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Galactomannan determination in biological samples of non-hematological patients with invasive aspergillosis caused by different *Aspergillus* spp.

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Objectives To evaluate the sensitivity of "Platelia *Aspergillus* EIA" test for galactomannan (GM) determination in non-hematological patients with invasive aspergillosis caused by different *Aspergillus* spp. **Methods** The investigation included 115 clinical samples collected during 1999–2014 years. from 60 non-hematological patients with diagnosis of invasive aspergillosis (IA) confirmed by classical mycological methods according to EORTC/MSG, 2008. The age of patients varied from 7 months to 83 years (median –36 year). The main underlying diseases were: malignancies (22.4%), chronic sinusitis (15.5%), chronic pyelonephritis (12.5%), chronic obstructive pulmonary disease (8.6%), pneumonia (6.9%), pulmonary tuberculosis (6.9%), systemic diseases with pulmonary involvement (5.8%), chronic bronchitis (9.8%), vasculitis (1.7%), osteomyelitis (1.7%); no underlying disease – 3.5%. Totally 60 serum samples and 55 samples of broncho-alveolar lavage (BAL) were examined for GM *Aspergillus* spp. antigen by means of the "Platelia *Aspergillus* EIA" test (Bio-Rad Laboratories, USA) with calculating of the optical density index (ODI) of the antigen. The serum sample was considered as positive if ODI \geq 0.5 and BAL sample – if ODI \geq 1.0. All BAL samples were investigated mycologically including direct fluorescent microscopy with calcofluor white and culture on Sabouraud agar.

Results Cultures of 55 BAL samples from 60 patients with IA revealed various *Aspergillus* spp.: *A. fumigatus* (31%), *A. niger* (31%), *A. flavus* (11%), *A. ochraceus* (1%). From 20 patients (34%) two or more different species were isolated including rare species *A. ustus* (6%) and *A. versicolor* (3%). *Aspergillus* spp. often caused lung disease (68%), more rarely – sinusitis (22%), in 10% of patients > 2 sites of infection. GM test was positive in 45% of serum samples and 60% of BAL samples from patients with mycologically confirmed IA. In cases with *A. fumigatus* as the causative agent of IA sensitivity of GM test in serum was 47%, in BAL – 66%. In patients with IA caused by *A. niger* or *A. flavus* sensitivity of the tests was lower: 36% ($P = 0.02$) and 53% ($P = 0.03$) for *A. niger*; 16% ($P = 0.001$) and 40% ($P = 0.002$) for *A. flavus*. In biological samples of patients with IA caused by several species of *Aspergillus* GM test was positive in 42% (blood serum) and 70% (BAL) of cases. The results show species specificity of *Aspergillus* GM metabolism during the course of infection.

Conclusion The sensitivity of GM test in blood serum and BAL samples from non-hematological patients with invasive aspergillosis is lower than in hematological patients and depends on the type of biological sample and the species of the etiological agent.

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Brain Abscess due to *Cladophialophora bantiana*: first case in Portugal

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Objectives *Cladophialophora bantiana* is a highly neurotropic dematiaceous mold that is a rare cause of cerebral abscesses, particularly in immunocompetent patients, and is associated with a high mortality (up to 70%). The aim of the authors is to present a rare with a successful outcome case of a brain abscess caused by this species. Few cases have been reported in the literature world-wide, being this one the first reported in Portugal.

Methods and Results A 56 year-old male, with chronic hepatic disease (HCV and alcohol/drug abuse), HIV negative, referred to the neurosurgery department of our hospital with multiple neurological deficits and a space-occupying lesion in the left frontal lobe. The patient underwent multiple surgical interventions to remove an abscess and specimens were sent to the microbiology laboratory. The first abscess material sent for examination was "dura mater" and

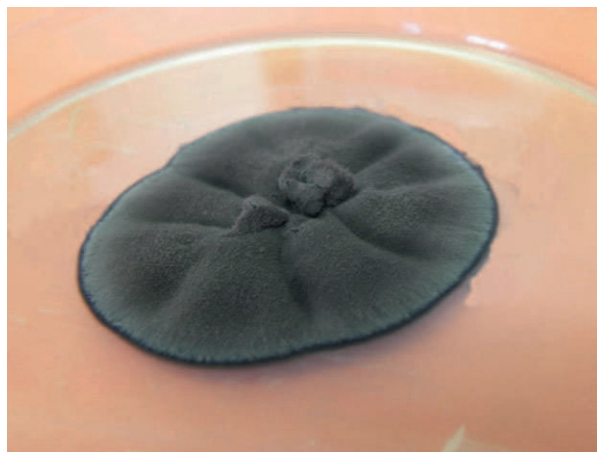


Figure 1

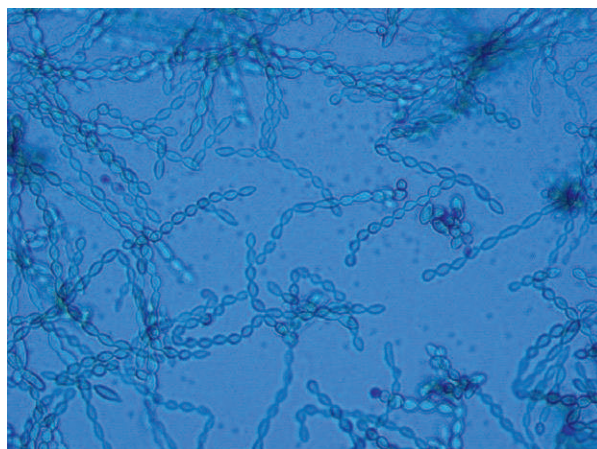


Figure 2

cultures revealed *Cladophialophora bantiana* and *Nocardia nova* (Maldi-TOF score 1.946) but in subsequent abscess material examined (pus) *C. bantiana* was the one persistently isolated. Samples were cultured on Sabouraud dextrose agar (SDA), SDA with gentamicin/chloramphenicol and SDA with actidione. After incubation velvety dark colonies were obtained with surface and reverse olive-gray to black color. Microscopic examination of a lactophenol blue mount preparation showed brown septate hyphae with long, sparsely branched conidiphores bearing wavy chains of smooth oval conidia without pigmented scars. The isolate was identified as *Cladophialophora bantiana* based on its morphological features growth at 42°C and cycloheximide resistance. Total genomic DNA was extracted. The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) of these isolates was amplified using the primer set ITS1 and ITS4. Sequencing of both strands was performed and nucleotide sequences were edited using the program Chromas2 and aligned using the program CLUSTAL X2. The obtained sequences were compared with sequences deposited in the GenBank and CBS-KNAW Fungal Biodiversity Centre databases: the isolate was identified as *C. bantiana* with 99% homology. Molecular results confirmed the microbiological diagnosis. Susceptibility testing was not performed. The patient was initially treated with vancomycin, meropenem and liposomal amphotericin B for 28 weeks but the poor clinical and imaging response lead to a switch to amoxicillin/clavulanic acid and voriconazole. After 16 months of the first brain abscess excision and after 5 months under therapy with voriconazol, the patient improved clinical and imagiologically, maintaining only minimal neurological deficits.

Conclusion New and emerging pathogens have renewed concern about the diagnosis and treatment of brain abscess and equally pose a challenge to clinical microbiologists. Given the fact that early and accurate diagnosis saves lives, such specimens should promptly be sent for microbiological analysis. Brain abscess is not only a neurosurgical emergency but also a microbiological emergency and a diagnostic challenge to both disciplines.

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Association of recalcitrant chronic rhinosinusitis with nasal polyposis and fungal finding in polyps single-cell suspension

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Objectives In recent years fungi are favoured as origin of recalcitrant chronic rhinosinusitis (CRS), especially with nasal polyps (wNP). The omnipresence of fungal spores makes the role of fungi in development of CRSwNP important, but difficult to determine. Data proving the hypothesis that fungi are involved in the pathogenesis of NP are limited mostly because of the difficulty to properly determine fungi and/or to interpret their finding from upper respiratory tract. Sensitive methods for fungal detection are still absent, therefore we used NP single-cell suspension for mycology investigations in aim to find out association of recalcitrant CRSwNP and fungal finding in patients that underwent functional endoscopic sinus surgery (FESS).

Methods A prospective case-series study and culture-based mycological examination was conducted in patients who underwent FESS for the first time (ft-FESS) and those with repeated FESS (re-FESS). The study was conducted in a tertiary Otorhinolaryngology Unit of Clinical Centre of Serbia and National Laboratory for Medical Mycology, Faculty of Medicine, University of Belgrade. A total of 43 consecutive patients with CRSwNP underwent FESS and 55 NP samples were analyzed by culture-based mycological examination. Samples were prepared as single-cell suspensions of NP tissue in Petri dish using a special mesh for the treatment of lymphatic tissue, under sterile conditions (Figure 1). Patient's co-morbidity data were collected.

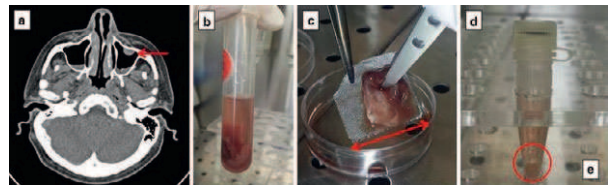


Figure 1. NP tissue processing algorithm for fungal detection

Results Our analyses found: (1) almost half of CRSwNP patients (19/43; 44%) underwent FESS more than once (re-FESS group); (2) fungi were detected (wF) in 10/43 (23.3%) patients (FESSwF group), representing 13/55 culture positive NP tissue (23.6%); (3) re-FESS group is the most often associated with asthma and aspirin intolerance (79; 58%, respectively) compared to ft-FESS group (25; 12%, respectively) ($P = 0.000$, $P = 0.002$; respectively); (4) mycology evaluation of single-cell suspension of NP tissue showed significant higher percentage of fungal finding in re-FESSwF than in ft-FESSwF group (42; 8%, respectively; $P = 0.01$); (5) we observed significantly longer duration of CRSwNP in FESSwF than in fungal negative patients (FESSsF group) (17.4 ± 10.9 ; 10.4 ± 7.0 years, respectively; $P = 0.033$) and (6) predominate strain was *Aspergillus flavus* detected in 6/10 patients.

Conclusion This is the first study which analysed association of fungi in single-cell suspension of NP tissue and recurrent CRSwNP. We demonstrate significantly higher percentage of positive fungal finding in re-FESSwF than in ft-FESSwF group. Although the role of fungi in CRSwNP is still controversial, we gave new insight in this field showing correlation between fungi and prolonged duration of CRSwNP and fungi and relapse of NP after FESS. Further studies are needed to determine if the nasal lavage with commercial or natural antifungals may have negative impact on the fungal growth in upper respiratory tract and benefit for patients with CRSwNP.

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Construction of a new diagnostic tool for the human pathogenic members of the *Fusarium fujikuroi* species complex

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Objectives Construct a new fast, accurate and reliable diagnostic tool for members of the *Fusarium fujikuroi* species complex: *Fusarium* species are emerging agents of superficial, locally invasive and systemic infections in humans. Especially members of the *Fusarium fujikuroi* species complex (FFSC) can cause disseminated fusariosis in immunocompromised – particularly leukemic – patients. Such disseminated fusarioses result in a high mortality rate of patients but patient survival may increase with rapid diagnosis. While most *Fusarium* species have high levels of resistance to antifungal compounds, they vary in their susceptibility towards commonly used antifungal drugs, like amphotericin B, voriconazole and posaconazole.

Methods The DNA-based Luminex assay we selected as platform is a microsphere phase array-based analysis, that combines a multiplex PCR with hybridization to probes linked to fluorescent microspheres, followed by analysis with a flow cytometer (Luminex Molecular Diagnostics, Toronto, Canada). This technique can detect up to 500-1000 different microsphere-linked probes simultaneously, making it possible to screen for a wide set of pathogens in one reaction, while enabling the build-in of safeguards and redundancy.

The FFSC harbours notorious plant pathogens of crops, like maize, rice and sugar cane. At least 13 species of the 34 species within the