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## Factors associated with coinfections in invasive aspergillosis: a retrospective cohort study

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1 **Factors associated with coinfections in invasive aspergillosis: a retrospective cohort study**

2

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44 † Prof. Ermanno Candolfi is deceased (in 2019).

45

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49

50 **Running head:** Coinfections in invasive aspergillosis

51

52 Preliminary results have been presented as an oral communication at ECCMID 2019.

53

54 **Keywords:** Invasive Fungal Infections; Coinfections; Fungal; Leukaemia; Mortality; Risk Factors

55

56

57

58 **Abstract**

59 **Objectives:** To describe the coinfections in invasive aspergillosis, to identify factors associated with  
60 coinfections and to evaluate the impact of coinfection on mortality.

61 **Patients and methods:** We conducted a monocentric retrospective study of consecutive putative,  
62 probable, or proven invasive aspergillosis that occurred from 1997 to 2017. All coinfections, with  
63 an onset within 7 days before or after the first sign of aspergillosis, were identified. Factors  
64 associated with coinfections and mortality were analysed by multivariable analysis.

65 **Results:** Among the 690 patients with IA included in the study, median age was 57 years (range: 7  
66 days-90 years). A coinfection was diagnosed in 272/690 (39.4%, 95%CI [35.8-43.2]) patients. The  
67 location of this coinfection was pulmonary only in 131/272 patients (48%), bloodstream only in  
68 66/272 patients (24%) and other/multiple sites in 75/272 patients (28%). Coinfections were  
69 bacterial (n=110/272 patients, 40%), viral (n=58/272, 21%), fungal (n=57/272, 21%), parasitic  
70 (n=5/272, 2%) or due to multiple types of pathogens (n=42/272, 15%). Factors associated with a  
71 coinfection in adjusted analysis were: allogeneic haematopoietic stem cell transplantation (OR=2.3  
72 [1.2-4.4]), other haematological malignancies (OR=2.1 [1.2-3.8]), other underlying diseases  
73 (OR=4.3 [1.4-13.6]); lymphopenia (OR=1.7 [1.1-2.5]); C-reactive protein >180 mg/l (OR=1.9 [1.2-  
74 3.0]); fever (OR=2.4 [1.5-4.1]); tracheal intubation (OR=2.6 [1.5-4.7]); isolation of  $\geq 2$  different  
75 *Aspergillus* species (OR=2.7 [1.1-6.3]); and presence of non-nodular lesions on chest computed  
76 tomography (OR=2.2 [1.3-3.7] and OR=2.2 [1.2-4.0]). Coinfections were independently associated  
77 with a higher mortality at week 12 (adjusted HR 1.5 [1.1-1.9],  $p < 0.01$ ).

78 **Conclusions:** Coinfections were frequent in IA patients and were associated with higher mortality.

79

80 **Introduction**

81 Invasive aspergillosis (IA) affects mostly severely immunocompromised patients. Despite  
82 improvements in diagnosis and treatment, 12-week mortality exceeds 30% [1-6]. High rates of  
83 coinfections have been described in patients with IA in a few studies. Georgiadou *et al.* showed  
84 49% of documented pulmonary coinfections in 126 patients with haematological malignancy (HM)  
85 and IA [7]. In another monocentric study in Israel, 21% of the HM patients had a polymicrobial  
86 pneumonia diagnosed on bronchoalveolar lavage fluid (BALF) [8]. *Aspergillus* was the most  
87 common pathogen identified (87%) and in-hospital mortality was higher in the coinfecting group.  
88 Recently, IA occurring after influenza virus infections was shown to have a 50% mortality rate [9,  
89 10]. Despite their high frequency, the risk factors for coinfections in IA and the impact on mortality  
90 have not yet been well studied. Our aim was to describe the coinfections associated with IA in a  
91 large cohort of patients with putative, probable or proven aspergillosis, to identify the factors  
92 associated with coinfections and to analyse the mortality.

93  
94 **Methods**

95 We conducted a retrospective study of consecutive IA cases that occurred in the University  
96 Hospital of Strasbourg from 1997 to 2017. The study is part of a retrospective, non-interventional  
97 study on opportunistic infections in immunosuppressed and frail patients. Institutional ethical  
98 committee has approved the study. According to French regulations, the database has been  
99 declared to the *Commission Nationale de l'Informatique et des Libertés*. The study has been  
100 registered in ClinicalTrials.gov (NCT03920735). All patients alive were asked to give their consent  
101 after receiving an information letter. In agreement with ethical committee recommendations,  
102 informed consent was not requested from the relatives of patients who had died. Patients who

103 had been enrolled in a prospective epidemiologic, diagnostic, or therapeutic clinical trial at time of  
104 their infection had all signed the appropriate informed consent form.

105 Suspected cases of IA were identified from the mycology laboratory database and from the  
106 hospital diagnosis database. Selection process is detailed in supplementary material. Patients  
107 included in a previous study on prognosis factors in IA are also part of this study [11]. Patients  
108 were included if they had been diagnosed with IA meeting the updated European Organization for  
109 Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) criteria for proven or  
110 probable IA in cancer and severely immunosuppressed patients [12] or the clinical algorithm for  
111 putative IA in critically ill patients [13]. These criteria were modified by using a cut-off for  
112 galactomannan index of  $\geq 0.5$  in serum or BAL, based on manufacturer's recommendations (Bio-  
113 Rad, Marne-La-Coquette, France). Pulmonary IA were not included if no chest computed  
114 tomography (CT) was available.

115

116 Date of first clinical, radiological or mycological sign attributed to IA was defined as Day 0 (D0). We  
117 collected all variables available on D0 or at earliest time point around D0 with the exception of  
118 mycological data that were collected over the full course of the IA to allow a definite diagnosis of  
119 this infection. Two groups were considered and compared: IA without coinfection and IA with a  
120 coinfection. A coinfection was defined by the presence of clinical and/or radiological signs of  
121 infection and the identification of a relevant pathogen in significant amount, within 7 days before  
122 or after D0 of IA [7]. Details and definitions appear in supplementary material [6, 7, 12, 14-16].

123

124 Categorical variables are described with numbers and percentages. For the study of factors  
125 associated with coinfections, and as a patient could suffer several episodes of IA, comparisons  
126 between groups were made using a mixed logistic regression model including a random patient

127 effect. All variables with a p-value <0.2 in univariate analysis (Table 1) were included in a  
128 multivariable model. Continuous variables were dichotomized using clinically relevant thresholds.  
129 Results are presented as odds ratios (OR) with their 95% confidence intervals (95%CI) calculated  
130 using the Wald method. Multicollinearity was assessed using the variance inflation factor (VIF)  
131 ensuring that no factor had a VIF greater than 2. The number of events per variable was greater  
132 than 10. Goodness of fit of the multivariable logistic model was assessed using the Hosmer-  
133 Lemeshow test. The rate of missing data was low (6.5% for multivariable model) and no  
134 imputation method was used. The effect of coinfection on mortality was assessed using a  
135 multivariable Cox regression model including a random patient effect. The adjustment variables  
136 included in the multivariable model were those with a p-value <0.2 in univariate analysis.  
137 Proportional hazards assumption was assessed using Schoenfeld residuals. Results are presented  
138 as hazards ratios (HR). Confidence intervals were computed using a robust variance. A p-value  
139 <0.05 was considered statistically significant. All analyses were performed using R software  
140 version 3.6.0 (2019) (R Foundation for Statistical Computing, Vienna, Austria. URL [https://www.R-](https://www.R-project.org/)  
141 [project.org/](https://www.R-project.org/)).

142

## 143 **Results**

### 144 **1) Global population characteristics**

145 Among 1075 suspicions of IA, we have identified 690 IA [549/690 (80%) probable, 29/690 (4%)  
146 putative, and 112/690 (16%) proven] in 661 patients (Figure 1). Median age was 57 years (range 7  
147 days - 90 years) and 65% (n=451/690) of patients were men (Table 1). Primary underlying diseases  
148 and other predisposing factors are listed in Table 1.

149

150 Microscopy, culture, or histopathology was positive in 404/690 (59%) patients. Galactomannan  
151 detection test was performed in serum in 678/690 (98%) episodes and was positive in 395/678  
152 (58%). BAL was performed in 449 (69%) of the 651 lower respiratory tract IA. Galactomannan  
153 detection test was performed in BALF in 396 episodes and was positive in 266/396 (67%) of them.  
154 Overall BALF analysis (positive culture or galactomannan test) was the only mycological  
155 documentation of IA in 121 (19%) of the 651 lower respiratory tract IA.

156

157 Site of IA was lower respiratory tract only, another single organ and disseminated in respectively  
158 586/690 (85%), 28/690 (4%) and 76/690 (11%) cases. Among the 651 lower respiratory tract IA,  
159 chest CT findings were nodule(s) only in 138/651 (21%) episodes, nodule(s) with other lesions in  
160 329/651(51%) and other lesions without nodule in 174/651 (27%). Ten patients with endoscopy-  
161 confirmed bronchial or tracheal lesions without lung involvement had a normal CT.

162

## 163 **2) Coinfections characteristics**

164 A coinfection was diagnosed in 272/690 (39%) episodes (Table 2). The site of coinfection was  
165 pulmonary only in 131/272 patients (48%), bloodstream infection in 66/272 patients (24%) and  
166 other or multiple sites in 75/272 patients (28%). Coinfection with bacteria only was identified in  
167 110/272 patients (40%), viruses in 58/272 (21%), fungi in 57/272 (21%) and parasites in 5/272  
168 (2%). In 42/272 cases (15%),  $\geq 2$  different types of copathogens were involved. The descriptions of  
169 the pathogens involved in coinfections are presented in Table 2 and supplementary Table 2. The  
170 proportion of BAL performed in lower respiratory tract IA with or without coinfection was similar  
171 (72% (n=184/257) and 67% (n=265/394) respectively; p=0.26).

172 We stratified our data in two periods (<2009 and  $\geq 2009$ ), corresponding to the implementation of  
173 multiplex respiratory virus PCR. After 2009, 158 coinfections in 347 (46%) IA patients were



174 documented compared to 114 in 343 (33%) IA before 2009 ( $p < 0.01$ ) (Supplementary Table 1). Viral  
175 coinfections were more frequently diagnosed after 2009 ( $n = 66/347$ ; 19%) compared to before  
176 ( $n = 22/343$ ; 6%) ( $p < 0.01$ ) whereas no differences were observed for other type of pathogens. Due  
177 to these differences, the diagnosis period ( $< 2009$  versus  $\geq 2009$ ) was entered in the multivariable  
178 analysis.

179

### 180 **3) Factors associated with a coinfection**

181 Univariable analysis results appear in Table 1. In multivariable analysis, factors associated with  
182 more coinfections were alloHSCT, other HM, the group of other underlying diseases, presence of  
183 non-nodular lesions on CT, low lymphocyte count, high C-reactive protein levels, fever, tracheal  
184 intubation and isolation of  $\geq 2$  different species of *Aspergillus*. (Table 3). Age  $> 65$  years was  
185 associated with a lower rate of coinfection.

186

### 187 **4) Impact of coinfections on survival**

188 Survival in the global population was 56% ( $n = 306/690$  deaths) at week 12 and 35% ( $n = 446/690$   
189 deaths) at 1 year (Figure 1). Twelve-week survival rate was 46% ( $n = 146/272$  deaths) in patients  
190 with a coinfection compared to 62% ( $n = 160/418$  deaths) in patients without coinfection (Figure 2).  
191 In univariable analysis, coinfection with bacteria (HR 1.4, 95% CI [1.02–1.9], fungi (HR 1.8 [1.2–2.6],  
192 parasites (HR 5.8 [2.8–12.1]) or  $\geq 2$  types of copathogens (HR 2.9 [2.0–4.2]), but not viral  
193 coinfection (HR 1.0 [0.7–1.6]), had higher risk of death compared to the absence of coinfection.  
194 Pulmonary (HR 1.4 [1.03–1.8]), bloodstream (HR 1.6 [1.1–2.3]) and other/multiple sites (HR 2.2  
195 [1.6–3.0]) were also associated with an increased risk of death compared to no coinfection. In  
196 multivariable analysis, coinfection was independently associated with a higher twelve-week

197 mortality (adjusted HR 1.5 [1.1–1.9],  $p < 0.01$ ). Other factors associated with mortality are in  
198 Supplementary Table 3.

199

## 200 **Discussion**

201 We described a large case series of putative, probable, or proven IA over 21 years in a tertiary care  
202 centre. We documented a high rate (39%) of coinfections with a broad spectrum of copathogens.  
203 Coinfections were associated with increased mortality at week 12. We identified factors  
204 associated with coinfections.

205

206 This study describes one of the largest monocentric cohort of IA ever published [17-19]. Our  
207 analysis provides real-life data in non-selected patients with mycological documentation of IA.  
208 Underlying conditions predisposing to IA cover a large spectrum of diseases, broader than usually  
209 reported in randomised clinical trials which focus more on specific subpopulations [1, 20-22]. As  
210 expected, HM and HSCT or SOT are predominant conditions predisposing to IA. However, we also  
211 identified cases in patients with non-classical risk factors such as systemic autoimmune or  
212 inflammatory diseases, liver failure, trauma, surgery, chronic pulmonary disease, aspiration  
213 pneumonitis, near-drowning, and prematurity. More than half of these latter patients have  
214 received steroids or other immunosuppressive drugs or were neutropenic. Distribution of  
215 underlying conditions are in accordance with the multicentre surveillance study conducted  
216 between 2005 and 2007 in France with, however, a higher proportion of SOT in our cohort due to  
217 the large SOT activity in Strasbourg [23].

218

219 We identified that 39% of IA cases in this global population had concomitant coinfections. BAL was  
220 performed in 69% of the patients with a lower respiratory tract involvement. This rate is high

221 compared to published data and facilitated the identification of pulmonary coinfections in our  
222 cohort [24, 25]. These data confirm the critical role of BAL in the investigation of pulmonary CT  
223 abnormalities in immunocompromised patients: it improved the accurate diagnosis rate of  
224 pulmonary IA with one in five cases diagnosed by BALF analysis only and identified copathogens.

225

226 Our results on the prevalence of coinfections are in concordance with those found in specific  
227 populations. Georgiadou *et al.* showed that 62 (49%) of the 126 patients with HM diseases had  
228 documented pulmonary coinfections [7]. Bacteria and viruses were also the most frequent  
229 copathogens in their cohort. Even higher rates of coinfections (65% and 70%) have been reported  
230 in two paediatric series IA [26, 27]. Two autopsy series respectively over a 15-year period (1989-  
231 2003) and 20-year period (1989-2008) have shown 42% and 46% of bacterial and fungal  
232 coinfections [28, 29]. The impact of influenza virus infections on both the occurrence of IA and the  
233 outcome has been recently highlighted [9, 10].

234

235 In our study, we have included all coinfections irrespective of their location. Although the type of  
236 coinfections and the copathogens were therefore heterogeneous, it reflects real-life and  
237 underlines the severity of immunosuppression in patients developing IA. The proportion of viruses  
238 increased after the use of multiplex PCR in respiratory samples, notably in BALF.

239

240 Interestingly, we were able to identify factors that were associated with coinfections and that may  
241 help practitioners to identify these coinfections. Among underlying diseases, alloHSCT, other HM  
242 and other underlying diseases were associated with increased rates of coinfection compared to  
243 acute leukaemia patients. The lower risk in acute leukaemia patients may be explained by the

244 environmental protective methods, antimicrobial prophylaxis and early empiric antibacterial  
245 therapy in these patients.

246

247 Age  $\geq 65$  years was a protective factor, which may be linked to less aggressive chemotherapy and  
248 lower rate of alloHSCT in elderly patients. BAL was performed at the same frequency in patients  
249 younger or older than 65 years (66% and 65% respectively). Paediatric studies reported rates of  
250 coinfection up to 70% also suggesting lower age is more frequently associated with multiple  
251 infections [27].

252

253 Among the other factors, tracheal intubation may be a gateway for multiple lung infections but  
254 also a marker of disease severity. Low lymphocyte count reflects immunosuppression and might  
255 favour the coinfections. The coinfections were more symptomatic with more frequent fever and  
256 higher C-reactive protein levels. Chest CT patterns were associated with coinfections and should  
257 be used as a red flag to suspect coinfections. Indeed, presence of non-nodular lesions such as  
258 consolidations, micronodules, alveolar or interstitial infiltrates were more often associated with  
259 coinfection than presence of nodules only.

260

261 A study conducted in Israel in HM patients with 21% of polymicrobial pneumonia, identified  
262 alloHSCT and graft versus host disease as risk factors for polymicrobial infections [8]. Polymicrobial  
263 infections were also more symptomatic with more dyspnoea, haemoptysis and required more  
264 respiratory support. Another study focusing on coinfections in HM patients did not find any  
265 difference in terms of subpopulation and radiological characteristics between patients with or  
266 without coinfections [7]. In IA patients with fever, high C-reactive protein and pulmonary CT

267 showing other lesions than nodule(s), we suggest to actively search for coinfections to avoid delay  
268 in treatments. BAL is the cornerstone for an appropriate identification of multiple pathogens.

269

270 Coinfections were independently associated with increased mortality in our study. Hospital  
271 mortality was also higher in the polymicrobial group (49 versus 19%,  $p < 0,001$ ) in the study  
272 conducted in Israel [8]. In 67 patients with HM admitted to an intensive care unit in France for  
273 acute respiratory failure related to IA, concomitant bacterial infections were documented in 24  
274 (36%) patients and were associated with higher mortality [30].

275

276 There are limitations in our study. The analysis is retrospective over 21 years, and despite our  
277 efforts to identify the IA cases, it is likely we have missed a few of them. Clinical practices in  
278 diagnosis, classification and treatment have changed over year. We added the period of diagnosis  
279 of IA ( $< 2009$  and  $\geq 2009$ ) in the multivariable analysis to limit this bias. The EORTC/MSG  
280 classification has been updated in 2019. We have therefore retrospectively validated the cases  
281 with current criteria, modified by the use of a galactomannan cut-off above 0.5 according to  
282 manufacturer's recommendations. While classification of putative, probable, proven IA is based on  
283 strict criteria, the discrimination between bacterial coinfection and colonization is not always easy.  
284 To reduce this risk, we only accepted results from quantitative cultures of BALF and endotracheal  
285 aspirate and applied a cut-off of  $10^4$  CFU/ml and  $10^5$  CFU/ml, respectively. These cut-offs provide a  
286 high specificity for community acquired, hospital acquired and ventilator associated pneumonia  
287 [14-16]. Higher mortality in patients with co-infection may be explained by multiple factors  
288 including the treatment of this coinfection. Unfortunately, we did not collect data on therapy of  
289 the coinfection to assess its appropriateness. Finally, the monocentric design of the study limits its

290 extrapolation as the incidence and the diagnostic procedures for IA and coinfection might change  
291 across different sites.

292

293 In conclusion, this analysis in a large and global cohort of patients with IA confirmed the high rate  
294 of coinfections and their poor outcome. We identified factors associated with coinfection that  
295 should act as warning for the clinician about this potential risk, to avoid treatment delay.

296

297 **Collaborators:**

298 Pietro Francesco Addeo, Dominique Astruc, Mathieu Baldacini, Karin Bilger, Marie-Pierrette  
299 Chenard, Olivier Collange, Tristan Degot, Nadia Dhif, Elise Dicop, Samira Fafi-Kremer, Luc-Matthieu  
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303 Michele Porzio, Julien Pottecher, Anne-Claude Roche, Maleka Schenck-Dhif, Cécile Sonntag, Elise  
304 Toussaint, Anne Zilliox.

305

306 **Author's contribution:**

307 François Danion, Céline Duval, Raoul Herbrecht: **conception and design of the work, acquisition,**  
308 **analysis, and interpretation of data; drafting of the work; final approval; agreement to be**  
309 **accountable**

310 François Séverac: **analysis and interpretation of data; drafting of the work; final approval;**  
311 **agreement to be accountable**

312 Julie Denis, Valérie Letscher-Bru, Marcela Sabou: **acquisition and interpretation of data; critical**  
313 **review of the work; final approval; agreement to be accountable**

314 Philippe Bachellier, Ermanno Candolfi, Vincent Castelain, Raphael Clere-Jehl, Laurence Dillenseger,  
315 Eric Epailly, Justine Gantzer, Blandine Guffroy, Yves Hansmann, Jean-Etienne Herbrecht, Pierre  
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317 Catherine Paillard, Benjamin Renaud-Picard, Francis Schneider, Morgane Solis, Charles Tacquard,  
318 Emilie Talagrand-Reboul, Francis Veillon, Marie-Pierre Ledoux, and Célestine Simand: **acquisition**  
319 **of data; critical review of the work; final approval; agreement to be accountable**

320

321 **Conflict of interest:**

322 François Danion reports personal fees from Gilead outside the submitted work.

323 Justine Gantzer reports personal fees from Chugai and Ipsen outside of the submitted work.

324 Blandine Guffroy reports personal fees from Shire, outside the submitted work.

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343

344

345



346 **Figure legends**

347

348 **Figure 1.**

349 Patient flow diagram

350

351

352 **Figure 2.**

353 Twelve-week probability of survival: (a) all patients; (b) according to the presence or not of a  
354 coinfection; (c) according to the type of copathogen; (d) according to the site of coinfection.

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**Table 1. Characteristics of patients with and without a coinfection and factors associated with the presence of a coinfection in univariate analysis**

	Coinfection		p value
	No (n=418)	Yes (n=272)	
Age, years, n (%)			<b>0.05</b>
≤40	66 (16%)	46 (17%)	
41-64	223 (53%)	165 (61%)	
≥65	129 (31%)	61 (22%)	
Sex, male, n (%)	271 (65%)	180 (66%)	0.72
Active or history of smoking, n (%)	206 (49%)	130 (48%)	0.58
Prior respiratory disease, n (%)	125 (30%)	79 (29%)	0.81
Primary underlying disease, n (%)			<b>&lt;0.01</b>
Acute leukaemia	135 (32%)	50 (18%)	
Allogeneic HSCT	52 (12%)	46 (17%)	
Autologous HSCT	24 (6%)	9 (3%)	
Other haematological malignancy <sup>a</sup>	87 (21%)	69 (25%)	
Immunologic or benign haematological disease <sup>b</sup>	23 (6%)	16 (6%)	
Solid organ transplantation	67 (16%)	49 (18%)	
Solid tumour	20 (5%)	16 (6%)	
Other <sup>c</sup>	10 (2%)	17 (6%)	
Diabetes mellitus, n (%)	84 (20%)	67 (25%)	<b>0.16</b>
T-cell immunosuppressant, n (%)	142 (34%)	96 (35%)	0.72
Systemic steroids, n (%)	207 (50%)	151 (56%)	<b>0.13</b>
Previous invasive aspergillosis episode, n (%)	26 (6%)	21 (8%)	0.44
Antifungal prophylaxis, n (%)	211 (50%)	108 (39%)	<b>&lt;0.01</b>
Antibiotics, n (%)	277 (66%)	182 (67%)	0.86
Fever ≥38.5, n (%)	305 (76%)	215 (83%)	<b>0.02</b>
Tracheal intubation, n (%)	38 (9%)	64 (24%)	<b>&lt;0.01</b>
Neutrophils <500/μl, n (%)	225 (54%)	122 (45%)	<b>0.02</b>
Lymphocytes (/μl), n (%)			<b>0.18</b>
<500	216 (52%)	158 (58%)	
500-4000	182 (44%)	99 (36%)	
>4000	20 (5%)	15 (6%)	
Monocytes <100/μl, n (%)	206 (49%)	123 (45%)	0.30
C-reactive protein >180 (mg/l), n (%)	77 (19%)	76 (29%)	<b>&lt;0.01</b>
Fibrinogen ≥5 (g/l), n (%)	242 (59%)	133 (50)	<b>0.03</b>
Creatinine clearance <50 (ml/min), n (%)	79 (19%)	69 (26%)	<b>0.05</b>
Chest CT, n (%)			<b>&lt;0.01</b>
Nodule(s) only	105 (25%)	33 (12%)	
Nodule(s) with other lesion(s) <sup>d</sup>	188 (45%)	141 (52%)	
Other lesion(s) <sup>d</sup> without nodule	92 (22%)	82 (30%)	
Extra-pulmonary invasive aspergillosis <sup>e</sup>	33 (8%)	16 (6%)	
Pleural effusion	166 (40%)	139 (51%)	<b>&lt;0.01</b>
Isolation of <i>Aspergillus species</i> , n (%)			<b>&lt;0.01</b>
None	207 (50%)	105 (39%)	
<i>Aspergillus fumigatus</i> complex	170 (41%)	127 (47%)	
Other <i>Aspergillus</i> sp.	28 (7%)	19 (7%)	
≥2 different <i>Aspergillus</i> sp.	13 (3%)	21 (8%)	
Galactomannan in serum			0.67
Positive	245 (59%)	150 (55%)	
Negative	166 (40%)	117 (43%)	
Not done	7 (2%)	5 (2%)	

**Table 1 (continued)**

Galactomannan in BALF			0.70
Positive	156 (37%)	110 (40%)	
Negative	81 (19%)	49 (18%)	
Not done	181 (43%)	113 (42%)	
Proven invasive aspergillosis, n (%)	69 (17%)	43 (16%)	0.81
Site of invasive aspergillosis, n (%)			0.23
Lower respiratory tract only <sup>e</sup>	360 (86%)	226 (83%)	
Other single sites	17 (4%)	11 (4%)	
Disseminated	41 (10%)	35 (13%)	
Diagnosis after the year 2009, (%)	189 (45%)	158 (58%)	<b>&lt;0.01</b>

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Abbreviations: BALF: bronchoalveolar lavage fluid; CT: computed tomography; HSCT: haematopoietic stem cell transplantation.

<sup>a</sup> Lymphoma, chronic lymphocytic leukaemia, multiple myeloma, chronic myeloproliferative neoplasm, non-transformed myelodysplastic syndrome.

<sup>b</sup> Systemic auto-immune or inflammatory diseases (13), aplastic anaemia (6), other auto-immune or toxic cytopenia (7), inherited immune deficiency (5), HIV infection (4), other acquired immune deficiency (2), macrophage activation syndrome (2).

<sup>c</sup> Trauma or post-surgery (7), liver failure (6), chronic pulmonary disease (5), aspiration pneumonitis (3), near-drowning (2), prematurity (2), diabetes (1), steroid therapy for chronic sinusitis (1).

<sup>d</sup> Other lesions included consolidations, ground-glass opacities or micronodules.

<sup>e</sup> Ten patients had only endoscopy-confirmed bronchial or tracheal lesions with normal chest CT.

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**Table 2. Copathogens and sites of the coinfections in 272 episodes of invasive aspergillosis**

<b>Characteristics</b>	<b>Number (%)</b>
<b>Sites of coinfections <sup>a</sup></b>	272 (100%)
Pulmonary only	131 (48%)
Bloodstream only	66 (24%)
Other or multiple sites	75 (28%)
<b>Types of coinfections <sup>a</sup></b>	272 (100%)
Bacterial coinfection only	110 (40%)
Viral coinfection only	58 (21%)
Fungal coinfection only	57 (21%)
Parasitic coinfection only	5 (2%)
≥2 types of coinfection	42 (15%)
<b>Pathogens involved in coinfections <sup>a</sup></b>	378 (100%)
<b>Bacteria</b>	178 (47%)
<i>Staphylococcus aureus</i>	17 (4%)
Coagulase-negative staphylococci	26 (7%)
<i>Streptococcus</i> and <i>Enterococcus</i>	28 (7%)
<i>Escherichia coli</i>	27 (7%)
<i>Klebsiella pneumoniae</i>	11 (3%)
Other enterobacteria	18 (5%)
<i>Pseudomonas aeruginosa</i>	23 (6%)
Other non-fermenting Gram-negative bacilli	9 (2%)
Other bacteria	13 (3%)
Mycobacteria	6 (2%)
<b>Viruses</b>	101 (27%)
Influenza virus	24 (6%)
Other respiratory viruses	33 (9%)
Cytomegalovirus	12 (3%)
Herpes simplex virus	21 (6%)
Other	11 (3%)
<b>Fungi</b>	89 (23%)
<i>Candida</i> spp.	26 (7%)
<i>Cryptococcus</i> spp.	4 (1%)
Other yeasts	2 (1%)
Mucorales	25 (7%)
<i>Fusarium</i> spp.	5 (1%)
<i>Scedosporium</i> spp.	4 (1%)
Other filamentous fungi	8 (2%)
<i>Pneumocystis jirovecii</i>	15 (4%)
<b>Parasites</b>	10 (3%)
<i>Toxoplasma gondii</i>	8 (2%)
<i>Plasmodium falciparum</i>	2 (1%)

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<sup>a</sup> A patient could have more than one copathogen, more than one site of coinfection and more than one species of bacteria, virus or fungus.

378 **Table 3. Multivariable analysis of factors associated with coinfection**

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	OR	IC95	p value
Age (years)			
≤40	ref		
41-64	0.99	0.59–1.67	0.984
≥65	<b>0.47</b>	<b>0.25–0.90</b>	<b>0.022</b>
Underlying disease			
Acute leukaemia	ref		
<b>Allogeneic HSCT</b>	<b>2.30</b>	<b>1.21–4.37</b>	<b>0.011</b>
Autologous HSCT	1.10	0.44–2.78	0.836
<b>Other haematological malignancy</b>	<b>2.12</b>	<b>1.17–3.84</b>	<b>0.013</b>
Immunologic or benign haematological disease	1.95	0.78–4.84	0.153
Solid organ transplantation	1.04	0.45–2.37	0.929
Solid tumour	1.03	0.42–2.54	0.949
<b>Other</b>	<b>4.28</b>	<b>1.35–13.55</b>	<b>0.013</b>
Diabetes mellitus	1.05	0.67–1.66	0.823
Systemic steroids	0.98	0.64–1.49	0.918
Antifungal prophylaxis	0.77	0.50–1.17	0.217
<b>Fever ≥38.5</b>	<b>2.45</b>	<b>1.45–4.12</b>	<b>&lt;0.001</b>
<b>Tracheal intubation</b>	<b>2.64</b>	<b>1.47–4.72</b>	<b>0.001</b>
Neutrophils <500/μl	0.82	0.51–1.34	0.434
Lymphocytes (/μl)			
500-4000	ref		
<b>&lt;500</b>	<b>1.67</b>	<b>1.11–2.50</b>	<b>0.014</b>
>4000	1.74	0.73–4.17	0.210
<b>C-reactive protein &gt;180 mg/l</b>	<b>1.90</b>	<b>1.21–2.97</b>	<b>0.005</b>
Fibrinogen ≥5 g/l	0.77	0.52–1.13	0.182
Creatinine clearance <50 ml/min	1.16	0.72–1.87	0.548
Chest CT-scan			
Nodule(s) only	ref		
<b>Nodule(s) with other lesion(s)</b>	<b>2.23</b>	<b>1.34–3.71</b>	<b>0.002</b>
<b>Other lesion(s) without nodule</b>	<b>2.22</b>	<b>1.25–3.96</b>	<b>0.007</b>
Extra-pulmonary invasive aspergillosis	1.60	0.65–3.99	0.309
Pleural effusion	1.19	0.81–1.74	0.388
Isolation of <i>Aspergillus species</i>			
None	ref		
<i>Aspergillus fumigatus complex</i>	1.08	0.71–1.65	0.720
Other <i>Aspergillus sp.</i>	1.33	0.62–2.83	0.465
<b>≥2 different <i>Aspergillus sp.</i></b>	<b>2.67</b>	<b>1.12–6.35</b>	<b>0.026</b>
<b>Diagnosis after the year 2009</b>	<b>1.88</b>	<b>1.26–2.80</b>	<b>0.002</b>

380 Abbreviations: Abbreviations: BAL: bronchoalveolar lavage; CT: computed tomography; HSCT:

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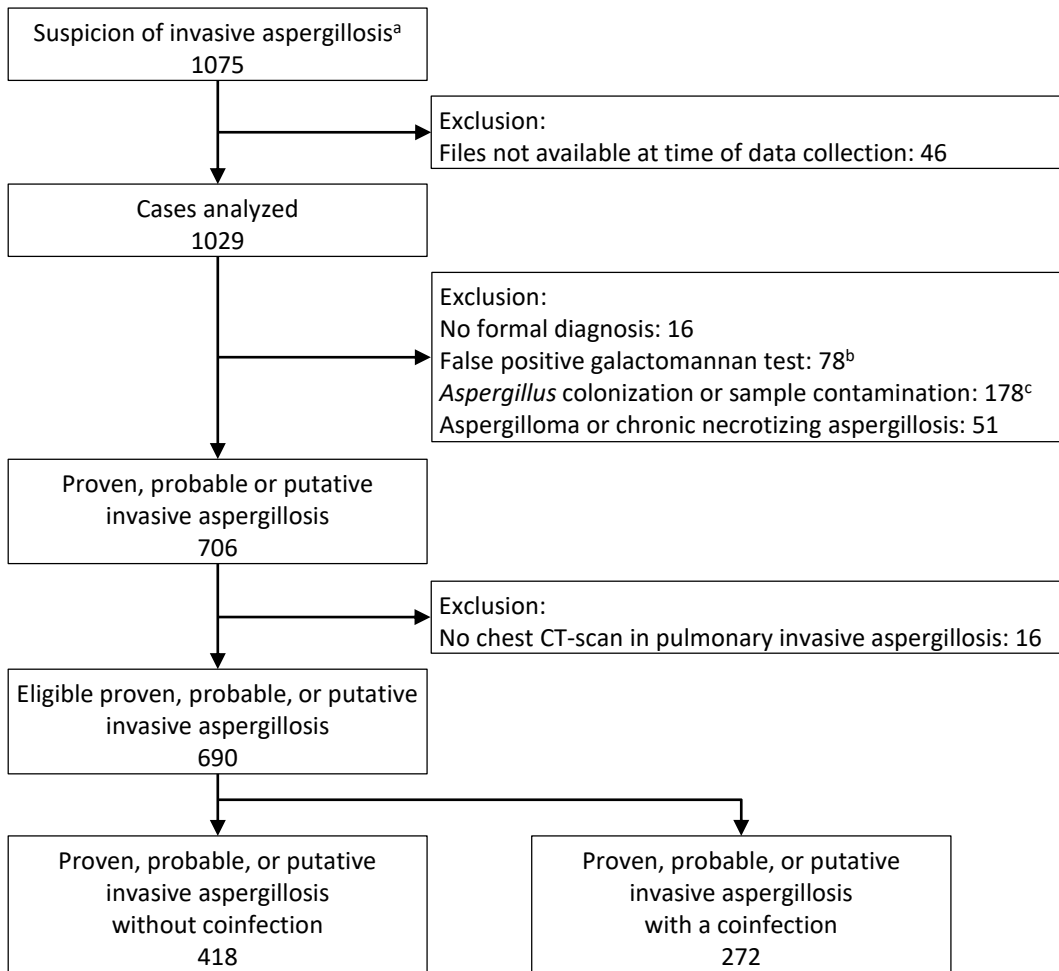
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385 References

386

- 387 1 Maertens JA, Raad, II, Marr KA, et al. Isavuconazole versus voriconazole for primary  
388 treatment of invasive mould disease caused by aspergillus and other filamentous fungi  
389 (secure): A phase 3, randomised-controlled, non-inferiority trial. *Lancet*. 2016; 387: 760-  
390 769.
- 391 2 Herbrecht R, Patterson TF, Slavin MA, et al. Application of the 2008 definitions for  
392 invasive fungal diseases to the trial comparing voriconazole versus amphotericin b for  
393 therapy of invasive aspergillosis: A collaborative study of the Mycoses Study Group (MSG  
394 05) and the European Organization for Research and Treatment of Cancer Infectious  
395 Diseases Group. *Clin Infect Dis*. 2015; 60: 713-720.
- 396 3 Ledoux MP, Guffroy B, Nivoix Y, Simand C, Herbrecht R. Invasive pulmonary  
397 aspergillosis. *Semin Respir Crit Care Med*. 2020; 41: 80-98.
- 398 4 Chai LY, Kullberg BJ, Johnson EM, et al. Early serum galactomannan trend as a predictor  
399 of outcome of invasive aspergillosis. *J Clin Microbiol*. 2012; 50: 2330-2336.
- 400 5 Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of  
401 galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive  
402 aspergillosis: Variables that affect performance. *J Infect Dis*. 2004; 190: 641-649.
- 403 6 Herbrecht R, Guffroy B, Danion F, Venkatasamy A, Simand C, Ledoux MP. Validation by  
404 real-life data of the new radiological criteria of the revised and updated consensus definition  
405 for invasive fungal diseases. *Clin Infect Dis*. 2020.
- 406 7 Georgiadou SP, Kontoyiannis DP. Concurrent lung infections in patients with hematological  
407 malignancies and invasive pulmonary aspergillosis: How firm is the aspergillus diagnosis? *J*  
408 *Infect*. 2012; 65: 262-268.
- 409 8 Hardak E, Avivi I, Berkun L, et al. Polymicrobial pulmonary infection in patients with  
410 hematological malignancies: Prevalence, co-pathogens, course and outcome. *Infection*.  
411 2016; 44: 491-497.
- 412 9 Schauwvlieghe A, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients admitted  
413 to the intensive care unit with severe influenza: A retrospective cohort study. *Lancet Respir*  
414 *Med*. 2018; 6: 782-792.
- 415 10 van de Veerdonk FL, Kolwijck E, Lestrade PP, et al. Influenza-associated aspergillosis in  
416 critically ill patients. *Am J Respir Crit Care Med*. 2017; 196: 524-527.
- 417 11 Nivoix Y, Velten M, Letscher-Bru V, et al. Factors associated with overall and attributable  
418 mortality in invasive aspergillosis. *Clin Infect Dis*. 2008; 47: 1176-1184.
- 419 12 Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus  
420 definitions of invasive fungal disease from the European Organization for Research and  
421 Treatment of Cancer and the Mycoses Study Group Education and Research Consortium.  
422 *Clin Infect Dis*. 2019.
- 423 13 Blot SI, Taccone FS, Van den Abeele AM, et al. A clinical algorithm to diagnose invasive  
424 pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med*. 2012; 186: 56-  
425 64.
- 426 14 Baselski V, Klutts JS, Baselski V, Klutts JS. Quantitative cultures of bronchoscopically  
427 obtained specimens should be performed for optimal management of ventilator-associated  
428 pneumonia. *J Clin Microbiol*. 2013; 51: 740-744.
- 429 15 Rasmussen TR, Korsgaard J, Moller JK, Sommer T, Kilian M. Quantitative culture of  
430 bronchoalveolar lavage fluid in community-acquired lower respiratory tract infections.  
431 *Respir Med*. 2001; 95: 885-890.
- 432 16 Torres A, Niederman MS, Chastre J, et al. International ERS/ESICM/ESCMID/ALAT  
433 guidelines for the management of hospital-acquired pneumonia and ventilator-associated  
434 pneumonia. *Eur Respir J*. 2017; 50.

- 435 17 Ramos ER, Jiang Y, Hachem R, Kassis C, Kontoyiannis DP, Raad I. Outcome analysis of  
436 invasive aspergillosis in hematologic malignancy and hematopoietic stem cell transplant  
437 patients: The role of novel antimold azoles. *Oncologist*. 2011; 16: 1049-1060.
- 438 18 Parody R, Martino R, Sanchez F, Subira M, Hidalgo A, Sierra J. Predicting survival in  
439 adults with invasive aspergillosis during therapy for hematological malignancies or after  
440 hematopoietic stem cell transplantation: Single-center analysis and validation of the Seattle,  
441 French, and Strasbourg prognostic indexes. *Am J Hematol*. 2009; 84: 571-578.
- 442 19 Contou D, Dorison M, Rosman J, et al. Aspergillus-positive lower respiratory tract samples  
443 in patients with the acute respiratory distress syndrome: A 10-year retrospective study. *Ann  
444 Intensive Care*. 2016; 6: 52.
- 445 20 Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for  
446 primary therapy of invasive aspergillosis. *N Engl J Med*. 2002; 347: 408-415.
- 447 21 Cornely OA, Maertens J, Bresnik M, et al. Efficacy outcomes in a randomised trial of  
448 liposomal amphotericin B based on revised EORTC/MSG 2008 definitions of invasive  
449 mould disease. *Mycoses*. 2011; 54: e449-455.
- 450 22 Marr KA, Schlamm HT, Herbrecht R, et al. Combination antifungal therapy for invasive  
451 aspergillosis: A randomized trial. *Ann Intern Med*. 2015; 162: 81-89.
- 452 23 Lortholary O, Gangneux JP, Sitbon K, et al. Epidemiological trends in invasive aspergillosis  
453 in France: The SAIF network (2005-2007). *Clin Microbiol Infect*. 2011; 17: 1882-1889.
- 454 24 Lopez-Medrano F, Silva JT, Fernandez-Ruiz M, et al. Risk factors associated with early  
455 invasive pulmonary aspergillosis in kidney transplant recipients: Results from a  
456 multinational matched case-control study. *Am J Transplant*. 2016; 16: 2148-2157.
- 457 25 Racil Z, Weinbergerova B, Kocmanova I, et al. Invasive aspergillosis in patients with  
458 hematological malignancies in the Czech and Slovak republics: Fungal Infection Database  
459 (FIND) analysis, 2005-2009. *Int J Infect Dis*. 2013; 17: e101-109.
- 460 26 Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: A multicenter  
461 retrospective analysis of 139 contemporary cases. *Pediatrics*. 2008; 121: e1286-1294.
- 462 27 Abbasi S, Shenep JL, Hughes WT, Flynn PM. Aspergillosis in children with cancer: A 34-  
463 year experience. *Clin Infect Dis*. 1999; 29: 1210-1219.
- 464 28 Chamilos G, Luna M, Lewis RE, et al. Invasive fungal infections in patients with  
465 hematologic malignancies in a tertiary care cancer center: An autopsy study over a 15-year  
466 period (1989-2003). *Haematologica*. 2006; 91: 986-989.
- 467 29 Lewis RE, Cahyame-Zuniga L, Leventakos K, et al. Epidemiology and sites of involvement  
468 of invasive fungal infections in patients with haematological malignancies: A 20-year  
469 autopsy study. *Mycoses*. 2013; 56: 638-645.
- 470 30 Burghi G, Lemiale V, Seguin A, et al. Outcomes of mechanically ventilated hematology  
471 patients with invasive pulmonary aspergillosis. *Intensive Care Med*. 2011; 37: 1605-1612.
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<sup>a</sup> based on a positive culture, galactomannan in serum, bronchoalveolar lavage fluid or cerebrospinal fluid, or PCR in serum, bronchoalveolar lavage fluid or tissue and at least one of the following condition : proven aspergillosis, presence of an host factor as defined by the EORTC /MSG criteria or hospitalized in an intensive care unit.

<sup>b</sup> False positive galactomannan in serum (n=71) or in bronchoalveolar lavage fluid (n=7).

<sup>c</sup> 185 species in 178 patients.



