

P1652

Paper Poster Session

Fungal diagnosis: from culture to molecular techniques

Calcofluor white: a useful tool for the diagnosis of invasive pulmonary aspergillosis in the molecular diagnosis era

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Background: Invasive Pulmonary Aspergillosis (IPA) is a serious disease with a very high morbidity and mortality rate. Diagnosis of IPA remains troublesome and challenging despite the many diagnostic tools available today (e.g., radiological, culture, serological, and molecular methods).

Direct examination of clinical specimens under fluorescence microscope using calcofluor white staining (CFWS) is simple, fast, and reliable. The aim of the study was to evaluate the utility of CFWS as a rapid and cheap tool for the diagnosis of IPA.

Material/methods: We reviewed retrospectively microbiology databases and clinical reports of patients hospitalized at Hospital General Universitario Gregorio Marañón from January 1st, 2008 to December 31st, 2012 for cases of Aspergillosis.

Patients were classified as having IPA if they fulfilled the criteria of proven, probable, or possible IPA according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group. They were classified as colonized if they did not fulfill these criteria.

We included all cases of Aspergillosis with an *Aspergillus*-positive culture and CFWS performed. CFWS was considered positive or negative according to the visualization of septate hyphae.

Results: Charts from 227 patients (71.4% men, mean age 65.9 ± 14.9 years [range, 0-90 years]) were examined during a 5-year period (2008-2012). Of these patients, 45 (19.8%) fulfilled the criteria for IPA, and 182 (80.2%) were considered colonized.

Aspergillus fumigatus was the most commonly isolated species in patients with IPA (64.0%) followed by *A. flavus* (10.7%), *A. niger* (10.7%), and *A. terreus* (2.7%). The remaining 12.0% included several *Aspergillus* spp.

A total of 316 clinical samples (75 from IPA patients and 241 from colonized patients) were available for detailed study. The origin of the respiratory samples was: sputum (n=209, 66.1%), bronchoalveolar lavage (n=55, 17.4%) and tracheal aspirate (n=52, 16.5%).

The performance of CFWS revealed septated hyphae in a total of 145 respiratory samples and yielded negative results in 171. In detail, among the 75 samples obtained from IPA patients, CFWS revealed that 60 samples (80.0%) were positive for fungal elements and 15 (20.0%) yielded negative results. Among the 241 samples obtained from colonized patients, CFWS revealed that 85 samples (35.3%) were positive for fungal elements and 156 (64.7%) yielded negative results. Sensitivity was 80%, specificity 64%, positive predictive value 41%, and negative predictive value 91%.

Conclusions: When *Aspergillus* species is present in the respiratory tract, a negative calcofluor result is suggestive of colonization with a high negative predictive value. Direct examination of clinical respiratory specimens using calcofluor white continue to be, in the molecular diagnosis era, a rapid, cheap and useful tool that could help in the diagnosis of IPA. This study was partially supported by FIS (P113/02783).