

Methods Biofilm formation was determined by inoculating each strain of yeast species into a glass tube containing Sabouraud Dextrose Broth and other glass tube containing Brain Heart Infusion. Tubes were incubated at 37°C for 24–48 h without agitation. After incubation, the culture broth in the tube was aspirated and the tubes were washed once with distilled water. The walls of the tubes were stained with safranin after media and yeast cells were discarded. Hemolysin production was evaluated using a plate assay. Each strain of yeast species was inoculated on a Sabouraud dextrose agar supplemented with 5% sheep blood. The plates were incubated at 37°C for 48 h. The presence of a distinct translucent halo around the inoculum site, viewed with transmitted light, indicated positive hemolytic activity. Results. 228 isolates were obtained from leukemia, cancer, tuberculosis, fibrocystic disease of breast, meningocele and Fallot's tetralogy patients. The isolates were cultured from urine ($n = 108$) and respiratory specimens ($n = 120$). The yeast strains including 132 *Candida albicans*, 44 *C.tropicalis*, 21 *C.parapsilosis*, 11 *C.glabrata*, 10 *C.lusitaniae*, 6 *Trichosporon asahii* and 4 *C.krusei*. A total of 132 (57.9%) of 228 yeast species isolates obtained were biofilm positive. Biofilm production was most frequently observed for isolates of *C.krusei* (100%, 4 of 4); *C.tropicalis* (93.2%, 41 of 44); *T.asahii* (83%, 5 of 6); and *C.parapsilosis* (71.4%, 15 of 21). At 48 h postinoculation, alpha hemolysis and beta hemolysis could be observed circumscribing the yeast colony. A total of 153 (67.1%) of 228 yeast species isolates obtained produced hemolytic activity. Hemolysin production was most frequently observed for isolates of *C.krusei*, *C.parapsilosis*, *C.glabrata* and *C.tropicalis*.

Conclusions Even though non-*albicans* species are considered less invasive and virulent than *C.albicans*, some species are inherently less susceptible to common antifungals and some species such as *tropicalis*, *glabrata* and *parapsilosis*; have the ability to produce biofilm and hemolysins as components of fungal virulence and cause disrupting host cell membranes.

P340

Galactomannan antigen detection as a biomarker for invasive mycosis in immunosuppressed pediatric host

V. Lora, G. López, M. L. Pérez, A. Bonifaz and C. Velasco
Hospital para el Niño Poblano, Puebla, Mexico

The diagnosis of Invasive Mycosis is based on clinical suspicion, imaging studies, and collection from affected tissue for pathologic and microbiologic examination. Because the prognosis of the disease improves with early commencement of specific therapy, diagnosis should be made as quickly as possible. The detection of galactomannan antigen in pediatric patients immunosuppressed serum samples used in conjunction with other diagnostic procedures can support the diagnosis of Invasive Mycosis.

Objective Galactomannan antigen test in serum for neutropenia leukemia and immunosuppressed pediatric patients to support invasive mycosis diagnosis.

Method Immunosuppressed pediatric patients galactomannan antigen test was used by immunoenzymatic sandwich microplate assay in serum samples. A first serum sample was tested for galactomannan antigen as individual reference value for each patient and 72 hours after a second serum sample tested was compared with the first value due to galactofuranose in cereals foods as interference probable. Serum samples subsequent were tested weekly. Invasive Mycosis diagnosis established with related host, clinical, microbiologic and histopatologic criteria. **Results** Serum samples were studied for detection of galactomannan antigen in 121 pediatric patients; 83 (68.6%) male and 38 (31.4%) female; 98 of 121 (81%) with neutropenia in leukemia and oncosis patients; 10 of 121 (8.3%) immune-deficiency syndromes; 9 of 121 (7.4%) Fallot's tetralogy; 3 of 121 (2.5%) cystic fibrosis; and 1 of 121 (0.8%) hemolytic-uremic syndrome. Galactomannan antigen was detected in 54/121 patients (44.6%) with >0.500 ng/ml. Only 15 (27.8%) of 54 patients were true positives results in conjunction with mycological culture and

clinical evidence; 39 (72.2%) of 54 patients were false positives results because sepsis evidence and presence of galactofuranose in cereals foods. *Aspergillus fumigatus* was in 11 of 15 invasive mycosis with detected galactomannan antigen 0.622–4.101 ng/ml; *Aspergillus versicolor* with 0.598 ng/ml antigen Galactomannan; *Penicillium* spp with 1.225 ng/ml, *Fusarium solanii* with 1.071 ng/ml, and *Candida tropicalis* with 0.880 ng/ml as antigen value results. The death rate was 33.3% (5 of 15) and 66.7% (10 of 15) for successful medical treatment with Voriconazole and Caspofungin acetate. *Aspergillus fumigatus* was etiological agent for invasive mycosis in 4 of 5 dead patients, 2 Fallot's tetralogy, 1 fibrocystic disease of breast, and 1 hemolytic-uremic syndrome. *Fusarium solanii* was etiological agent for invasive mycosis related to Fallot's tetralogy in 1 of 5 dead patients only.

Conclusion Utilizing biopsy to diagnose Invasive Mycosis is hampered by the physiologic condition of the patients, as most cannot tolerate invasive procedures due to thrombocytopenia, coagulation disorders and/or their critical condition. Positive test results for galactomannan antigen with no clinical signs have been reported, especially in young children; however, current data support the usefulness of the galactomannan antigen detection as a biomarker for Invasive Mycosis to be used in conjunction with other diagnosis test as radiographic procedures and clinical findings.

P341

Disseminated mucormycosis in a 6 month old infant following congenital heart defect corrective surgery: Case report

P. Brogueira,¹ N. Carvalho,² J. Batista,¹ E. Goncalves,¹ R. F. P. Sabino,³ C. Verissimo,³ K. Mansinho,¹ R. Anjos² and C. Toscano¹

¹Hospital Egas Moniz, Lisboa, Portugal; ²Hospital de Santa Cruz, Lisboa, Portugal and ³National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal

Objectives (Introduction) Invasive fungal infections in children are becoming more frequent primarily due to an increased survival of children with primary or secondary immune deficiencies. Although uncommon, mucormycosis has been increasingly identified among immunocompromised patients and carries a high fatality rate. There are several risk factors specially related to an immunodeficiency state such as hematologic malignancy with prolonged neutropenia and bone marrow or solid organ transplant, illustrating the role of phagocytic capacity as well as cellular immunity in the prevention of mucosa and tissue invasion.

Methods (Clinical case) A 6 month old infant was admitted to our intensive care unit due to central cyanosis and heart failure. He had past history of prematurity (35 week and 6 days), neonatal cholestasis, atrial dysplasia with multifocal atrial tachycardia, right ventricle hypoplasia with ventricular tachycardia and inferior vena cava interruption with continuity to superior vena cava through azygos. He was submitted to medical and surgical correction and was on mechanical ventilation and central venous catheterization. During hospital stay he suffered from several nosocomial infections treated with large spectrum antibiotics. On day 48 he was diagnosed with sepsis. A *Rhizomucor pusillus* was isolated from urine and 4 days later it was also detected from several tracheal aspirates. Due to his clinical condition, tissue biopsy was not an option. He was treated with liposomal amphotericin B for 40 days. Caspofungin was added for 27 days. Also vesical irrigations with amphotericin B deoxycholate was done as initial therapy. He was on peritoneal dialysis because of acute renal injury AKIN (Acute Kidney Injury Network) III. Immunological study revealed decreased lymphocyte absolute number, mainly due to CD8 subpopulation. Renal echography showed 2 mm bilateral nodules suggestive of fungal etiology. He was extubated on day 90 with success. Due to clinical recovery but persistence of dialysis dependence, he was transferred to a pediatric nephrology unit after 122 days.