UMPhST Târgu Mureş - Doctoral School
Abstract of the PhD thesis

Aspects regarding the oxidative burst of phagocytes in the newborn induced by *Escherichia coli* and *Candida albicans*. Trends in the distribution and resistance phenotypes of invasive isolates of Candida spp.

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The ability of the neutrophils and monocytes to generate reactive oxygen species (ROS), the so called respiratory bust, is mandatory for the removal of invading bacteria and fungi. Alterations in burst oxidation may reflect an increased susceptibility of the neonates to infections and could be useful for their early detection. The invasive fungal infections (IFI) caused by *Candida* species have known an ascending incidence over the last decades as a result of increasing use of invasive procedures, immunosupressive tratments and the large use of broad spectrum antibiotics. The incidence rates of the IFI, as well as the distribution and antifungal susceptibility pattern of the invasive isolates of *Candida spp.* present a substantial regional and temporal variability often because of different patient populations or study methodology.

The first aim of the thesis was to identify the impact immaturity in preterm and term neonates has on the ability of their peripheral blood phagocytes (PBPs) to generate ROS in the presence of various stimuli when compared to healthy adults. The second aim was to assess the incidence of the IFI, the trend of species distribution and the antifungal susceptibility patterns of all invasive strains *of Candida spp*. isolated over a 6-year period in an Eastern European University hospital.

Study1. The aim of this study was to verify in our laboratory conditions the performance criteria of a commercial kit (*Phagoburst™*, Glycotope Biotechnology) as described by the producers and international standards, and validate the use of a new non-opsonized *Candida albicans* ATCC 10321 stimulus, in addition to the opsonized *Escherichia coli* stimulus provided by the manufacturer. The intra-assay imprecision as well as the ranges of neutrophil and monocyte burst activation triggered by various stimuli (the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) and the protein kinase C ligand phorbol 12-myristate 13-acetate (PMA)) were assessed. The granulocyte and monocyte activation range for *E. coli* was similar to the one described by the producer (granulocytes: 78.45-99.43% vs. 99.6-99.95%, average CV% of 1.53% vs. 0.1%; monocytes: 54.63-92.33% vs. 81.80-96.67%, average CV% 6.92% vs. 1.1%), though presenting a broader inferior limit in our laboratory conditions. We had similar activation ranges as the producer's for fMLP and PMA in the case of granulocytes, while no data were available for comparison for monocytes and the above mentioned stimuli. Our results showed an acceptable variation of the average CV% (6.46% activated cells and 7.54% mean fluorescence intensity) for granulocytes in the case of the burst activation triggered by the non-opsonized *C. albicans*. Due to a low monocyte activation after the 20 minutes of incubation time, the resulting average CV% had a higher, but acceptable value for validation (8.80% activated cells and 11.61% for mean fluorescence intensity).

Study 2. The production of ROS, as well as the assessment of leucocyte subclasses in healthy preterm and term neonates were performed using healthy adults as controls. Burst activation of PBPs was quantified by using a flow cytometry method (*Phagoburst™,* Glycotope) that implied incubation of PBPs with various stimuli that included an opsonized *Escherichia coli*

and a non-opsonized *Candida albicans* yeast suspension. Lymphocyte subclasses were assessed by flowcytometry (FACSCalibur, BD Biosciences). We identified a significantly higher absolute count of white blood cells in preterm and term neonates when compared to adults (p=0.029, and p=0.003 respectively) and a higher neutrophil absolute count in term neonates compared to adults (p=0.015). The absolute lymphocyte and monocyte counts were significantly higher in the preterm and term neonates than in adults (both p<0.001), while NK lymphocyte frequencies were higher in preterm neonates compared to adults (p=0.001). We identified a larger percentage of spontaneously (saline stimulus) activated granulocytes in the preterm and term neonates than in adults (p<0.05). A smaller population of activated granulocytes was observed after exposure to *E. coli* in premature neonates than in term neonates (41.19±20.63 vs. 50.01±26.50, p=0.472) and control adults (41.19±20.63% vs. 58.32±33.46%, p=0.058). Monocyte burst activation triggered by *E. coli*, although lower in preterm than in control adults was not significantly different (38.97±20.73% vs. 46.82±21.05%, p>0.05). Incubation with non-opsonized *C. albicans* led to a similar low granulocyte and monocyte activation in all studied groups.

Study 3. The present study aimed to evaluate the incidence of IFI, the trend of species distribution and the antifungal susceptibility patterns of all invasive strains of *Candida spp*. isolated over a 6-year period in an Eastern European University hospital. A total number of 156 isolates were reidentified by MALDI-TOF and tested for susceptibility by Sensititre™ YeastOne™. Isolates were assigned as non-susceptible or belonging to the wild type according to the new CLSI (2017, 2018) break points and epidemiological cut-off values*. C. parapsilosis* (37.82%) was the most frequently isolated yeast, followed by *C. albicans* (26.28%). The general tendency of the species distribution during the surveyed period shifted towards an increase in the non-*albicans* isolates, specifically *C. parapsilosis* and *C. lusitaniae*. Fluconazole resistance was present in 23.52% of *C. glabrata*, 5.08% of *C. parapsilosis* and in none of the *C. albicans, C. tropicalis* and *C. lusitaniae isolates*. Echinocandin resistance was present only in 1(5.88%) *C. glabrata* isolate. No multiresistant or cross-resistant isolates were identified.

Conclusions

The intra-assay precision obtained in our laboratory conditions, as well as the ranges of activated leukocytes, are comparable to the ones described by the producer when using *E. coli* as a stimulus. An extra fungal stimulus for the assessment of burst oxidation could provide additional information regarding the non-specific cellular immune response, particularly in patients at risk for candidemia.

The preterm granulocytes showed a lower capacity to activate în order to produce ROS than the adult ones. The preterm and term, as well as the adult PBPs presented an almost similar low oxidative burst when exposed to *C. albicans*, in spite of evoked functional immaturity of the preterm neonate phagocytes.

The non-*albicans Candida* isolates dominated the etiology of IFI during each of the six observed years with *C. parapsilosis* being the most frequently identified yeast. All *C. albicans* strains were *in vitro* susceptible to all the 9 tested antifungals. *C. glabrata* presented a high non-susceptibility to fluconazole (23,52%), while a large number of isolates displayed a non-wild-type phenotype to voriconazole (64,7%) and posaconazole (35,29%). Therefore, local studies on the epidemiology of IFI isolates and their antifungal sensitivity should be a priority in order to continuously monitor the occurrence of *Candida* species with low sensitivity or intrinsic resistance.