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Paper Poster Session
Fungal diagnosis: from culture to molecular techniques

Evaluation of two homemade ELISA assays for monitoring exacerbations in patients with allergic broncho-pulmonary aspergillosis

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Background: ABPA is an immunologic bronchopulmonary inflammation due to immune response of the lower respiratory tract against *Aspergillus fumigatus*. The evolution of ABPA comprises five stages going through exacerbation episodes. Early detection of exacerbations is of major importance due to negative effects on patients, resulting in degradation of health status.

Material/methods: A prospective follow-up over a 24 months period of a cohort of 18 ABPA patients with initial ABPA diagnosis allowed us to screen serums for IgE levels, circulating precipitins by immunoelectrophoresis (gold standard method) and *A. fumigatus*-specific antibodies by IgG ELISA Serion. Clinical outcomes from each patient were also collected and analyzed. In parallel, homemade Elisa assays using semi-purified antigens to detect IgG isotypes (ELISA1) and IgG4 (ELISA2) were evaluated. Western blot using the same antigen was used to explore the antibody response. A control group of serums from healthy subjects (n= 30) and patients diagnosed with atopic dermatitis (n= 5), cystic fibrosis (n= 3), COPD (n=9), and candidiasis (n=4) was screened to conclude about the specificity of our homemade-ELISA assays.

Results: During the course of the study, 84 serums from 18 ABPA patients were included for detection of antibodies. Ten exacerbation episodes were notified for 10 patients. Through healthy subjects, a cut-off was established for ELISA1. All the exacerbations well correlated with high ELISA1 antibody levels above the cut-off, whereas often associated with negative precipitins. Exacerbation episodes were also characterized by a high level of anti-IgG4 and a special signature (a band of 10KDa) in Western blot. Some patients who were in remission still had a high level of ELISA1 antibodies that could be discriminated from a persistent exacerbation by ELISA2 and Western blot. Monitoring IgG isotype and IgG4 levels for patients from the control group showed that the cut-off is highly selective since all healthy subjects, atopic dermatitis and COPD patients are under the cut-off . However, one of the 3 patients with invasive candidiasis was above the cut-off for ELISA1 but easily excluded as a false positive by ELISA2. Cystic fibrosis patients without ABPA but positive with the gold standard method and ELISA Serion also have a very high level of IgG by ELISA1. Performing ELISA2 and Western blot allowed us to confirm negativity for two of these patients.

Conclusions: The ELISA1 assay evaluated in this study appeared to be efficient to point out exacerbation episodes in patients with ABPA. False positive results obtained for cystic fibrosis and candidiasis patients could be ruled out performing anti-IgG4 ELISA2 assay or Western blot.