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1 **Impact of domestic mould exposure on *Aspergillus* biomarkers and lung**  
2 **function in patients with chronic obstructive pulmonary disease**

3

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20

21 **ABSTRACT**

22 Patients with chronic obstructive pulmonary disease (COPD) are frequently colonised or  
23 sensitised by *Aspergillus*, but clinical significance remains unclear. Furthermore, little is  
24 known on the impact of indoor mould exposure during COPD. In this study, we assessed the  
25 relationship between domestic mould exposure, *Aspergillus* biomarkers and COPD severity  
26 during acute exacerbation and at stable state. *Aspergillus* section *Fumigati* culture in sputum  
27 and anti-*Aspergillus* antibodies detection (IgG and precipitins) were followed up in COPD  
28 patients that were prospectively recruited during exacerbation (n=62), and underwent a visit at  
29 stable state after 18 months (n=33). Clinical characteristics were collected at inclusion.  
30 Electrostatic dust collectors (EDCs) were used to measure domestic mould contamination.  
31 *Aspergillus* section *Fumigati* was more frequently detected during exacerbation (16.9%) than  
32 at stable state (4.0%), but the frequency of patients presenting with anti-*Aspergillus* antibodies  
33 was similar (32.2% and 33.3%, respectively). *Aspergillus* section *Fumigati* detection was  
34 associated with a higher body-mass index (BMI) during exacerbation, whereas patients with  
35 anti-*Aspergillus* antibodies presented a lower BMI and forced expiratory volume in 1 second,  
36 as well as a higher frequency of inhaled corticoids and higher total mould and *Penicillium*  
37 exposure at final visit (P<0.05). The frequency of patients with anti-*Aspergillus* antibodies  
38 was higher for total mould counts >30 CFUs/cm<sup>2</sup> (P=0.03). Aspergillosis was diagnosed in 2  
39 patients (6.1%) who presented increased levels of antibodies. Our data suggest that anti-  
40 *Aspergillus* antibodies are associated with chronic lung function alteration and/or domestic  
41 mould exposure, thereby supporting the consideration of indoor mould contamination and  
42 anti-*Aspergillus* antibodies kinetics in COPD management.

43 **Keywords:** COPD, *Aspergillus*, fungi, environment, anti-*Aspergillus* antibodies

44

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51

## 52 **1. Introduction**

53           Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease,  
54 which has a global mean prevalence of 12.4% in subjects aged  $\geq 40$  years in Europe (Blanco et  
55 al., 2018), and is mainly caused by inhalation of cigarette smoke. The hallmark feature of this  
56 disease is airflow limitation, but a substantial number of patients also suffer from acute  
57 exacerbations (AECOPD), which are a major cause of morbidity, mortality and hospital  
58 admission (Soler-Cataluña et al., 2005). AECOPD is mainly triggered by infections due to  
59 bacteria or viruses (Domenech et al., 2013), which have been shown to be facilitated by  
60 chronic inflammation and impaired lung immune defences (Le Rouzic et al., 2017; Pichavant  
61 et al., 2015, 2014). In addition, COPD patients are also at risk for invasive pulmonary  
62 aspergillosis (IPA). IPA has been increasingly reported with incidences varying from 1.6 to  
63 3.9%, probably under-estimated, and is associated with a high mortality rate (72-100%)  
64 (Delsuc et al., 2015; Guinea et al., 2010).

65           Increasing evidence supports the role of filamentous fungi in stable COPD. Indeed,  
66 *Aspergillus* section *Fumigati* and anti-*A. fumigatus* antibodies are frequently detected in  
67 COPD patients at stable state (Agarwal et al., 2010; Bafadhel et al., 2014). Although patients  
68 with or without *Aspergillus* section *Fumigati*-positive culture show no difference in  
69 exacerbation frequency or respiratory function, *Aspergillus* sensitisation was found to be  
70 associated with a lower forced expiratory volume in 1 second (FEV1) (Bafadhel et al., 2014)  
71 or bronchiectasis (Everaerts et al., 2017). Indoor mould exposure, which is involved in the  
72 development and evolution of other respiratory diseases (Caillaud et al., 2018; French Agency  
73 for Food, Environmental and Occupational Health & Safety (ANSES), 2016) could also play  
74 a role in the pathophysiology of COPD. However, little data is available to assess this  
75 possibility. One study evaluated fungal contamination using electrostatic dust fall collectors

76 (EDCs) and showed no difference of fungal exposure between COPD patients and controls  
77 (Barrera et al., 2019). Nevertheless, another study has recently shown that indoor  
78 environments were associated with greater COPD symptoms and lower FEV1 at stable state  
79 (Tiew et al., 2020). The presence of indoor visible moulds was also identified as an  
80 independent risk factor of persistent bronchial obstruction (Ciebiada et al., 2014), and  
81 elevated indoor dust fungal DNA levels were found to be associated with low FEV1 or forced  
82 vital capacity in asthmatic patients (Shendell et al., 2012). Furthermore, visible moulds were  
83 found to be associated with FEV1 decrease in the general population (Devien et al., 2018).  
84 Altogether, these data suggest mould exposure could contribute to airflow limitation during  
85 COPD.

86 Here, our aim was to address the specific topic of mould exposure during COPD by  
87 using EDCs, which are innovative tools used to perform quantitative and qualitative  
88 characterisation of fungal flora (Ege et al., 2011; Dauchy et al., 2018; Viegas et al., 2019). We  
89 assessed the relationship between domestic mould exposure measured with EDCs, *Aspergillus*  
90 biomarkers (*Aspergillus* culture in sputum and anti-*Aspergillus* antibodies detection), and  
91 COPD severity (lung function and frequency of exacerbation episodes) in patients who were  
92 prospectively recruited during severe acute exacerbation requiring hospitalisation, and  
93 underwent a final visit at stable state after 18 months.

94

## 95 **2. Methods**

### 96 *2.1. Patient recruitment and follow-up, data collection, clinical and environmental sampling*

97 Patients with acute exacerbation of COPD, who had no other pulmonary diseases  
98 (tuberculosis, cancer, diffuse bronchiectasis, asthma, etc.) and had not received antifungal  
99 systemic treatment within the 6 previous months, were prospectively recruited in the

100 Pneumology, Intensive Care or Post-Emergency services of Lille University Hospital (France)  
101 from August 2011 to November 2015. Age, sex, GOLD stage, FEV1 at stable state, frequency  
102 of exacerbation episodes in the 2 previous years, tobacco status, and corticoid treatments were  
103 collected at inclusion. The results of the cytobacteriological examination of sputum and viral  
104 analyses were also collected to determine the origin of exacerbation. Patients were asked to  
105 attend a visit at stable state 18 months after inclusion.

106 Sputum and serum samples were collected at inclusion and after 18 months, at stable  
107 state, for detection of *Aspergillus* section *Fumigati* and anti-*Aspergillus* antibodies. Home  
108 mould exposure was assessed using EDCs that were exposed for 10 weeks in the patient's  
109 bedroom.

110 The study was approved by the local ethics committee (North-West Ethics Committee,  
111 University of Rouen, France, referral number 2010-031) and was registered at  
112 ClinicalTrials.gov (NCT02318524).

113

## 114 2.2. *Mycological analysis of sputa and anti-Aspergillus antibodies detection*

115 Sputum samples were half diluted with a solution of acetylcysteine (Digest-EUR,  
116 Eurobio), and 10 µL were cultured on half-diluted Sabouraud agar medium with 0.5 g/L  
117 amikacin (SAB) at 30°C, chromogenic agar medium at 37°C and erythritol agar medium at  
118 24°C for 7 days. Anti-*Aspergillus* IgG were detected using the Platelia™ *Aspergillus* IgG kit  
119 (Bio-Rad, Marne-La-Coquette, France), and an in-house Ouchterlony double  
120 immunodiffusion method was used for detection of anti-*A. fumigatus* precipitins. Patients  
121 were considered to have anti-*Aspergillus* antibodies when levels over 9 UA/mL using ELISA,  
122 or at least 2 precipitin arcs were detected.

123

### 124 2.3. Sampling and mycological analysis of indoor dust

125 Domestic fungal exposure was assessed from EDCs that were analysed by culture.  
126 EDC washing consisted of shaking for 10 minutes in 20 mL of sterile NaCl 0.9% with 0.1%  
127 Tween 80 in a Stomacher® 80 Biomaster (Life System), as previously described (Dauchy et  
128 al., 2018; Scherer et al., 2014), and 60 µL of the collected solution were cultured on SAB at  
129 30°C, dichloran - rose Bengal – chloramphenicol (DRBC) - benomyl and dichloran - glycerol  
130 (DG18) agar media (Oxoid, Dardilly, France) at 24°C for 7 days. The choice of DG18  
131 medium was based on the recommendation by the ISO 16000-17 norm for analysis of air  
132 samples by culture (ISO 16000-17, 2008). The SAB medium and 30°C incubation were  
133 selected for optimal detection thermophilic pathogenic moulds, especially *Aspergillus* spp.  
134 The DRBC – benomyl medium was added for its inhibitory properties on microtubule growth  
135 and hyphal elongation, which allows detection of slow-growing fungi (Jochová et al., 1993).  
136 Moulds were identified by macroscopic and microscopic (morphology of conidiophores,  
137 conidia and hyphae) characteristics (ANOFEL, 2018; de Hoog et al., 2001), and CFUs were  
138 counted on the 3 media for each genus or species. When moulds were detected on several  
139 culture media, the final number of colonies corresponded to the results from the medium with  
140 the highest number of colonies for a given species/genus (ISO 16000-17, 2008).

141

### 142 2.4. Statistical analysis

143 Data are presented as mean±standard error mean. Comparison of FEV1 and mould  
144 contamination levels in EDCs was assessed using the t-test or Mann–Whitney U-test in  
145 GraphPad Prism 5 for parametric or nonparametric groups, respectively. For comparison of  
146 three groups or more, the Kruskal–Wallis test was used, with Dunn’s multiple comparison test



147 as post-test to compare individual pairs of groups. The Fisher's exact test was used for  
148 proportions. A p-value of <0.05 was considered statistically significant.

149

### 150 **3. Results**

#### 151 *3.1. Patients' characteristics at inclusion and origin of exacerbations*

152 Sixty-two patients meeting inclusion criteria and no exclusion criteria were recruited.  
153 The sex ratio was 2.6 (46 men and 17 women), and the mean age was 63.3±10.4 years-old.  
154 Nine patients had mild (GOLD II stage), 28 had severe (stage III) and 25 had very severe  
155 COPD (stage IV). Overall, the mean FEV1 reached 35.3±2.0% of predicted and the mean  
156 body-mass index (BMI) was 25.2±0.8 kg/m<sup>2</sup>. All the patients had a previous history of  
157 smoking. Mean pack years was 63.4±4.2, whereas 17/62 patients (27.4%) were current  
158 smokers. In the 2 previous years, 33% presented no exacerbation episode, whereas 24%  
159 presented at least 3 episodes. Inhaled corticoids were used as maintenance treatment in 80.6%  
160 of patients. During exacerbation, a bacterial infection was confirmed for 21 patients (33.9%),  
161 and among them the most frequent bacteria were *Streptococcus pneumoniae* and *Haemophilus*  
162 *influenzae* (both isolated in 6 sputa, i.e. 28.6% of patients with documented bacterial  
163 exacerbation). *Pseudomonas aeruginosa* was isolated in 5 patients (23.8%), and *Moraxella*  
164 *catarrhalis* in 3 patients (14.3%). Five patients (8.1%) presented a viral infection.

165

#### 166 *3.2. Frequency and clinical significance of Aspergillus positive biomarkers during* 167 *exacerbation and at stable state*

168 At inclusion, *Aspergillus* section *Fumigati* was detected in 10 out of the 59 patients  
169 who were able to expectorate (16.9%). Twenty patients (32.2%) presented anti-*Aspergillus*  
170 antibodies, with IgG levels varying from 2 to over 80 UA/mL and 0 to 8 anti-*A. fumigatus*

171 precipitins. Five patients had both positive culture and anti-*Aspergillus* antibodies (8.1%). No  
172 patient presented current *Aspergillus* infection, but 3 patients (N°45, N°52 and N°60) had a  
173 history of *Aspergillus* infection 3 to 6 years before inclusion. Patients N°52 and N°60, but not  
174 N°45, received voriconazole. Mortality reached 19.4% during the 18-month follow-up period.

175         Thirty-three patients underwent the final visit at stable state. Sputa were sampled for  
176 25 out of 33 patients, and *Aspergillus* section *Fumigati* was detected in 1/25 patients (4.0%),  
177 yielding a lower frequency at stable state than at inclusion, but this difference was not  
178 significant. The frequency of patients with anti-*Aspergillus* antibodies reached 33.3%, being  
179 similar at stable state and at inclusion. Only one patient had both positive culture and anti-  
180 *Aspergillus* antibodies (3.0%). When comparing the individual evolution of *Aspergillus*  
181 biomarkers, the 4 patients with positive culture became negative at stable state, the only  
182 patient who had positive culture at final visit was negative at inclusion, and 20 negative  
183 patients remained negative (Fig. 1). Anti-*Aspergillus* antibodies were detected in 3 out of 23  
184 patients who were negative at inclusion. Among the 10 patients with anti-*Aspergillus*  
185 antibodies at inclusion, 8 remained positive, 3 having stable antibody levels and 5 increased  
186 antibody levels at the final visit. Aspergillosis was diagnosed at final visit in 2 patients  
187 (6.1%), who had increased anti-*Aspergillus* antibodies (initial diagnosis in patient N°10 and  
188 recurrence in patient N°52). Both patients presented clinical (worsening respiratory  
189 insufficiency in spite of appropriate antibiotic therapy and ventilatory support), and  
190 radiological features of aspergillosis (abnormal medical imaging by CT scan). But only  
191 patient N°10 had positive *Aspergillus* section *Fumigati* sputum culture. For patient N°10,  
192 evolution was favorable after a 6 month treatment with voriconazole. A decrease in anti-  
193 *Aspergillus* antibodies was observed after the end of the treatment (Fig. 2). Patient N°52 was  
194 initially treated with voriconazole, which was replaced with liposomal amphotericin B after 1

195 month. However, he died after 20 days. No aspergillosis was diagnosed in any of the other  
196 patients, even when significant levels of anti-*Aspergillus* antibodies were detected.

197

### 198 3.3. Relationship between *Aspergillus* biomarkers and patients characteristics at inclusion

199 Mean FEV1 tended to be lower in patients with *Aspergillus* section *Fumigati* positive  
200 culture (P=0.06; Table 1, Fig. 3A) than without, and was significantly lower in patients with  
201 (P=0.01; Table 1, Fig. 3B) than without anti-*Aspergillus* antibodies. Frequency of inhaled  
202 corticoid treatment was significantly higher in patients than in patients with than without anti-  
203 *Aspergillus* antibodies. Mean BMI was significantly higher in patients with *Aspergillus*  
204 section *Fumigati* positive culture, and lower in patients with anti-*Aspergillus* antibodies.  
205 Smoking status and frequency of exacerbation with confirmed bacterial origin were similar in  
206 patients with or without anti-*Aspergillus* antibodies, or in patients with positive or negative  
207 culture (Table 1).

208

### 209 3.4. Assessment of domestic mould contamination and relationship with *Aspergillus* 210 biomarkers at inclusion and final visit

211 EDCs were sent to the laboratory by 51 out of 62 patients. Assessment of domestic  
212 mould contamination yielded 0.6 to 98.1 CFUs/cm<sup>2</sup>, representing 1 to 13 genera or species.  
213 Mould frequencies and counts were higher on DG18 for most genera and species, except for  
214 *Aspergillus* section *Fumigati* and mucorales which were more frequently detected on SAB  
215 and Benomyl, respectively (Supplementary Table). Overall counts resulting from the 3 media  
216 showed that *Penicillium* spp., *Cladosporium* spp., *Aspergillus* section *Fumigati*, *Aspergillus*  
217 series *Versicolores* and Mucorales were the most frequent moulds (in 90.2%, 64.7%, 49%,  
218 39.2% and 33% of EDCs, respectively; Table 2). *Aspergillus* section *Nigri*, *Fusarium* spp. and

219 *Scopulariopsis* spp., which are thermotolerant pathogenic moulds, were also frequently  
220 isolated (17.6-25.5%). *Aspergillus* genera represented 1.5-92.5% of total fungal flora (median  
221 22.2%), with *Aspergillus* section *Fumigati* corresponding to 0-100% of *Aspergillus* spp.  
222 (median 16.7%) (Supplementary Fig.1). Overall mould contamination level and *Penicillium*  
223 spp. concentrations tended to be higher in patients with positive vs. negative culture or  
224 detection of anti-*Aspergillus* antibodies at inclusion (Fig. 4A-B, 4D-E), and were significantly  
225 higher in patients with positive vs. negative detection of anti-*Aspergillus* antibodies at the  
226 final visit ( $P < 0.05$ ; Fig. 4C, 4F). Furthermore, when we analysed the repartition of total  
227 mould contamination level, we identified a group of 8 dwellings (15.7%) presenting a higher  
228 contamination level,  $>30$  CFUs/cm<sup>2</sup> (Supplementary Fig. 2). When comparing the frequency  
229 of patients with or without anti-*Aspergillus* antibodies for dwellings with total contamination  
230 level of  $\leq$  or  $>30$  CFUs/cm<sup>2</sup>, we found that the frequency of patients with anti-*Aspergillus*  
231 antibodies tended to be higher at inclusion and was significantly higher at final visit in  
232 dwellings with contamination levels  $>30$  CFUs/cm<sup>2</sup> ( $P = 0.03$ ; Table 3). Since *Aspergillus*  
233 section *Fumigati* was only detected in a single patient's sputum at the final visit, the impact of  
234 domestic mould contamination on this biomarker could not be assessed. We found no  
235 significant difference in mould contamination level in patients with positive culture or anti-  
236 *Aspergillus* antibodies when *Aspergillus* section *Fumigati*, *Aspergillus* spp., or *Cladosporium*  
237 spp. concentrations were compared (Supplementary Fig. 3). No threshold yielding significant  
238 differences between the frequencies of patients with positive or negative culture or detection  
239 of anti-*Aspergillus* antibodies could be identified for these specific moulds or for *Penicillium*  
240 spp.

241

242 *3.5. Relationship between domestic mould contamination and COPD stage or exacerbation*  
243 *frequency*

244 Total mould counts tended to be lower in stage-II patients than in stage-III or -IV  
245 patients, and *Aspergillus* section *Fumigati* counts tended to be higher in stage-IV than in  
246 stage- II or -III patients (Fig. 5A), but these differences were not significant. *Aspergillus* spp.,  
247 *Penicillium* spp. and *Cladosporium* spp. counts were similar in stage-II, -III and -IV patients  
248 (Supplementary Fig. 4A). Total mould counts, *Aspergillus* section *Fumigati*, *Aspergillus* spp.,  
249 *Penicillium* spp. and *Cladosporium* spp. counts were also similar in patients with 0, 1, 2 or  
250 more than 3 exacerbations during the previous 2 years (Fig. 5B, Supplementary Fig. 4B).

251

#### 252 **4. Discussion**

253 Our study showed a 16.9% frequency of *Aspergillus* section *Fumigati* detection in  
254 sputum during AECOPD and 4.0% at stable state. These frequencies were lower than those  
255 reported previously (28%-37%) (Bafadhel et al., 2014). However, lower quantities of sputum  
256 were used in our study. A limitation could also be the absence of repeated samples to detect  
257 *Aspergillus* section *Fumigati* colonisation in our study. The higher frequency of *Aspergillus*  
258 section *Fumigati* we found at inclusion is consistent with impaired immune response during  
259 exacerbation (Berenson et al., 2014), which could facilitate *Aspergillus* colonisation.

260 Conversely, the frequencies of patients with anti-*Aspergillus* antibodies were similar  
261 during exacerbation (32.2%) and at stable state (33.3%). Both frequencies were higher than  
262 those reported in previous studies which used skin prick test and/or *A. fumigatus* IgE or IgG  
263 detection by ImmunoCAP (8.5-18% in Agarwal et al., 2010; Bafadhel et al., 2014; Everaerts  
264 et al., 2017), whereas IgG detection by ELISA and precipitins were used in our study. These  
265 similar frequencies during exacerbation and at stable state suggest that anti-*Aspergillus*  
266 antibodies are more related to chronic lung-function alteration and/or environmental  
267 exposures than acute disorders during COPD. This hypothesis is also supported by the

268 association of anti-*Aspergillus* antibodies with a lower FEV1, and the higher frequency of  
269 inhaled corticoids in patients with anti-*Aspergillus* antibodies. The lower BMI in patients with  
270 anti-*Aspergillus* antibodies is consistent with the association of a lower BMI with chronic  
271 *Aspergillus* infections (Ohara et al., 2016). Lower BMI was also identified among the risk  
272 factors of pneumonia during COPD, together with lower FEV1 or higher inhaled  
273 corticosteroid dose (Agusti et al., 2018; Crim et al., 2009). The higher BMI in patients with  
274 positive detection of *Aspergillus* section *Fumigati* is surprising. Since *A. fumigatus*  
275 colonisation in COPD patients with acute exacerbation is a risk factor for invasive  
276 aspergillosis (Blot et al., 2012; Guinea et al., 2010; Khasawneh et al., 2006), this result could  
277 be related to a higher risk of developing invasive aspergillosis during exacerbation for patients  
278 with higher BMI.

279         Anti-*Aspergillus* antibody detection was used as a diagnostic criterion for the 2  
280 patients who developed aspergillosis in our study. Although a positive *A. fumigatus* serum test  
281 has been proposed as a criterion for IPA diagnosis (Bulpa et al., 2007), the clinical  
282 significance of anti-*Aspergillus* antibodies during COPD remains unclear, and this marker is  
283 not among the criteria that were proposed by Blot et al. for critically ill patients (Blot et al.,  
284 2012). In our study, 3 other patients presented increased anti-*Aspergillus* antibodies or  
285 became positive, and were not diagnosed with *Aspergillus* infection in the absence of clinical  
286 and radiological criteria for aspergillosis. Since elevated IgG against *A. fumigatus* are usually  
287 considered to indicate *Aspergillus* infection (Uffredi et al., 2003), these data underscore the  
288 need for further studies on the kinetics of *Aspergillus* IgG and precipitins during COPD to  
289 evaluate its clinical significance and usefulness for the diagnosis of acute infections.

290         The use of EDCs for assessment of mould contamination yielded the first data on  
291 domestic fungal contamination in our region, showing frequencies of *Penicillium*,  
292 *Cladosporium*, and *Aspergillus* series *Versicolores* that were similar to those reported in other

293 studies in France (Dallongeville et al., 2015; Reboux et al., 2009). However, higher  
294 frequencies of pathogenic thermophilic moulds, such as *Aspergillus* section *Fumigati* and  
295 Mucorales were observed in our study. Our data confirm that this easy-to-use method, which,  
296 contrary to air sampling, does not require specific equipment, and is representative of fungal  
297 exposure over several weeks, is a promising alternative for quantitative measurement of  
298 mould contamination in dwellings (Fréalles et al., 2017).

299 An interesting finding in our study was the higher domestic mould exposure (total  
300 counts and *Penicillium* spp. concentrations) of patients with anti-*Aspergillus* antibodies,  
301 which supports the idea that mould exposure is a risk factor for development of anti-  
302 *Aspergillus* antibodies during COPD. The association between anti-*Aspergillus* antibodies and  
303 increased *Penicillium* (but not other mould concentrations) could be related to the  
304 predominance of this fungal genus in indoor environments, and to increased indoor  
305 concentrations of *Penicillium* in dwellings with indoor air-quality problems (Cooley et al.,  
306 1998). The tendency to a higher total mould contamination level in dwellings from stage-III  
307 and stage-IV patients and *Aspergillus* section *Fumigati* counts in stage-IV patients is  
308 consistent with previous reports showing association between mould exposure and airway  
309 obstruction in asthmatic patients or healthy subjects (Ciebiada et al., 2014; Devien et al.,  
310 2018; Shendell et al., 2012), and supports the recent finding of an association between the  
311 abundance of fungal allergens in indoor environments and greater COPD symptoms or lower  
312 FEV1 (Tiew et al., 2020). Beyond the significant association between anti-*Aspergillus*  
313 antibodies and total fungal counts in EDCs, the higher frequency of patients with anti-  
314 *Aspergillus* antibodies in dwellings with total mould contamination levels of >30 CFUs/cm<sup>2</sup>  
315 suggests that this threshold could be used to define abnormal indoor mould exposure, as well  
316 as the need for patient's home assessment by a professional (such as a medical indoor-  
317 environment counsellor) and remediation measures. Since the lack of threshold values is a

318 major problem for assessment of mould exposure, and there are currently no guidelines on  
319 acceptable levels of fungal exposure in dust, this definition of a threshold for EDCs represents  
320 an important finding for the further use of this method in practical assessment of dwellings.

321 The absence of association between *Aspergillus* section *Fumigati* in EDCs and  
322 *Aspergillus* section *Fumigati* detection in sputum or antibody detection could be due to the  
323 fact that, although high concentrations of airborne *Aspergillus* section *Fumigati* can be found  
324 in dwellings (Paluch et al., 2019) or near specific reservoirs such as compost (Williams et al.,  
325 2019), this species is usually not predominant in the environment (Reboux et al., 2009;  
326 Vesper et al., 2007). The establishment of a relationship between *Aspergillus* section *Fumigati*  
327 exposure and colonisation/anti-*Aspergillus* antibodies could also be biased by the  
328 pathogenicity of these thermophilic species, which can yield transient colonisation, making  
329 low doses have a higher effect than exposure to high concentrations of non-pathogenic species  
330 (Eduard, 2009). The fact that differences were found only when total counts or *Penicillium*  
331 spp. concentrations were compared (according to anti-*Aspergillus* antibodies status)  
332 underscores the difficulty of assessing the pathogenic effect of moulds which are usually  
333 present in low concentrations in dwellings. Using total fungal counts, which mainly reflect the  
334 usual predominant species (i.e. *Penicillium* and *Cladosporium*), as a global indicator of fungal  
335 risk could allow us to indirectly assess the risk related to minority species (such as *Aspergillus*  
336 section *Fumigati*) or to species poorly detected by culture (such as *Stachybotrys chartarum*).  
337 The use of real-time PCR for DNA quantification of these species could also be helpful in  
338 further studies.

339 Our study was limited by the quite low number of patients. Nevertheless, this number  
340 is close to the one that was reported in several other studies where COPD patients were  
341 included during exacerbation (which is more limiting than inclusion at stable state), and  
342 sputum was analysed for bacterial and fungal culture (Bafadhel et al., 2014). Home evaluation



343 with EDCs was a supplementary cause of refusal and further limited the recruitment. Despite  
344 this quite low number of patients, we were able to confirm data that were observed in other  
345 studies (such as a lower FEV1 or higher frequency of inhaled corticoids in patients with anti-  
346 *Aspergillus* antibodies (Bafadhel et al., 2014; Everaerts et al., 2017)), and provided new data  
347 on the relationship between mould exposure and bioclinical parameters during COPD.  
348 However, having a greater number of patients would have been helpful to confirm some  
349 tendencies that were observed (e.g., relationship between mould exposure and COPD stage),  
350 or to assess more reliably the relationship between mould exposure and exacerbation  
351 frequency.

352 Despite this limitation, our data suggest that *Aspergillus* colonisation could be  
353 facilitated by impaired immune response during COPD exacerbation, whereas anti-  
354 *Aspergillus* antibodies are rather associated with chronic lung-function alteration. The  
355 association of mould exposure with anti-*Aspergillus* antibodies and association of anti-  
356 *Aspergillus* antibodies with a lower FEV1 suggest abnormal indoor mould contamination  
357 could contribute to the alteration of lung function during COPD, thereby supporting the  
358 consideration of domestic mould exposure in the management of COPD patients. Further  
359 studies are needed to assess the clinical significance of anti-*Aspergillus* antibody kinetics and  
360 the potential role of mould exposure in the development of aspergillosis during COPD.

361

#### 362 **Declaration of competing interest**

363 E. Fréalle reports research grants from Gilead, MSD and Pfizer, and travel grants for  
364 congress and conference attendance by Gilead. The other authors have no conflicts of interest  
365 to disclose with the submitted work.

366

#### 367 **Credit author statement**

368 EF, GR, PG and SF conceived the study and designed the project. OLR, NB, MCW,  
369 JD, SN and SF recruited the patients and collected the data. EF, GR and SF analysed the data.  
370 EF wrote the manuscript draft. All authors critically revised the manuscript and approved the  
371 submitted version.

372

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543  
544

545 **Figure legends**

546 **Fig. 1.** Flow diagram of patient recruitment and follow-up

547

548 **Fig. 2.** Kinetics of anti-*Aspergillus* antibodies in patients with aspergillosis diagnosed at the  
549 final visit

550

551 **Fig. 3.** Association between FEV1 and *Aspergillus* biomarkers

552 FEV1 was compared between patients with positive or negative *Aspergillus* section *Fumigati*  
553 culture in sputum (A) and between patients with or without anti-*Aspergillus* antibodies (B) at  
554 inclusion.

555

556 **Fig. 4.** Association between domestic mould contamination levels (total or *Penicillium* spp.  
557 counts) and *Aspergillus* biomarkers

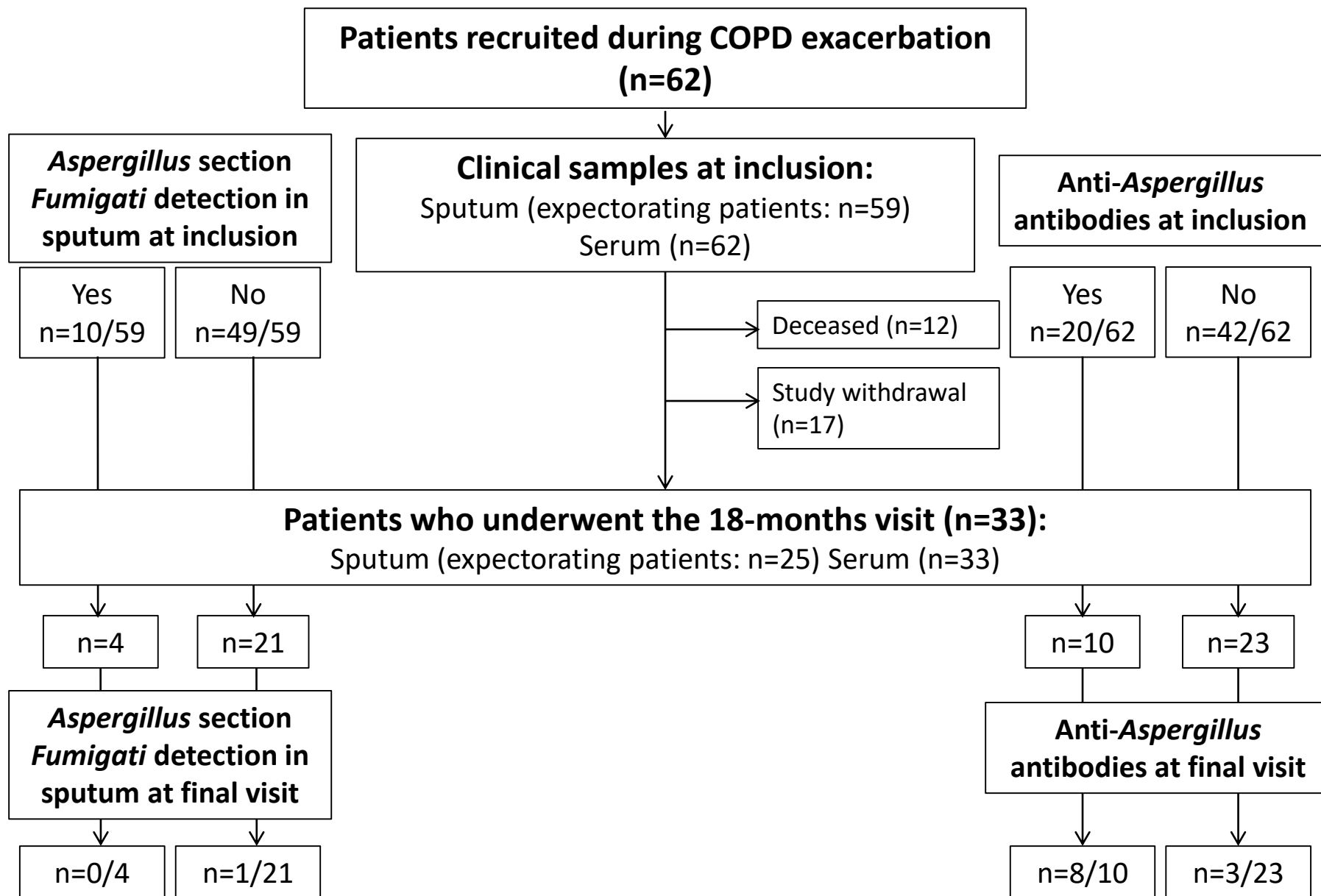
558 Total mould (A-C) and *Penicillium* spp. (D-F) concentrations in EDCs were compared  
559 between patients with positive or negative *Aspergillus* section *Fumigati* culture in sputum at  
560 inclusion and between patients with or without anti-*Aspergillus* antibodies at inclusion, and at  
561 final visit.

562

563 **Fig. 5.** Association between domestic mould contamination levels (total or *Aspergillus* section  
564 *Fumigati* counts) and COPD stage (A) or number of exacerbations (B)

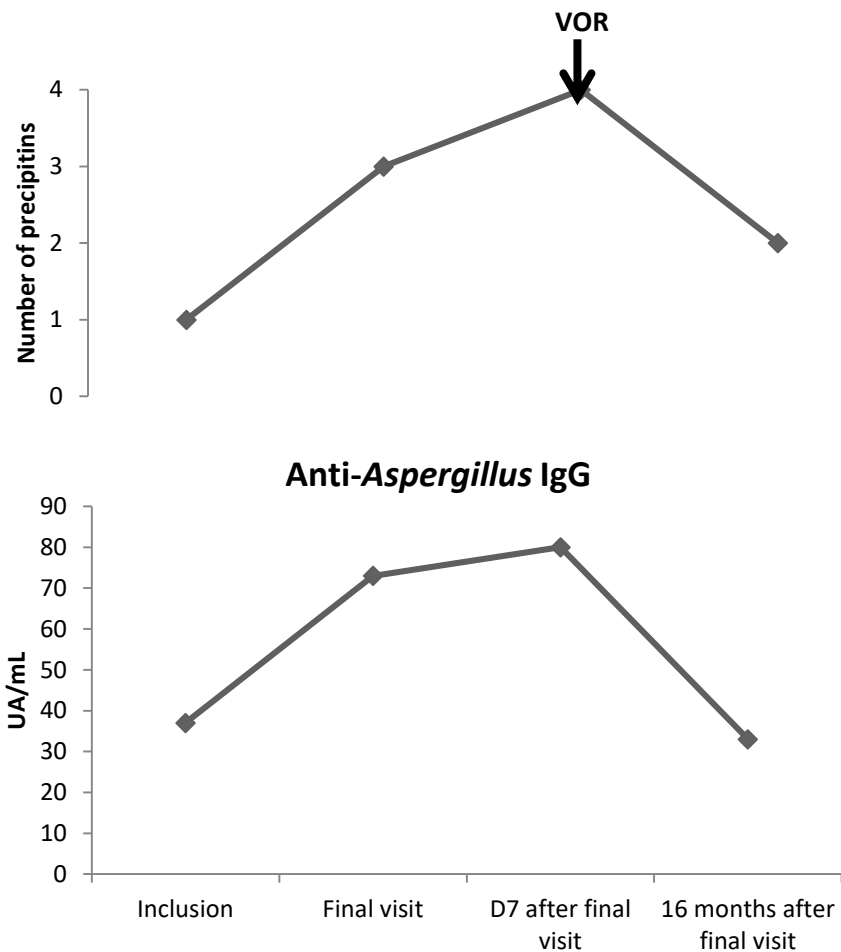
565

**Fig. 1**



**Fig. 2**

**N°10**  
**Anti-*A. fumigatus* precipitins**



**N°52**  
**Anti-*A. fumigatus* precipitins**

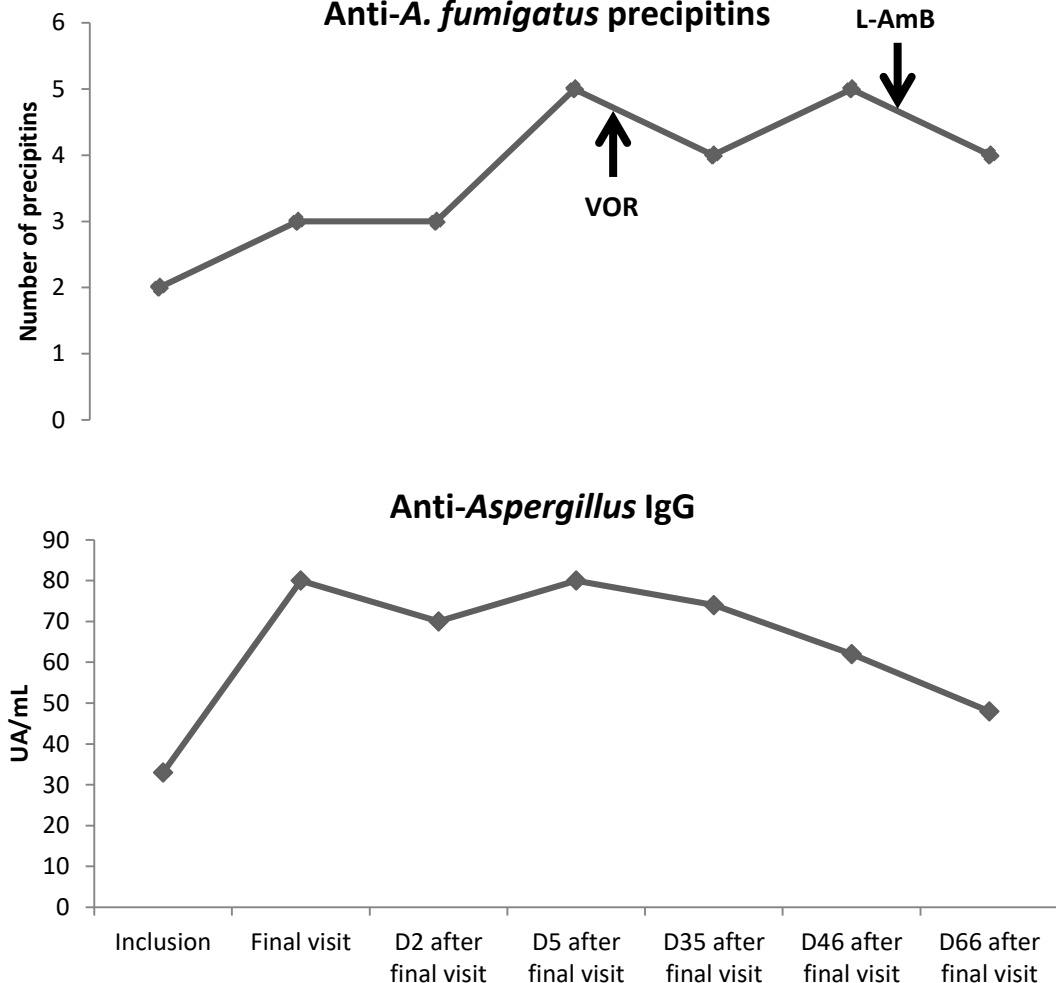
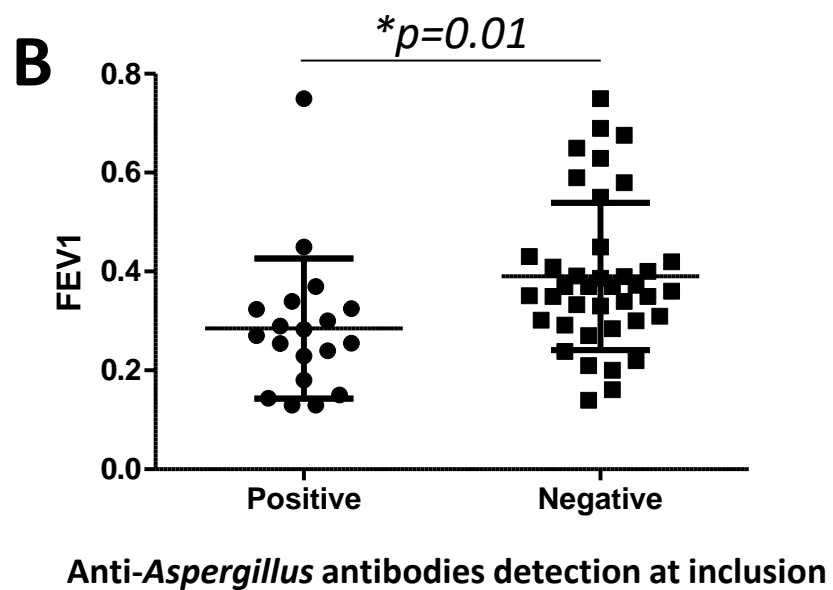
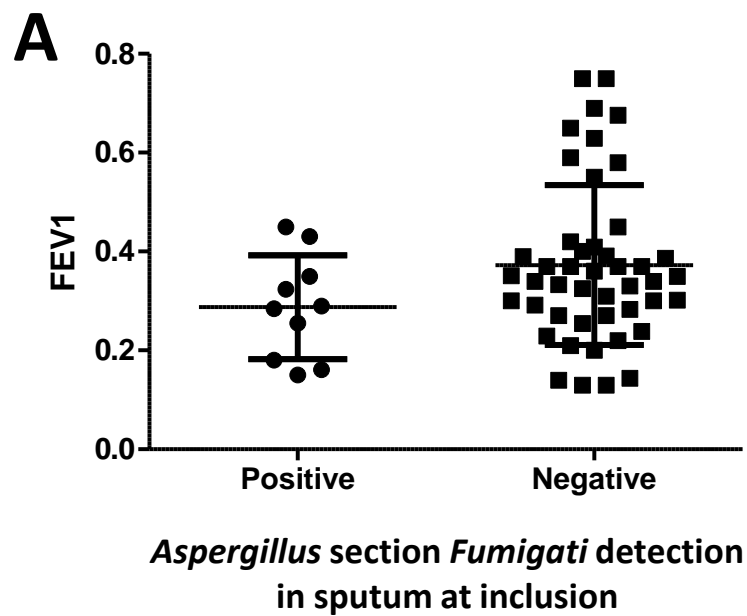


Fig. 3



**Fig. 4**

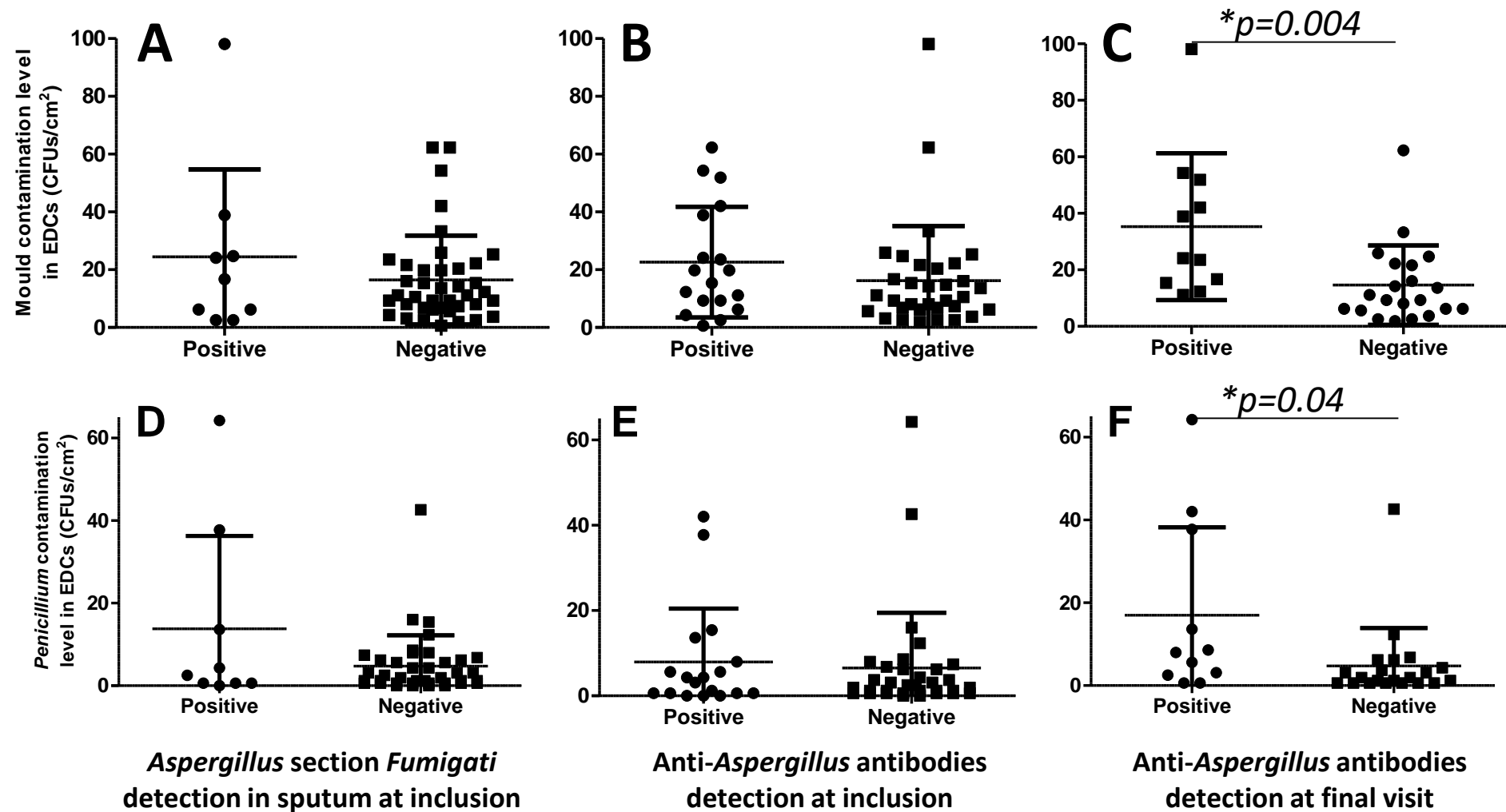
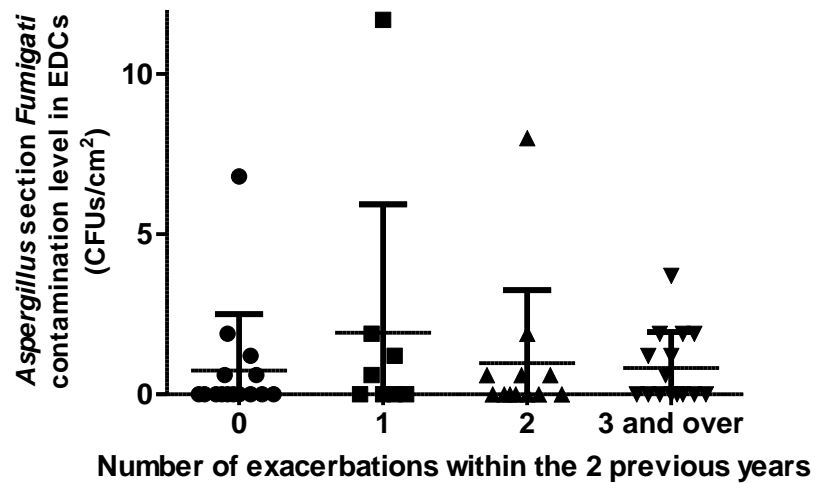
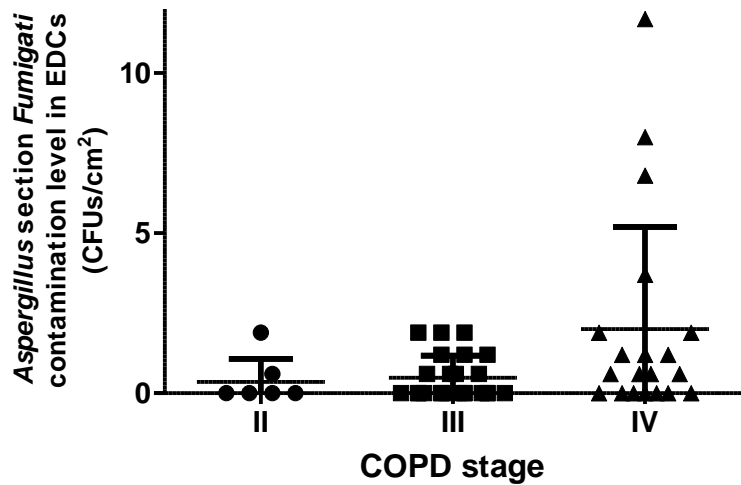
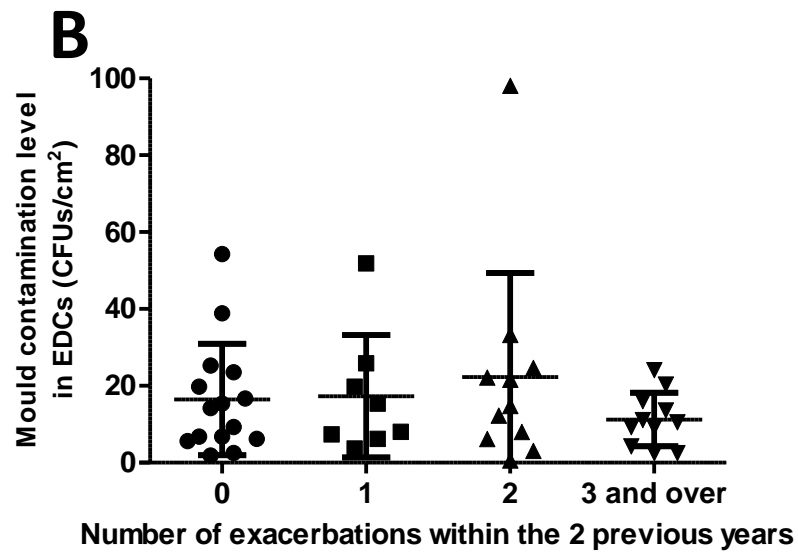
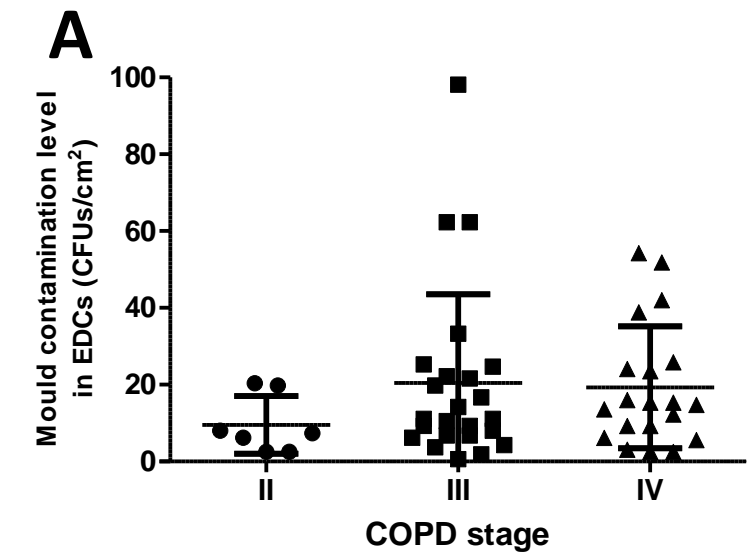


Fig. 5



**Table 1**

Predictors of *Aspergillus* section *Fumigati* positive sputum culture or anti-*Aspergillus* antibody detection during exacerbation

	<i>Aspergillus</i> section <i>Fumigati</i> detection in sputum			Detection of anti- <i>Aspergillus</i> antibodies		
	Positive	Negative	P-values	Positive	Negative	P-values
Patients (n)	10	49		20	42	
Men (%)	80.0	69.4	0.40	75.0	71.4	0.51
Age	61.1±2.4	63.9±1.7	0.23	61.5±1.7	64.6±1.9	0.14
Body mass index (BMI) (kg/m <sup>2</sup> )	28.7±1.6	24.4±0.9	<b>0.01</b>	23.0±1.1	26.2±1.1	<b>0.03</b>
Pack years	67.9±12.1	61.5±4.7	0.29	71.8±6.7	59.3±5.3	0.08
Current smokers (%)	30.0	26.5	0.55	20.0	30.9	0.28
≥ 1 exacerbation with hospitalization/year in the 2 previous years (%)	66.7	66.7	0.64	64.3	70	0.63
GOLD II/III/IV	0/50.0/50.0	18.4/44.9/36.7	0.32	5.0/35.0/60.0	19.0/50.0/31.0	0.07
Inhaled corticosteroid (%)	70	81.6	0.33	95.0	71.4	<b>0.03</b>
FEV1 (mean %)	28.7 ± 3.3	37.3 ± 2.4	0.06	28.5 ± 3.3	39.1 ± 2.4	<b>0.01</b>
Confirmed bacterial exacerbation (%)	40.0	32.7	0.52	45.0	28.6	0.16

Data are presented as n (%), mean±SEM, unless otherwise stated. Bold type represents statistical significance at p<0.05.



**Table 2**

Mould species frequency and number of colonies in electrostatic dust collectors

Species	Frequency (%)	Median CFUs/cm <sup>2</sup> (min-max)
<i>Penicillium</i> spp.	90.2	3.1 (0.6-64.2)
<i>Cladosporium</i> spp.	64.7	1.9 (0.6-15.4)
<i>Aspergillus</i> section <i>Fumigati</i>	49.0	1.2 (0.6-11.7)
<i>Aspergillus</i> series <i>Versicolores</i>	39.2	0.9 (0.6-24.7)
<i>Mucorales</i>	33.3	1.2 (0.6-9.3)
- <i>Mucor</i> spp.	15.7	0.9 (0.6-1.9)
- <i>Rhizopus</i> spp.	13.7	0.6 (0.6-1.2)
- <i>Lichtheimia</i> spp.	2.0	0.6 (0.6-0.6)
- <i>Cunninghamella</i> spp.	2.0	0.6 (0.6-0.6)
- Other mucorales	7.8	0.9 (0.6-9.3)
<i>Aspergillus</i> section <i>Nigri</i>	25.5	1.2 (0.6-6.2)
<i>Aspergillus</i> spp.	25.5	1.2 (0.6-54.9)
<i>Alternaria</i> spp.	25.5	1.2 (0.6-4.3)
<i>Fusarium</i> spp.	19.6	1.5 (0.6-34.6)
<i>Scopulariopsis</i> spp.	17.6	0.6 (0.6-1.9)
<i>Wallemia</i> spp.	11.8	0.9 (0.6-3.7)
<i>Geotrichum</i> spp.	11.8	0.6 (0.6-1.2)
<i>Aspergillus</i> section <i>Flavi</i>	9.8	3.1 (0.6-7.4)
<i>Paecilomyces</i> spp.	9.8	0.6 (0.6-0.6)
<i>Trichoderma</i> spp.	9.8	0.6 (0.6-0.6)
<i>Botrytis</i> spp.	5.9	0.6 (0.6-1.2)
<i>Ulocladium</i> spp.	5.9	0.6 (0.6-0.6)
<i>Aspergillus</i> series <i>Nidulantes</i>	3.9	0.9 (0.6-1.2)
Other fungi	≤2.0	NA

**Table 3**

Frequency of *Aspergillus* section *Fumigati* detection in sputum and anti-*Aspergillus* antibodies in patients with total mould contamination level of  $\leq$  or  $>$  30 CFUs/cm<sup>2</sup> at inclusion and final visit

	Total counts		
	$\leq$ 30 CFUs/cm <sup>2</sup>	$>$ 30 CFUs/cm <sup>2</sup>	P-values
<i>Aspergillus</i> section <i>Fumigati</i> detection in sputum			
Inclusion (n=48)	7/41 (17%)	2/7 (29%)	0.39
Final visit (n=25)	0/20 (0%)	1/5 (20%)	0.20
Detection of anti- <i>Aspergillus</i> antibodies			
Inclusion (n=51)	13/43 (30%)	5/8 (63%)	0.09
Final visit (n=32)	6/25 (24%)	5/7 (71%)	<b>0.03</b>

Bold type represents statistical significance at  $p < 0.05$ .