott - Gomori's technique. Fungal DNA was extracted from clinical samples by a chloroform-isoamyl extraction method. DNA amplification was performed using *Aspergillus* - and *Mucormycetes* - specific primers pairs separately and EvaGreen based mHRM-RT-PCR on Rotor-Gene 6000 cycler.

Results

The mHRM-RT-PCR allows to identify In clinical samples from patients with aspergillosis and mucormycosis the representatives of *Aspergillus* to the genus and *Mucormycetes* to the species level: *Rhizopus arrhizus, Rhizopus microsporus, Mucor racemosus, Rhizomucor pusillus, Lichtheimia corymbifera* (Fig.1).

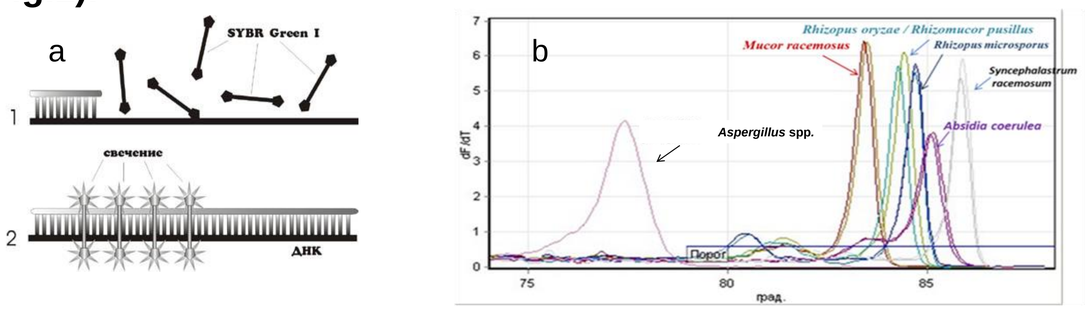

Fig.1. a: Real time PCR with use SYBR GREEN;

b: Melting temperature of PCR - products: Tm *Aspergillus sp.* - 76 - 79° ,

Tm *Mucormycetes* - small thermal spices – 81 – 82,5° , big - 84 – 87°.

In patients with mucormycosis direct microscopy of 8 native tissue samples was positive in 75% cases. *Lichtheimia corymbifera* was isolated in 12,5% and *Rhizopus arrhizus* - in 37,5% cases.

Results

In 22 formalin-fixed paraffin-embedded tissue non-septate mycelium of *Mucormycetes* spp. was detected. The mHRM-RT-PCR was positive in native and formalin-fixed paraffin-embedded tissue samples of all patients with mucormycosis. PCR assay allowed to identify the representatives of *Mucormycetes* spp.: *Rhizopus arrhizus* in 13, *Lichtheimia corymbifera* in 4, *Rhizomucor pusillus* in 2 and *Rhizopus microsporus* in 2 from 26 samples. In biological specimens of 4 patients (13%) the PCR assay detected a mixed infection by *Mucormycetes* and *Aspergillus* spp.: *Rhizopus microsporus* (50%) + *Aspergillus* spp. and *Rhizopus arrhizus* + *Aspergillus* spp. (50%).The positive results of PCR assay in patients with mucormycosis in 100% of cases correlated with traditional methods. In 21 control tissue samples PCR test was negative.

<u>Example 1.</u>The investigation of the biopsy material of liver tissue from patient S. with leukemia and mucormycosis.

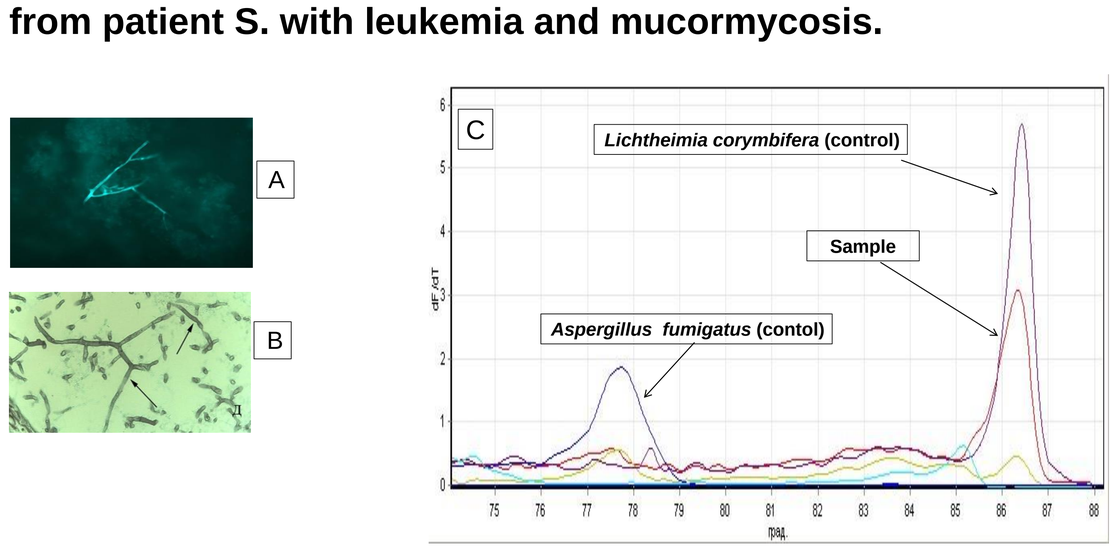

Fig.2 . A-direct microscopy: non-septate mycelium; B-histologic study (Gomori – Grocott) - non-septate mycelium; C –mHRM-RT-PCR –revealed DNA *Lichtheimia corymbifera.*

<u>Example 2.</u>The investigation of formalin-fixed paraffin-embedded tissue from patient M. with Hodgkin's lymphoma and mixed mycosis of lungs.

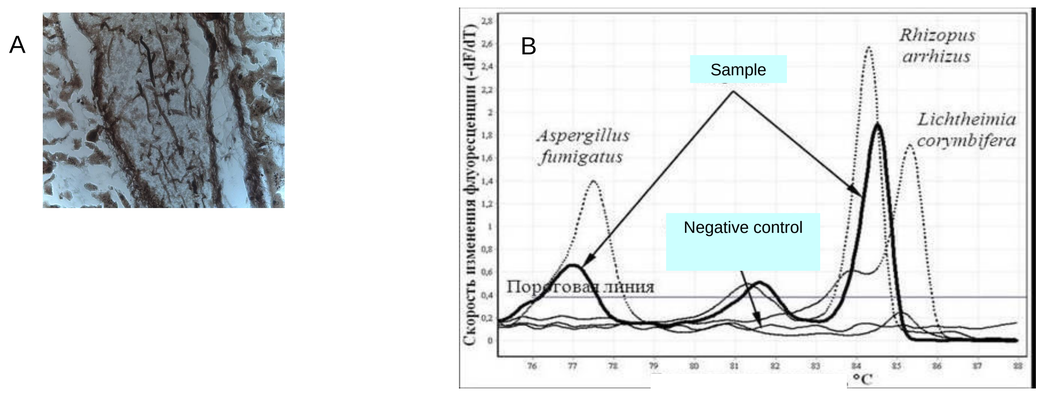

Fig.3 . A – histologic study (Gomori – Grocott) – non-septate mycelium; B – mHRM-RT-PCR – revealed DNA *Rhizopus arrhizus* + *Aspergillus fumigatus.*

Conclusions

The multiplex RT-PCR has high sensitivity and specificity in patients with mucormycosis. This study indicated that the mHRM-RT-PCR may be a very useful tool for detection of etiologic agents of mycoses, particularly in the case of a mixed infection caused by *Aspergillus* spp. and of the order *Mucorales*.

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