Pathogenicity and immunogenicity of clinical *Paracoccidioides* spp. isolates and their correlation with disease severity

Pereira, B.A.S[1]; Marçon, C.[1]; Amorim B.C.[1]; Silva, J.F.[1]; Cavalcante, R.S.[1]; Carvalho, L.R.[2]; Bagagli, E.[3]; Fabris, V.E.[1]; Venturini, J.[1]; Mendes, R.P.[1]

[1] Universidade Estadual Paulista (UNESP), Faculdade de Medicina de Botucatu, Botucatu, São Paulo– Brazil,
[2] Departamento de Bioestatística, Instituto de Biociências, Universidade Estadual Paulista - UNESP, Brasil;
[3] Departamento de Microbiologia e Imunologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brasil.

Introduction. Paracoccidioidomycosis (PCM) is a systemic mycosis caused by thermally dimorphic fungi of the *Paracoccidioides* genus, which pathogenicity, immunogenicity and the correlation between virulence and patients' severity have been seldom evaluated. *Methodology*. We compared the pathogenicity and immunogenicity of four clinical isolates from different and well-defined clinical forms of PCM and three collection isolates, after their genotyping, in experimental infection of BALB/c mice. Pathogenicity was evaluated by lethal dose 50% (LD50) estimation, by histopathological evaluation of lung and spleen, and by colony-forming unit (CFU) counts in the same organs. Immunogenicity was evaluated by determining the specific antibodies serum levels by double agar gel immunodiffusion test (DID), and the pulmonary concentrations of interleukins and vascular endothelial growth factor (VEGF). Pb234 and Pb417 were isolated from patients with the chronic moderate form, Pb326 from an acute severe form and Pb531 from a chronic severe form. *Results*. The strains Pb417 and Pb326 joined the strains identified as *P. brasiliensis* S1a, Pb531 as *P. brasiliensis* S1b, and Pb234 and Pb192 as *P. restrepiensis* (PS3). Distribution of the isolates regarding virulence showed Pb531 strain as high, Pb326 as intermediate and the others - Pb192, Pb234, Pb417, Pb01, and Pb8334 as low. Pb531 (S1b) showed the lowest LD50, the highest fungal load in lung and spleen, and the greatest intensity of histopathological alterations both in lung and spleen. Specific serum levels of antibodies were detected only in animals infected with the Pb326 strain, at the sixth week post-infection. Patients' severity showed direct correlation with pulmonary and splenic fungal load, and pulmonary TNF-α concentration, in addition to an indirect correlation with pulmonary concentration of IL-2 and VEGF, and a tendency to indirect correlation with LD50. *Conclusions*. Our study demonstrated the following findings: a) the strains present differences in the degree of pathogenicity; b) there is a direct correlation between virulence, evaluated by this methodology and patients' severity; c) the evaluation of the pulmonary fungal load in BALB/c mice receiving $5x10^6$ viable yeast cells intraperitoneally and sacrificed at the sixty week of infection is available, easy to perform and shows correlation with virulence and patients' severity.

Keywords. Paracoccidioidomycosis, systemic mycoses, virulence, severity, murine model.