New Commercially Available IgG Kits and Time-Resolved Fluorometric IgE Assay for Diagnosis of Allergic Bronchopulmonary Aspergillosis in Patients with Cystic Fibrosis

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Allergic bronchopulmonary aspergillosis (ABPA) is difficult to diagnose; diagnosis relies on clinical, radiological, pathological, and serological criteria. Our aim was to assess the performance of two new commercially available kits and a new in-house assay: an Aspergillus fumigatus enzyme-linked immunosorbent assay (ELISA) IgG kit (Bordier Affinity Products), an Aspergillus Western blotting IgG kit (LDBio Diagnostics), and a new in-house time-resolved fluorometric IgE assay (dissociation-enhanced lanthanide fluorescent immunoassays, or DELFIA) using recombinant proteins from an Aspergillus sp. recently developed by our laboratory for ABPA diagnosis in a retrospective study that included 26 cystic fibrosis patients. Aspergillus fumigatus-specific IgG levels measured by a commercial ELISA kit were in accordance with the level of precipitins currently used in our lab. The ELISA kit could accelerate and help standardize ABPA diagnosis. Aspergillus fumigatus-specific IgE levels measured by ImmunoCAP (Phadia) with A. fumigatus M3 antigen and by DELFIA with a purified protein extract of A. fumigatus were significantly correlated (P < 10^-6). The results with recombinant antigens glucose-6-phosphate isomerase and mannitol-1-phosphate dehydrogenase were encouraging but must be confirmed with sera from more patients. The DELFIA is an effective tool that can detect specific IgE against more fungal allergens than can be detected with other commercially available tests.
ferent studies, the efficacy of these recombinant antigens is variable, and a consensus test to find specific IgE levels has yet to be defined.

Several bacterial and fungal proteins involved in antigen-antibody interactions in hypersensitivity pneumonitis have been identified in our laboratory using an immunoproteomic approach that includes sorting by IgG Western blot analysis with sera from hypersensitivity pneumonitis patients (16). Five immunoreactive proteins of an *Aspergillus* sp. (NAD-dependent formate dehydrogenase AcIA/Fdh [NAD], glucose-6-phosphate isomerase [G6Pi], Glu/Leu/Phe/Val dehydrogenase [GLPV], mannotol-1-phosphate dehydrogenase [Man1P], and enolase) were produced as recombinant antigens, and two of them, G6Pi and GLPV, were particularly efficient for diagnosing hypersensitivity pneumonitis by ELISA IgG (17). Apart from Man1P, these proteins were also identified by Singh et al. using an immunoproteomic approach, including sorting by IgE Western blot analysis with sera from ABPA patients (6, 18). Two of them, G6Pi and NAD, were highlighted as ABPA specific (6, 18).

Dissociation-enhanced lanthanide fluorescent immunoa-
say (DELFIA) is well known as a very sensitive method (19, 20) for evaluating the specific IgE level, as already demonstrated for the detection of specific IgE in other types of allergy (venom, mites) (20, 21). For this study, we developed an in-house DELFIA to measure IgE levels against several *Aspergillus* antigens (*A. fumigatus* purified protein extract [PPE] and five recombinant antigens, including those previously highlighted by Singh et al. [6, 18]).

The aim of this study was to assess the performance of three new serological tests available in our laboratory: (i) new commercialized ELISAs (*Aspergillus fumigatus* ELISA IgG kit; Bordier Affinity Products, Crissier, Switzerland), (ii) new commercialized Western blot assays (*Aspergillus* Western blotting IgG kit; LDBio Diagnostics, Lyon, France), and (iii) the in-house DELFIA IgE assay with PPE and recombinant antigens, for serodiagnosis of ABPA in CF patients.

This study is a part of a larger study on the monitoring of ABPA in CF patients (22).

**MATERIALS AND METHODS**

**Sera.** Twenty-six adult CF patients were recruited by the pneumology department of the university hospital of Besançon starting in June 2011, when they visited the department for their annual appointments. All patients gave written consent.

Patients were classified according to the consensus conference of the CF Foundation that defined minimum criteria required for ABPA diagnosis in CF (4), including (i) acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, change in pulmonary function or increased sputum production) not attributable to another etiology; (ii) total serum IgE concentration of >500 IU/ml if the patient is not treated by corticosteroids; (iii) immediate cutaneous reactivity to *Aspergillus* or in vitro demonstration of IgE antibody to *A. fumigatus*; and (iv) one of the following: (a) precipitins to *A. fumigatus* or in vitro demonstration of IgG antibody to *A. fumigatus* or (b) new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest computed tomography (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy.

In our study, *Aspergillus* IgE measured by ImmunoCAP techniques and *Aspergillus* precipitins measured by electroosmosis (ES) were used for criteria iii and iv, respectively (Table 1).

In preliminary experiments, we checked the reproducibility of the DELFIA technique and the correlation between the DELFIA technique and the ImmunoCAP system (Phadia, Uppsala, Sweden) using 53 anonymous serum samples. These sera had been previously tested using the ImmunoCAP system in routine practice in the laboratory of Clinical Biochemistry, Besançon University Hospital (France). Of the 53 anonymous sera, 31 were IgE specific, measured by ImmunoCAP (Phadia), under 0.35 kIU/liter, and the 22 others were IgE specific between 0.37 to 28 kIU/liter.

**Immunoprecipitation techniques to detect precipitin arcs.** Both immunoprecipitation techniques were performed with metabolic and somatic antigens of *A. fumigatus* (Bio-Rad, Marnes-la-Coquette, France).

**Electroosmosis (ES).** Electroosmosis (ES) was performed with an ES apparatus on a cellulose acetate sheet (Sartorius, Goettingen, Germany). After 10 min in a bath containing buffered Tris glycerine solution (TGS) (pH 8.8), cellulose acetate sheets were placed in the electrophoresis vat filled with TGS. Next, 15 μl of each serum sample was placed on three spots at the anode side, and a 15-μl line of antigen was placed at the cathode side. A 110-V current was applied for 2 h 15 min. After washing, the cellulose acetate sheets were stained with Coomassie blue (23).

**Immunoelectrophoresis on agar gel.** Immunoelectrophoresis (IEP) was done in two phases. First, the antigen (15 μl) was separated on 1% agar plate using electrophoresis in an electrophoresis vat containing a diethylamylanolurea sodium-buffered solution (pH 8.75) (110 V for 2 h). The second phase consisted of passively diffusing (48 h) the serum sample (200 ml) placed in a trough cut into the gel along the zone where the antigens were separated using electrophoresis. After the washing and staining steps, the arcs were enumerated. This process took 5 days. IEP was performed only when one arc was obtained by the electroosmosis method, and sensitivity was established when two or more arcs were obtained.

**Measurement of *A. fumigatus*-specific IgG.** The specific IgG level of *A. fumigatus* was evaluated with an *Aspergillus fumigatus* ELISA IgG kit (Bordier Affinity Products, Crissier, Switzerland) commercialized in line with supplier recommendations. This kit was made with a mix of somatic, metabolic, and recombinant antigens from *A. fumigatus* (dipeptidyl peptidase type V [chymotrypsin] and RNase [mitogillin]).

Commercial strips for the *Aspergillus* Western blotting IgG kit (LDBio Diagnostics, Lyon, France) were used with serum samples from patients, as recommended by the supplier. Briefly, a serum sample or positive control was added with sample buffer at a concentration of 1/600 on a strip on each channel of the incubation rack and then incubated for 90 min under agitation. Strips were washed three times, and the IgG conjugate was added for 60 min. After a second wash step, substrate was incubated for 60 min. Strips were then dried at room temperature, and colored bands were compared with the positive control. The Western blot tests were positive when at least two specific bands out of four were observed (16 kDa, 18 to 20 kDa, 22 kDa, and 30 kDa).

**Measurement of specific IgE.** (i) **ImmunocAP system.** Specific IgE were assayed by ImmunoCAP (Phadia) with crude antigens of *A. fumigatus*, named M3 by the supplier. The threshold recommended by the manufacturer was 0.35 kIU/liter.

(ii) **Time-resolved fluorometric assay: DELFIA.** Preparation of PPE of *A. fumigatus* (IHEM 22670) was obtained by culture for 7 days on a DG18 solid medium, and the conidia were then gently scraped off the agar. Conidia were resuspended in phosphate-buffered saline (PBS), crushed, sonicated, and purified, as described in detail by Roussel et al. (24). Five recombinant antigens—NAD, G6Pi, GLPV, Man1P and enolase—were produced, as previously described by Millon et al. (25), and used for DELFIA. Presence of the recombinant proteins was controlled by an SDS-PAGE (10% acrylamide gel stained by BioSafe Coomassie [Bio-Rad]). Image acquisition was performed with a GS800 calibrated densitometer (Bio-Rad) and was analyzed with Quantity One software, and the obtained optical density per square milimeter showed a purity between 95% and 98% (Fig. 1). Microtiter plates (PerkinElmer, Waltham, MA) were coated with 100 μl of PPE of *A. fumigatus* or with the five recombinant antigens (NAD,
<table>
<thead>
<tr>
<th>Status</th>
<th>Code</th>
<th>Age, yr (gender)</th>
<th>Clinical symptom(s)</th>
<th>$A.\ fumigatus$-specific IgE level (kIU/liter)</th>
<th>Total IgE level (IU/ml)</th>
<th>Radiographic criteria</th>
<th>Major diagnostic criteria</th>
<th>Minor diagnostic criteria</th>
<th>Somatic antigens of $A.\ fumigatus$ (no. of arcs)</th>
<th>Total eosinophil count (cells/μl)</th>
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<td>No ABPA</td>
<td>PN1</td>
<td>19 (M)</td>
<td>Normal</td>
<td>&lt;0.1</td>
<td>2.9</td>
<td>TT opacities</td>
<td>Mucoid impaction, Nod, TT opacities</td>
<td>0</td>
<td>0</td>
<td>200</td>
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<td></td>
<td>PN2</td>
<td>32 (F)</td>
<td>Cough</td>
<td>&lt;0.1</td>
<td>20.9</td>
<td>TT opacities</td>
<td>Mucoid impaction, Nod, TT opacities</td>
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<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>PN3</td>
<td>27 (M)</td>
<td>Cough</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263</td>
<td>Nod, TT opacities</td>
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<td>100</td>
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<td>&lt;0.1</td>
<td>&lt;20</td>
<td>TT opacities</td>
<td>TT opacities</td>
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<td>0</td>
<td>200</td>
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<td>PN5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>20 (M)</td>
<td>Deterioration of general condition, cough, expectoration</td>
<td>0.2</td>
<td>54.9</td>
<td>Nod, TT opacities</td>
<td>TT opacities</td>
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<td>ND</td>
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<td>35 (M)</td>
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<td>1.9</td>
<td>59.1</td>
<td>Mucoid impaction, Nod, TT opacities</td>
<td>TT opacities</td>
<td>0</td>
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<td>PN7</td>
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<td>Normal</td>
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<td>TT opacities</td>
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<td>200</td>
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<td>PN8</td>
<td>20 (M)</td>
<td>Normal</td>
<td>&lt;0.1</td>
<td>8.6</td>
<td>Nod, TT opacities</td>
<td>TT opacities</td>
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<td>0</td>
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<td>PN9</td>
<td>22 (F)</td>
<td>Cough, dyspnea, expectoration</td>
<td>0.3</td>
<td>310</td>
<td>Nod, TT opacities</td>
<td>TT opacities</td>
<td>1</td>
<td>0</td>
<td>200</td>
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<tr>
<td></td>
<td>PN10</td>
<td>20 (F)</td>
<td>Cough, expectoration</td>
<td>&lt;0.1</td>
<td>18.2</td>
<td>Mucoid impaction, Nod, TT opacities</td>
<td>TT opacities</td>
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<td>ND</td>
<td>100</td>
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<td>PS1</td>
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<td>NR</td>
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<td>Nod, TT and FIG opacities</td>
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<td>4</td>
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<td></td>
<td>PS2</td>
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<td>Cough, expectoration</td>
<td>&lt;0.1</td>
<td>111</td>
<td>Nod, TT opacities</td>
<td>Mucoid impaction, TT opacities</td>
<td>3</td>
<td>4</td>
<td>200</td>
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<tr>
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<td>PS3</td>
<td>25 (F)</td>
<td>Expectoration</td>
<td>0.7</td>
<td>381</td>
<td>Nod, TT opacities</td>
<td>Mucoid impaction, TT opacities</td>
<td>3</td>
<td>4</td>
<td>30</td>
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<tr>
<td></td>
<td>PS4</td>
<td>25 (F)</td>
<td>Normal</td>
<td>&lt;0.1</td>
<td>&lt;2</td>
<td>Nod, TT opacities</td>
<td>TT opacities</td>
<td>4</td>
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<tr>
<td></td>
<td>PS5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>43 (M)</td>
<td>Expectoration</td>
<td>0.2</td>
<td>28.6</td>
<td>Nod, TT and FIG opacities</td>
<td>Nod, TT and FIG opacities</td>
<td>3</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>PS6&lt;sup&gt;g&lt;/sup&gt;</td>
<td>35 (M)</td>
<td>Cough, expectoration</td>
<td>&lt;0.1</td>
<td>18.7</td>
<td>Nod, TT opacities</td>
<td>TT opacities</td>
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<td>0</td>
<td>400</td>
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<td></td>
<td>PS7&lt;sup&gt;g&lt;/sup&gt;</td>
<td>22 (M)</td>
<td>Normal</td>
<td>&lt;0.1</td>
<td>3,986</td>
<td>TT opacities</td>
<td>TT opacities</td>
<td>5</td>
<td>5</td>
<td>0</td>
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<td>PS8</td>
<td>17 (F)</td>
<td>Normal</td>
<td>&lt;0.1</td>
<td>3.3</td>
<td>TT opacities</td>
<td>TT opacities</td>
<td>5</td>
<td>3</td>
<td>220</td>
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<tr>
<td></td>
<td>PS9&lt;sup&gt;g&lt;/sup&gt;</td>
<td>22 (M)</td>
<td>Cough, dyspnea, expectoration</td>
<td>&lt;0.1</td>
<td>75</td>
<td>Mucoid impaction, Nod, TT opacities</td>
<td>Nod, TT opacities</td>
<td>3</td>
<td>4</td>
<td>360</td>
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<tr>
<td></td>
<td>PS10</td>
<td>22 (M)</td>
<td>Not done</td>
<td>0.1</td>
<td>65</td>
<td>Nod, TT opacities</td>
<td>TT opacities</td>
<td>4</td>
<td>2</td>
<td>100</td>
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<td>PS11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21 (F)</td>
<td>Deterioration of general condition, cough, fever, dyspnea</td>
<td>0.54</td>
<td>189</td>
<td>TT, FIG, fleeting opacities</td>
<td>Nod, TT, FIG, fleeting opacities</td>
<td>3</td>
<td>3</td>
<td>400</td>
</tr>
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</table>

**TABLE 1** Characteristics of CF patients and criteria for diagnosis of ABPA

<sup>a</sup>M, male; F, female.

<sup>b</sup>Other minor criterion requested by the ISHAM recommendations.

<sup>c</sup>TT, tram track; Nod, nodules; FIG, finger-in-glove.

<sup>d</sup>In bold are values exceeding the threshold set by Stevens et al. (4): elevated IgE levels against $A.\ fumigatus$ (>0.35 kIU/liter), elevated total IgE level (>500 IU/ml), and presence of precipitins against $A.\ fumigatus$.

<sup>e</sup>ND, not determined.

<sup>f</sup>Patients had been treated for ABPA with antifungal medication.

<sup>g</sup>Patients with history of ABPA.

<sup>h</sup>Patients were closely monitored for ABPA by clinicians but not yet diagnosed.

| FIG 1 SDS-PAGE (10% acrylamide) Coomassie stain illustrating recombinant proteins. w1: enolase, 47.3 kDa; w2: GLPV, 49.3 kDa; w3: Man1P, 42.9 kDa; w4: G6Pi, 61.4 kDa. Analysis of NAD, 45.7 kDa, was not done. | G6Pi, GLPV, Man1P, and enolase) at 10 μg/ml in coating buffer (50 mM NaHCO<sub>3</sub>, pH 9.6) at 4°C overnight. After washing five times with washing buffer (wash concentrate, PerkinElmer, Waltham, MA), plates were blocked for 1 h at room temperature under gentle shaking with purified bovine serum albumin (PerkinElmer) and diluted at 0.5% in assay buffer (PerkinElmer). After a second washing step, 50 μl of each serum diluted at 1/10 in assay buffer was added in triplicate, and plates were incubated for 2 h at room temperature with shaking. The plates were washed again and incubated for 1 h further at room temperature with shaking, with 100 μl of an anti-human IgE ε chain (Life Technologies, Frederick, MD), and diluted at 1/15,000 in assay buffer. Plates were washed (5 times) and then incubated for 30 min at room temperature under shaking with 100 μl europium-labeled streptavidin (1/1,000 in assay buffer). A final washing step with eight successive washes was performed, and then 100 μl of enhancement solution (Perkin- |
Five patients had ABPA (PA). The 21 other patients did not have ABPA: 10 patients were PPN, and 11 patients were PPP. Three patients had a history of ABPA (patients PPN5, PPP5, PPP6) but were in remission at the time of the study, and two patients (PPP7 and PPP11) were being closely monitored for ABPA because of a deterioration in clinical or biological conditions suggestive of ABPA onset. The mean age of these patients was 27 years (standard deviation, ± 10 years) (Table 1).

Measurement of A. fumigatus-specific IgG by ELISA and Western blot analysis and of precipitins by ES and IEP in CF patients. Specific levels of IgG estimated by the Aspergillus fumigatus ELISA kit (Bordier) were in accordance with the electrosyneresis results (Tables 1 and 2). Levels were elevated in only two cases (PPN1 and PPP3), while the number of arcs by electrosyneresis was low. In two precipitin-positive patients, PPP5 and PPP6, the opposite was observed, i.e., low levels in ELISA while two to three arcs were observed. A Spearman’s correlation coefficient of 868 ($P < 10^{-3}$) was obtained by comparing the ELISA Bordier kit results to the number of arcs obtained with the somatic antigens.

The results using the Western blot kit by LDBio Diagnostics were positive for all patients with ABPA and for precipitin-positive patients (Table 2). The test was also positive for two patients, PPN2 and PPN10, while immunoprecipitation techniques and ELISA were negative. Two other patients, PPN1 and PPP5, appeared positive using both the Western blot and the ELISA technique but were not positive with immunoprecipitation techniques. In this case, no significant correlation was highlighted between Western blot and the other techniques.

**Evaluation of the DELFIA technique and comparison with ImmunoCAP.** Correlations between ImmunoCAP and DELFIA tests were evaluated with the 53 anonymous serum samples and with PPE from A. fumigatus. Results obtained by ImmunoCAP and DELFIA were significantly correlated (Spearman’s rank test, $P < 10^{-6}$). This led us to conclude that a good correlation existed between levels of specific IgE in sera evaluated by ImmunoCAP and europium counts (Fig. 2).

Low intraassay variability was shown, with an average of 7.9%. The interassay coefficient of variance was evaluated at 25% based on results obtained with 42 serum samples tested in triplicate 18 times with DELFIA.

**RESULTS**

**Patients.** Five patients had ABPA (PA). The 21 other patients did not have ABPA: 10 patients were PPN, and 11 patients were PPP. Three patients had a history of ABPA (patients PPN5, PPP5, PPP6) but were in remission at the time of the study, and two patients (PPP7 and PPP11) were being closely monitored for ABPA due to clinical or biological conditions suggestive of ABPA onset. The mean age of these patients was 27 years (standard deviation, ± 10 years) (Table 1).

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![FIG 2 Correlation between ImmunoCAP assay and DELFIA (Spearman’s rank test, $P < 10^{-6}$).](http://cviasm.org/.../2017/10/28/)
Measurement of specific IgE by DELFIA in CF patients. Indices obtained by DELFIA for each patient are shown in Fig. 3 and Table 3. ROC analyses showed that DELFIA IgE with recombinant G6Pi, Man1P, and PPE of A. fumigatus could discriminate between patients with ABPA and patients without ABPA with an AUC up to 0.75 and with specificities above 86% and sensitivities up to 60% (Table 4). DELFIA IgE tests using NAD, GLPV, and enolase were not efficient enough to diagnose ABPA (AUC < 0.75).

The combination of Man1P and PPE allowed us to obtain a sensitivity of 100% and a specificity of 86%. Results obtained for the recombinant G6Pi provided no additional information and reduced the specificity (a combination of G6Pi and PPE had a sensitivity of 100% and a specificity of 76%).

The five patients with a history of ABPA or who were being closely monitored (PPN5, PPP5, PPP6, PPP7, and PPP11) obtained a low level of DELFIA indices for all antigens tested.

DISCUSSION
We showed a very good correlation between the level of IgG determined by the new ELISA Bordier test and the number of precipitin arcs obtained using ES or IEP techniques. We also
showed a good correlation between the levels of A. fumigatus-specific IgE determined by the ImmunoCAP and the DELFIA techniques. PPE of A. fumigatus and recombinant Man1P seem to be the best combination of antigens, using DELFIA, to identify CF patients with ABPA, with a sensitivity of 100% and a specificity of 86%.

In this study, we used the Stevens classification (4) to categorize ABPA patients, despite the fact that the International Society for Human and Animal Mycology (ISHAM) ABPA complicating asthma working group (3) recently proposed new diagnostic criteria, including (i) predisposing conditions (bronchial asthma or cystic fibrosis); (ii) high levels of total IgE (>1,000 IU/ml) and specific IgE against A. fumigatus (or a positive skin test); and (iii) at least two of the three following criteria: presence of precipitating or IgG antibodies against A. fumigatus, chest radiographic features consistent with ABPA, and an eosinophil count above 500 cells/μl. If we had used these criteria, diagnosis of ABPA would have been excluded for two patients, because they had a total IgE level lower than the cutoff of 1,000 IU/ml (Table 1), and none of the patients had an eosinophil count above 500 cells/μl.

Results of ELISA by Bordier were in accordance with the immunoprecipitation techniques classically used in our laboratory. This Bordier ELISA kit could replace the immunoprecipitation techniques, because ELISA can be automated, fast, and reproducible and because this kit used a mixture of antigens, including recombinant antigens, providing some standardization. In contrast, results from Western blotting developed by the LDBio Diagnostics supplier were not statistically in accordance with the other techniques (IEP, ES, and ELISA).

Our study also supports the good correlation between DELFIA and ImmunoCAP using Aspergillus antigens. DELFIA IgE was efficient for detecting ABPA patients using the new recombinant antigens G6Pi and Man1P. Unlike the use of PPE, the use of recombinant antigens allows for standardization of the test. However, these results should be interpreted cautiously because of the small number of patients.

DELFIA appears to be an alternative tool to the ImmunoCAP system by Phadia to determine the titer of specific IgE against multiple fungal antigens involved in fungal allergy and antigens.
that are unavailable with the ImmunoCAP system. For example, if DELFIA could be developed to detect IgE directed against other filamentous fungi involved in allergic bronchopulmonary mycosis, such as other *Aspergillus* species (*Aspergillus terreus, Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, Aspergillus versicolor*) or *Scedosporium apiospermum* or *Exophiala dermatitidis* (26–28), it could help us to better manage fungal complications in CF patients.

The cutoff for specific IgE defined by suppliers is 0.35 kIU/liter for ImmunoCAP Phadia, and this is probably too low to identify ABPA in CF patients (five false-positive patients [see Table 1]). A cutoff specifically evaluated in such patients may be more appropriate. For patients with ABPA complicating asthma, a recent study fixed the cutoff at 1.91 kIU/liter (29). The specific cutoff for DELFIA IgE assays should also be defined with a larger cohort of patients.

Agarwal et al. (3) warn of the complexity in diagnosing ABPA in CF patients, especially in view of the existence of silent forms of ABPA and of remission periods (1). The new ELISA, Western blot, or DELFIA techniques used in this study could not identify patients PPP5, PPP5, and PPP6 who had a history of ABPA any more than ES, IEP, and ImmunoCAP could identify this type of patient.

In conclusion, our study argues for the specific IgG level ELISA kit from Bordier to replace the immunoprecipitation techniques that are less standardized. The DELFIA technique using a combination of PPE and recombinant antigen Man1P was able to identify ABPA in CF patients. The results of this study were limited by the number of patients and should be confirmed on a larger scale (e.g., with more patients and multicenter recruitment). However, DELFIA appears to be a promising tool to determine the titer of (e.g., with more patients and multicenter recruitment). However, DELFIA appears to be a promising tool to determine the titer of

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