**S10.1d Molecular diagnosis and antifungal susceptibility of Colombian clinical isolates of the Sporothrix spp complex**

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**S10.1 Antifungal dosing in children and adolescents, September 24, 2022, 10:30 AM - 12:00 PM**

**Objective:** To detect by chitinase PCR and Species-Specific PCR (SS PCR) the DNA of Sporothrix spp in fresh tissue and Formalin Fixed Paraffin Embedded (FFPE) tissue from patients with sporotrichosis and to evaluate the susceptibility to antifungal of Sporothrix spp isolates obtained from patients diagnosed with sporotrichosis in the Medical Mycology group of the Faculty of Medicine at the University of Antioquia.

**Materials & Methods:** At the Medical Mycology group service, 24 patients with suspected sporotrichosis were evaluated between 2019 and 2022. Samples of the skin lesions (fresh tissue) were taken for molecular and microbiological culture.

Regarding the FFPE tissue, 87 samples stored by different histopathological diagnosis centers from 1976 to 2022 were chosen: 45 samples had a histopathological diagnosis of sporotrichosis, and 42 samples of other diseases with involvement in subcutaneous tissue, were used as controls.

DNA extracts previously from fresh tissue and FFPE tissues were used to perform chitinase nested PCR and SS nested PCR. The chitinase nested PCR amplifies a 209 bp fragment for the genus Sporothrix. For species identification, species-specific primers were used (SS PCR), which amplify a 311 bp sequence for S. schenckii s. s. and 245 bp for S. globosa of the calmodulin gene. For the SS PCR in tissues, a nested PCR was implemented using the primers CAL1 and CAL2 for the external sequence and the species-specific primers for the internal sequence.

The antifungal susceptibility tests were performed according to the Clinical and Laboratory Standards Institute (CLSI) M27-A3 protocol for years. Six antifungal drugs were used: itraconazole, terbinafine, voriconazole, posaconazole, amphotericin B, and thio-tegavenone.

Results: The culture was positive for Sporothrix spp in 15 (65%) patients and the chitinase nested PCR was positive in 14 of these, with a sensitivity of 93% and a specificity of 91%. In 11 patients, both the culture and the chitinase nested PCR were negative.

SS nested PCR was applied to the 14 DNAs with positive chitinase PCR, of which seven were positive for S. schenckii s. s. and one for S. globosa. For the other six samples, the results of this PCR were negative. The results of these PCR were confirmed by the identification of species from the isolates recovered in culture: 15 were identified as S. schenckii s. s. and 1 as S. globosa.

Of the 45 FFPE tissue samples with histopathological diagnosis of sporotrichosis, the chitinase PCR was positive for 25 (55%) of these, and all FFPE tissue samples were negative for SS nested PCR.

Conclusions: Chitinase nested PCR had a sensitivity of 93% and a specificity of 91% with respect to culture, in samples from fresh tissue. This PCR also has a good performance when it is applied to good-quality DNA obtained from FFPE tissue, hence, PCR positivity decreased in samples stored for >15 years. The results of the nested SS PCR are encouraging, however, it
Fungal beta-glucans and mannan performances in HIV-associated histoplasmosis

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Objective: Diagnosis of histoplasmosis in people living with HIV (PLWHIV) remains challenging despite developments in Histoplasma antigen and molecular detection tools. Fungal markers such as Beta(1-3,1-6)-glucans (BDG) and galactomannan (Ag) are widely available, but the performance is limited during PURHIV workup for suspicion of histoplasmosis. Our objective was to evaluate and compare BDG and GM performances for the diagnosis of HIV-associated histoplasmosis.

Methods: We performed a diagnostic accuracy study using primary serum samples stored frozen in a certified biorepository (CRB Amazonia-DC-2021-4648). Samples consisted of consecutive hospitalized PURHIV unexposed to oral antifungals during the previous month (IDESAPHIS study-NCT01004779). All patients gave consent for biobanking and ancillary studies on fungal markers.

Histoplasmosis cases, proven (EORTC/MSG criteria) and probable (polyclonal or monoclonal Histoplasma antigen detections in urine or serum), as well as negative controls, were randomly selected. Patients with a proven or suspected Penicillium marneffei infection were excluded. Following manufacturers’ instructions, samples were blindly tested for BDG and GM using Fungitell® and Platelia® Aspergillus Ag assays, respectively.

Gold-standard definition used these scenarios: EORTC scenario (cases and controls defined according to the EORTC/MSG 2020 criteria for endemic mycoses); strict scenario with proven cases restricted to those positive to all three Histoplasma antigen detections, and controls confirmed negatively for all methods; and a large scenario with proven or probable cases and controls negatives for all methods.

Results: We included 121 samples, 92 HIV-associated histoplasmosis cases (54 proven and 38 probable), and 29 negative controls. Compared with controls, histoplasmosis cases were significantly younger and advanced in the course of HIV disease (median CD4 count levels: 551 vs 116; p < 0.0001).

BDG and GM median detection levels were significantly higher among histoplasmosis cases compared with controls across all scenarios [568 (174-441) pg/mL vs 142 (89-211) for BDG and 2.5 (1.3-4.8) vs 0.19 (0.14-0.56) for GM, in cases vs controls of the strict scenario, respectively].

In the strict scenario, at 150 pg/mL and 0.3 for BDG and GM respectively, sensitivity, specificity, positive and negative likelihood ratios were respectively: 97.5% [95% confidence interval (CI), 85-100] vs 90% [77-100], 52% [34-70] vs 83% [49-97], 2 [1.4-3.5] vs 5.5 [2.6-12.0], and 0.1 [0.01-0.7] vs 0.12 [0.05-0.45]. ROC curves found AUCs of 0.92 (0.86-0.98) vs 0.92 (0.86-0.99), and optimal thresholds at 28 Pg/mL and 1.29, for BDG and GM, respectively (Fig. 1). Poor test probabilities showed best performances at the lowest thresholds for negative testing of both BDG and GM, and at the 0.7 thresholds for a positive test (Fig. 2).

Conclusion: BDG and GM may not be used for the same objective when searching for HIV-associated histoplasmosis. Although a negative BDG test at the lower thresholds should rule out histoplasmosis in a screening context, limitations of a positive BDG test, even at the higher thresholds, call for a consecutive positive GM test before starting patients on anti-fungal therapy targeting histoplasmosis. Still, when considering the highest cost of BDG testing, higher balanced diagnostic performances, and lower costs of GM testing alone, one may favor the use of GM, notably in resources-limited settings.