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Abstract

Introduction

Invasive aspergillosis (IA) is a fungal infection that particularly affects immunocompromised hosts. Recently, several reports have indicated a high incidence of IA in intensive care unit (ICU) patients. However, few data are available on the epidemiology and outcome of patients with IA in this setting.

Methods

An observational study including all patients with a positive *Aspergillus* culture during ICU stay was performed in 30 ICUs in eight countries. Cases were classified as proven IA, putative IA or *Aspergillus* colonization according to recently validated criteria. Demographics, microbiological and diagnostic data were collected. Outcome was recorded 12 weeks after *Aspergillus* isolation.

Results

A total of 563 patients were included of whom 266 were colonized (47%), 203 had putative IA (36%) and 94 proven IA (17%). The lung was the most frequent site of infection (94%) and *Aspergillus fumigatus* the most commonly isolated species (92%). Patients with IA had more incidences of cancer and organ transplantation than those with colonization; they were also more frequently diagnosed with sepsis on ICU admission and more frequently received vasopressors and renal replacement therapy (RRT) during the ICU stay than other patients. Mortality was 38% among colonized patients, 67% in those with putative IA and 79% in proven IA (P <0.001). Independent risk factors for death among patients with IA included older age, history of bone marrow transplantation, and mechanical ventilation, RRT and higher Sequential Organ Failure Assessment (SOFA) scores at diagnosis.

Conclusions

IA among critically ill patients is associated with high mortality. Patients diagnosed with proven or putative IA had greater severity of illness and more frequently needed organ support than those with *Aspergillus* spp colonization.

Introduction

Invasive aspergillosis (IA) is a serious opportunistic infection that mainly affects immunocompromised patients, such as those with prolonged neutropenia and cancer [1]. As such, most research on the epidemiology and clinical impact of *Aspergillus* spp. infection has been conducted in patients with hematological malignancies or after stem cell and solid organ transplantation [2,3]. However, several reports have shown that *Aspergillus* spp. can cause invasive disease in other categories of patients, including those admitted to intensive care units (ICUs) [4-8]. In this setting, clinical diagnosis of IA is a real challenge, as standard diagnostic definitions were developed and have been validated only for patients with cancer or after hematopoietic stem cell transplants and cannot necessarily be extrapolated to critically ill patients, who lack specific host factors as defined by the European Organization for Research and Treatment of Cancer (EORTC) [9].

Although IA has been considered a rare condition among critically ill patients [10-12], recent data indicate that it should be reconsidered as an emerging and devastating infectious disease in this population. Indeed, in an 18-month surveillance program involving 18 Italian ICUs, only 12 cases of IA occurred in 5561 patients (0.2%); mortality among these patients was 60% [13]. Also, another study reported 7% of patients with IA, adding up to a mortality rate of 91%; interestingly, 70% of these patients had no predisposing factors for invasive fungal disease [14]. Moreover, in ICU patients, IA may potentially affect multiple organs, evolving into a disseminated disease, which remains largely under-diagnosed and is associated with poor outcome [15].

In clinical practice, a diagnosis of IA is frequently suspected when *Aspergillus* is isolated from non-sterile body sites, particularly tracheal and bronchial aspirates [16]. However, since *Aspergillus* spp. are ubiquitous, one must be cautious in ascribing a pathogenic role to the fungus obtained from these samples. Moreover, the impact of *Aspergillus* spp. isolates from respiratory cultures on the occurrence of IA has been extensively studied in immunocompromised patients but little is known about this effect in other populations,

including ICU patients [17,18]. The presence of other risk factors, such as chronic lung and liver disease or general debilitation, may strengthen the likely clinical relevance of a positive *Aspergillus* culture [15]; nevertheless, invasive diagnostic procedures, such as lung biopsy, which are necessary to confirm a diagnosis of *Aspergillus* infection, are often not feasible in patients with severe respiratory insufficiency and critical illness [19-21]. Moreover, non-invasive diagnostic tests, such as galactomannan (GM) determination, have been integrated into a diagnostic algorithm that has been validated only for patients with bone marrow transplant and cancer, and needs to be further studied in ICU patients [5,22].

The aim of this study was, therefore, to collect data from a large series of ICU patients with either *Aspergillus* colonization or invasive disease, in order to investigate the epidemiology of IA in this population.

Material and methods

Patients and setting

This was an international, multicenter (n = 30) observational study of ICU patients with evidence of either *Aspergillus* colonization or IA (*Asp*ICU Study). All consecutive adult (>18 years) ICU patients with a culture and/or direct examination and/or histopathological sample positive for *Aspergillus* spp. at any site between January 2000 and January 2011 were eligible for inclusion. Patients with a *post-mortem* diagnosis of IA were also eligible. Data were collected prospectively. However, because we anticipated a relative paucity of patients in which histopathology data were available we accepted patients from historical cohorts (as from January 2000) on the condition that none of the requested data was missing. Clinical suspicion of IA prior to ICU admission was an exclusion criterion. The study was approved by the local ethics committee/institutional review board of each participating center (Appendix 1) and, because of the observational nature of the study and the lack of any modification in the general management of these patients, the need for informed consent was waived. A complete and detailed description of the study methodology has been reported elsewhere [23].

Data collection and outcomes

Data were collected from patient medical records and submitted via a web-based registration system [24]. Collected patient data included: demographics (age, weight, height, sex); underlying diseases; and acute illness severity scores, including Acute Physiology And Chronic Health Evaluation (APACHE) II score on admission [25] and Sequential Organ Failure Assessment (SOFA) score [26] on the day of the positive *Aspergillus* culture. Acute respiratory distress syndrome (ARDS) was defined according to the 1994 Consensus Conference criteria [27]. Sepsis was defined according to standard criteria [28]. We also collected clinical data, including signs compatible with invasive fungal disease (i.e. refractory or recrudescent fever, pleuritic chest pain or rub, dyspnea, hemoptysis or worsening lung function). Sampling techniques and sites, as well as mycological tests (including GM measurements and *Aspergillus* PCR, whenever available) to support a diagnosis of IA, were recorded. Test results were interpreted as positive if they met international consensus criteria [5,9,29]; in particular, GM was considered abnormal with an optical density index >0.5. Organs affected by *Aspergillus* and species identification were recorded. Radiologic data included findings from chest X-rays or computed tomography (CT) scans from involved

organs (chest, sinuses, abdomen and central nervous system). Findings on chest CT-scan suggestive of invasive fungal disease were defined as "typical" with at least one of the following: wedge-shaped lesion, halo or air-crescent sign, lung cavitation or nodule [9].

The time of diagnosis of IA was considered as the date of the first positive *Aspergillus* culture or as the date of clinical deterioration compatible with fungal disease in case of *post-mortem* diagnosis. Although the "clinical" diagnosis of IA was reported in the database according to the judgment of the attending physicians, the final diagnosis was obtained using a central adjudication committee for the various diagnostic categories according to (a) the EORTC/Mycosis Study Group (MSG) (proven, probable, possible invasive aspergillosis or not-classifiable) criteria [9] and (b) "validated" criteria to discriminate *Aspergillus* colonization from IA in critically ill patients (putative or proven IA; Appendix 2) [23]. Among those patients with putative or proven IA, we separately analyzed those who had EORTC host-factors for immunosuppression (i.e. recent history of neutropenia (<500 neutrophils/mm^3); receipt of an allogeneic stem cell transplant; prolonged use of corticosteroids at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for 13 weeks; treatment with other recognized T-cell immunosuppressants; inherited severe immunodeficiency) [9] from others. Data were also collected on antifungal therapy and its duration as well as outcomes, including length of ICU stay, 12-week survival rates and use of mechanical ventilation and/or renal replacement therapy (RRT) or vasopressor agents, both at the time of the first *Aspergillus*-positive culture and during the ICU stay.

Statistical analysis

Statistical analyses were performed using the SPSS 18.0 for Windows NT software package (SPSS Inc. 2004, Chicago, IL, USA). Descriptive statistics were computed for all study variables; discrete variables are expressed as counts (percentage) and continuous variables as mean ± standard deviation or median [1^{st}–3^{rd} quartile]. Differences between the three study groups were assessed using chi-square test, Fisher's exact test, Student's t-test, or Mann–Whitney U test, as appropriate. A time-to-death analysis was performed until 84 days (12 weeks) after the first positive culture for *Aspergillus*. The Kaplan-Meier method with log-rank test was used to compare the survival curves of the three populations (colonization, putative IA and proven IA) over time. This long-rank test was then adjusted for all clinically relevant variables that could affect outcome (i.e., age; sex; medical admission; sepsis on admission; acute respiratory distress syndrome [ARDS] on admission; diagnostic category, APACHE II score on admission; bone marrow transplantation; comorbidities; SOFA score on the day of positive *Aspergillus* culture; lung involvement; comorbidities; EORTC criteria for IA; antifungal therapy; vasopressors; mechanical ventilation; RRT). The hazard ratio for death (HR) and 95% confidence intervals (CI) were also reported. Multivariable logistic regression analysis with mortality as the dependent variable was performed in patients with putative/proven IA; only variables associated with a higher risk of 12-week mortality ($p < 0.2$) on a univariate basis were introduced in the multivariate model. Co-linearity between variables was excluded prior to modeling. Odds ratios (OR) with 95% CI were computed. All tests were two-sided and a $p < 0.05$ was considered as statistically significant.

Results

Characteristics of the study cohort

A total of 563 patients were included in the study from 30 ICUs in eight countries (Belgium, France, Brazil, China, Spain, Greece, India and Portugal). Characteristics of the cohort are shown in Table 1. Most patients were medical admissions (n = 392, 70%). The most common reasons for ICU admission were respiratory (n = 222, 39%) and cardiovascular (n = 147, 26%) disease. The most common comorbid conditions were chronic obstructive pulmonary disease (COPD, n = 174, 31%) and diabetes (n = 92, 16%); 59 (11%) patients were receiving immunosuppressive therapy and 256 (45%) corticosteroids, with 168 (30%) on prolonged (>30 days) corticosteroid therapy. Sepsis and ARDS were diagnosed on admission in 222 (39%) and 57 (10%) patients, respectively. Fifty-six patients (10%) had undergone solid organ transplantation. Neutropenia was present in 40 patients (6%), but prolonged neutropenia (>10 days) in only 3 patients.

Table 1 Characteristics of the study cohort on ICU admission

	All patients (n = 563)	**Proven IA (n = 94)**	**Putative IA (n = 203)**	**Colonization (n = 266)**
Age (years)	61 ± 17	60 ± 13	62 ± 16	61 ± 18
Male, n (%)	341 (61)	54 (57)	127 (63)	160 (60)
BMI (kg/m^2)	24 [21–27]	24 [21–26]	23 [20–27]	25 [22–28]
Underlying conditions				
No underlying disease, n (%)	76 (14)	4 (4) *	11 (5) *	61 (23)
COPD, n (%)	174 (31)	22 (23) #	80 (39) *	72 (27)
Chronic heart failure, n (%)	55 (10)	8 (9)	19 (9)	28 (11)
Diabetes, n (%)	92 (16)	19 (19)	33 (16)	40 (15)
Solid tumor, n (%)	58 (10)	13 (14)	21 (9)	24 (9)
Hematologic cancer/BMT, n (%)	48 (8)	15 (16) *	31 (15) *	6 (2)
Neutropenia, n (%)	40 (7)	5 (5) *	18 (9) *	3 (1)
Radiotherapy/chemotherapy, n (%)	53 (9)	12 (13) *	33 (16) *	8 (3)
Solid organ transplantation, n (%)	56 (10)	19 (20) *	28 (14) *	9 (4)
Immunosuppressive drugs, n (%)	59 (11)	27 (29) * #	25 (12) *	7 (3)
HIV, n (%)	5 (1)	1 (1)	1 (1)	3 (1)
Liver disease, n (%)	40 (7)	13 (14) *	14 (7)	13 (5)
Chronic hemodialysis, n (%)	22 (4)	3 (3)	8 (4)	11 (4)
Smoking, n (%)	88 (16)	15 (16)	28 (14)	45 (17)
Alcohol abuse, n (%)	54 (10)	9 (10)	16 (8)	29 (11)
Diagnostic category				
Medical admission, n (%)	392 (70)	79 (84) * #	94 (46) *	152 (57)
Cardiovascular	147 (26)	29 (30)	49 (24)	69 (26)
Respiratory	222 (39)	38 (41) * #	107 (53) *	77 (29)
Neurological	32 (6)	3 (3) *	3 (2) *	26 (10)
Intoxication	5 (1)	0	1 (1)	4 (2)
Gastro-intestinal	37 (7)	6 (7)	13 (6)	18 (7)
Endocrine	18 (3)	8 (9) *	10 (5) *	0
Post-operative	48 (9)	7 (8)	15 (7)	26 (10)
Trauma	47 (8)	2 (2) *	3 (2) *	42 (16)
Others	7 (1)	1 (1)	2 (1)	4 (1)
Severity scores & Main diagnoses				
APACHE II score on admission	23 [17–28]	24 [18–28]	25 [17–30]	23 [17–28]

Sepsis on ICU admission	222 (39)	47 (51) *	94 (46) *	81 (30)
ARDS on ICU admission	57 (10)	24 (26) * #	21 (10) *	12 (4)
Septic shock, n (%)	64 (11)	14 (15) *	29 (14) *	21 (8)
Traumatic brain injury, n (%)	28 (5)	0 *	1 (1) *	27 (10)
Pneumonia, n (%)	72 (13)	15 (16)	29 (14)	28 (11)
Other brain injuries, n (%)	23 (4)	1 (1) *	2 (1) *	20 (8)
Acute heart failure/CABG, n (%)	39 (7)	3 (3) *	6 (3) *	30 (11)
Pancreatitis, n (%)	7 (1)	1 (1)	1 (1)	5 (1)
Prophylactic antifungals, n (%)	32 (6)	8 (9) *	19 (9) *	5 (2)
Antifungal therapy, n (%)	285 (51)	78 (85) * #	147 (72) *	60 (22)
Duration of therapy, days $	13 [6–28]	10 [4–24]	12 [7–29]	18 [9–28]

* $p < 0.05$ vs. Colonization; # $p < 0.05$ vs. Putative.

$ calculated among those patients on therapy.

BMI = body mass index; COPD = chronic obstructive pulmonary disease; BMT = bone marrow transplantation; HIV = human immunodeficiency virus; APACHE = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit; ARDS = acute respiratory distress syndrome; RRT = renal replacement therapy; CABG = coronary artery bypass graft.

From the cohort of 563 patients, 94 had proven IA (17%), 203 had putative IA (36%), and 266 were colonized (47%) when "validated" criteria were used. It would not have been possible to classify 438 (77%) of the patients if only the EORTC/MSG criteria had been used.

Aspergillus fumigatus was the most commonly isolated species (n = 519, 92%).

Clinical signs and medical imaging

Clinical and radiological findings are reported in Table 2. Chest computed tomography (CT) was performed in 223 (40%) patients, broncho-alveolar lavage (BAL) in 225 (40%) patients and serum or BAL GM measured in 151 (27%) patients. There were significantly more radiological findings typical of IA on chest CT-scan in patients with proven or putative IA than in those with colonization. CT-scan of the sinuses was performed in 24 patients and 15 of the scans were abnormal. Five of the 24 patients had sinus cultures positive for *Aspergillus*; all 5 had abnormal CT-scan findings, but 3 were diagnosed as colonization and 2 as putative IA. Abdominal CT-scan was performed in 38 patients and 24 of the scans were abnormal. Three of the 38 patients had positive *Aspergillus* cultures on abdominal samples (2 in hepatic and one in peritoneal samples); all had abnormal CT-scan findings and were diagnosed as proven IA. Cerebral CT-scan was performed in 45 patients and 30 of the scans were abnormal. Nine of the 45 patients had positive *Aspergillus* cultures on cerebral samples, either cerebrospinal fluid (CSF) or brain biopsy; all had abnormal CT-scan findings and were diagnosed as proven IA. One additional patient, in whom no cerebral CT was performed, had CSF cultures positive for *Aspergillus*.

Table 2 Clinical, radiological and microbiological findings related to *Aspergillus* diagnosis

	All patients (n = 563)	Proven IA (n = 94)	Putative IA (n = 203)	Colonization (n = 266)
ICU stay before first positive culture	4 [1–9]	4 [1–11]	4 [2–10]	4 [2–9]
SOFA II score on diagnosis	8 [4–12]	11 [7–14] * #	9 [6–12] *	5 [3–10]
Clinical signs				
At least of the following signs, n (%)	317 (56)	73 (77) *	141 (69) *	103 (39)
Refractory fever, n (%)	163 (29)	53 (57) * #	68 (34) *	42 (16)
Recrudescent fever, n (%)	18 (3)	4 (3)	7 (3)	7 (3)
Pleuritic chest pain, n (%)	18 (3)	5 (5)	8 (4)	5 (2)
Pleuritic rub, n (%)	6 (1)	3 (3)	0	3 (1)
Dyspnea, n (%)	257 (46)	45 (48) * #	129 (64)	83 (31)
Hemoptysis, n (%)	32 (6)	14 (15) * #	13 (6)	5 (2)
Worsening lung function, n (%)	219 (39)	61 (66) *	118 (58) *	40 (15)
Abnormal Radiologic Findings	487 (87)	94 (100) *	203 (100) *	191 (71)
Chest X-rays / CT-scan, n (%)	515 (91) / 223 (40)	86 (92) / 62 (67) * #	192 (95) / 96 (47) *	237 (89) / 65 (24)
Non-specific chest CT-scan findings	135/223	33/62 *	55/96 *	57/65
"Typical" chest CT-scan findings	84/223	29/62 *	41/96 *	4/65
Microbiological findings				
BAL/ETA, n (%)	477 (96)	79 (92)	182 (98)	216 (97)
BAL performed, n (%)	225 (40)	56 (60) *	112 (55) *	57 (21)
Positive GM, n/mes (%)	86/151 (57)	44/52 (84) *	31/37 (84) *	11/62 (18)
β-D-Glucan, n/mes (%)	3/6 (50)	0	3/5 (60)	0/1 (0)
PCR, n/mes (%)	4/4 (100)	0	3/3 (100)	1/1 (100)
Performed biopsy/autopsy, n (%)	72/61 (24)	93 (100) * #	15 (7)	25 (9)
Isolated species				
Aspergillus fumigatus, n (%)	519 (92)	83 (88)	182 (90)	254 (96)
Aspergillus flavus, n (%)	19 (3)	2 (2)	12 (6)	5 (2)
Aspergillus niger, n (%)	7 (1)	2 (2)	2 (1)	3 (1)
Others, n (%)	18 (3)	7 (8)	7 (3)	4 (2)
EORTC Host Factors				
EORTC host factor present on diagnosis, n (%)	249 (44)	65 (70) *	143 (70) *	41 (15)
Neutropenia, n (%)	34 (6)	8 (9) *	21 (10) *	5 (2)
Malignancy under cytotoxic therapy, n (%)	66 (12)	18 (19) *	37 (18) *	11 (4)
Glucocorticoid treatment, n (%)	256 (45)	59 (63) * #	156 (78) *	42 (15)
Inherited Severe Immunodeficiency, n (%)	11 (2)	3 (3)	6 (3)	0
Organ support at time of diagnosis				
Vasopressor therapy, n (%)	346 (61)	63 (67) *	138 (68) *	145 (55)
RRT, n (%)	155 (28)	48 (51) * #	61 (30) *	46 (18)
Mechanical ventilation, n (%)	482 (86)	92 (98) * #	177 (87)	214 (80)
Organ support during ICU stay				
Vasopressor therapy, n (%)	429 (76)	78 (83) *	168 (83) *	183 (69)
RRT, n (%)	182 (32)	57 (60) * #	71 (35) *	54 (21)
Mechanical ventilation, n (%)	506 (90)	86 (91)	187 (92)	233 (87)

* $p < 0.05$ vs. Colonization; # $p < 0.05$ vs. Putative.

ICU = intensive care unit; SOFA = Sepsis Organ Failure Assessment; CT = computed tomography; BAL = broncho-alveolar lavage; ETA = endotracheal aspirate; GM = galactomannan; PCR = polymerase chain reaction; EORTC = European Organization for Research and Treatment of Cancer; RRT = renal replacement therapy.

Affected site and diagnostic classification

The most commonly affected site was the lung/trachea (92%) (Figure 1). Only 16% of the patients with positive *Aspergillus* culture in the lung/trachea had proven IA, whereas almost all patients with positive cultures in the abdominal, brain and endovascular samples were diagnosed as proven IA. Two positive abdominal samples were considered as colonization as they originated from indwelling drains; the other 9 patients had liver (n = 5) or peritoneal (n = 4) involvement. Twenty-eight patients had more than one site affected (22 had two and 6 had three affected sites). Six patients with positive lung cultures were classified as "proven" because of non-pulmonary proven IA; 9/10 patients with brain involvement and 8/8 with endovascular IA had also pulmonary positive cultures. Three of the patients with positive abdominal cultures (i.e. proven disease) had also proven pulmonary IA. The seven patients with putative infection in the skin (n = 4) or the sinus (n = 3) were classified as such because of concomitant putative pulmonary localization; in particular, specific skin biopsy was not performed in any patient to confirm cutaneous aspergillosis, except in one (proven disease).

Figure 1 Number of sites affected by *Aspergillus* spp for the different diagnostic categories: PR = proven; PT = putative; CO = colonization – data are reported as number (%). In case of multiple sites being positive for *Aspergillus* spp, the patient has been counted more than once.

Patients with putative or proven IA were more likely to have hematological cancer and to have undergone bone marrow or solid organ transplantation than those with *Aspergillus* colonization, which likely explains the larger proportion of patients receiving chemotherapy, radiotherapy and immunosuppressive drugs in these groups. Patients with putative or proven IA were also more frequently admitted for a medical reason and were more likely to have a diagnosis of sepsis or ARDS on ICU admission. Although radiological findings were abnormal in all patients with proven or putative IA, no more than 40% had chest CT-scan findings that were considered "typical" of IA. Where available, serum or BAL GM measurements were abnormal in more than 80% of patients with proven or putative IA, compared to only 18% of patients with colonization. Patients with proven and putative IA had a higher SOFA score on the day of the first positive *Aspergillus* culture than colonized patients; these patients also had a greater requirement for mechanical ventilation, vasopressors or RRT during the ICU stay.

In patients with proven IA, COPD was less frequently observed than in putative IA patients; in addition, compared to patients with putative IA, patients with a proven diagnosis were more frequently treated with immunosuppressive drugs, more frequently admitted for medical reasons, had a greater incidence of respiratory diseases and ARDS on ICU admission, and higher SOFA scores on IA diagnosis. On the day of diagnosis, patients with proven IA had more compatible clinical signs (fever refractory to antibacterial therapy, dyspnea and hemoptysis) than those with putative IA, as well as more non-specific findings on CT-scan. Most of the extra-thoracic cases of *Aspergillus* were in patients with proven IA, on the basis of analysis of fluids from sterile body sites or biopsies. Finally, RRT therapy was more commonly used among patients with proven IA. Antifungal therapy was initiated in 285 (51%) patients; in 31 additional cases, therapy was not administered because either clinical decision or post-mortem diagnosis. The median time from positive *Aspergillus* culture to initiation of therapy was 2 [0–5] days for patients with *Aspergillus* colonization and 1 [0–3] days for both proven and putative IA.

Immunocompromised Patients

Among those patients with putative or proven IA, 208 (70%) had an immunosuppressive state, according to EORTC criteria (Table 3). Immunocompromised patients had similar severity of disease than others and presented more respiratory failure and fewer traumas as reasons for ICU admission. They also received more prophylactic antifungal therapy than others and were less likely to be on RRT on IA diagnosis.

Table 3 Main differences among patients with putative or proven IA, with regard to the presence of immunosuppression

	Immunosuppression (n = 208)	**No-Immunosuppression (n = 89)**	**p value**
Age (years)	61 ± 15	63 ± 15	0.15
Male, n (%)	127 (61)	53 (60)	0.92
BMI (kg/m^2)	23 [20–27]	24 [21–28]	0.77
Underlying conditions			
COPD, n (%)	67 (32)	35 (39)	0.23
Chronic heart failure, n (%)	14 (7)	13 (15)	0.06
Diabetes, n (%)	28 (14)	24 (27)	0.02
Liver disease, n (%)	16 (8)	11 (12)	0.19
Chronic hemodialysis, n (%)	6 (3)	5 (6)	0.25
Smoking, n (%)	24 (12)	19 (21)	0.03
Alcohol abuse, n (%)	12 (6)	15 (17)	0.002
Diagnostic category			
Medical admission, n (%)	170 (82)	69 (78)	0.08
Cardiovascular, n (%)	49 (24)	29 (33)	0.1
Respiratory, n (%)	112 (54)	33 (37)	0.008
Neurological, n (%)	4 (2)	2 (2)	0.85
Intoxication, n (%)	1 (1)	0	0.51
Gastro-intestinal, n (%)	11 (5)	8 (9)	0.23
Endocrine, n (%)	13 (6)	5 (6)	0.83
Post-operative, n (%)	18 (9)	4 (4)	0.21
Trauma, n (%)	0	5 (6)	0.006
Others, n (%)	2 (1)	1 (1)	0.89
Severity scores & Main diagnoses			
APACHE II score on admission	25 [17–30]	23 [17–28]	0.29
Sepsis on ICU admission	103 (50)	38 (42)	0.07
ARDS on ICU admission	33 (16)	22 (25)	0.07
Septic shock, n (%)	28 (13)	16 (18)	0.31
Traumatic brain injury, n (%)	1 (1)	0	0.51
Pneumonia, n (%)	27 (13)	17 (19)	0.17
Other brain injuries, n (%)	3 (1)	0	0.25
Acute heart failure/CABG, n (%)	1 (1)	8 (9)	<0.001
Pancreatitis, n (%)	2 (1)	0	0.35
Prophylactic antifungals, n (%)	24 (12)	3 (3)	0.03
Antifungal therapy, n (%)	159 (76)	66 (74)	0.67
Time from diagnosis to therapy, days	1 [0–3]	1 [0–4]	0.34
Duration of therapy, days [$]	11 [6–25]	14 [5–28]	0.83
ICU stay before first positive culture	4 [2–11]	4 [2–10]	0.76
SOFA II score on diagnosis	10 [6–13]	9 [6–12]	0.15
Organ support at time of diagnosis			
Vasopressor therapy, n (%)	139 (67)	62 (70)	0.63

RRT, n (%)	67 (32)	42 (47)	0.01
Mechanical ventilation, n (%)	188 (90)	81 (91)	0.87
Organ support during ICU stay			
Vasopressor therapy, n (%)	173 (83)	73 (82)	0.81
RRT, n (%)	83 (40)	45 (51)	0.09
Mechanical ventilation, n (%)	190 (91)	83 (93)	0.58
12-week Mortality, n (%)	151 (73)	59 (66)	0.27

BMI = body mass index; COPD = chronic obstructive pulmonary disease; APACHE = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit; ARDS = acute respiratory distress syndrome; RRT = renal replacement therapy; CABG = coronary artery bypass graft.

Outcomes

The length of ICU stay was similar in patients with proven IA, putative IA and colonization (15 [8-23,25-33] days vs. 17 [9-23,25-36] days vs. 14 [6-23,25-31] days, p = 0.07). Mortality at 12-weeks was significantly higher in patients with proven IA (74/94, 79%) than in those with putative IA (136/203, 67%, p = 0.03) or colonization (101/ 266, 38%, p < 0.001). Patients with pulmonary involvement had mortality rates of 55% (71% if proven IA, 68% if putative IA and 39% if *Aspergillus* colonization), which were similar to the rates in patients with skin/wound and sinus involvement (55% and 37%, respectively). The highest mortality rates were observed in patients with abdominal (81%), cerebral (90%) or endovascular (86%) involvement. Among patients with putative or proven IA, mortality was similar between those with EORTC criteria for immunosuppression and others (73% vs. 66%, p = 0.27). The use of antifungal therapy was not associated with differences in overall survival when compared to untreated patients. Also, the delay between the first positive *Aspergillus* culture and the initiation of antifungal therapy was not associated with outcome.

At 84 days, patients with putative and proven IA had significantly lower survival rates than those with colonization (Figure 2, log-rank p < 0.001). After adjustment for several confounders, this difference remained significant only for proven IA (Table 4). Among patients with proven or putative IA, independent risk factors for mortality were older age, bone marrow transplantation, a higher SOFA score and need for mechanical ventilation or RRT on the day of positive *Aspergillus* culture (Table 5).

Figure 2 Survival curves for different diagnostic categories, using the criteria of the clinical algorithm. Log-rank for survival distributions was p < 0.001 when putative or proven IA were compared to colonization. Log-rank for survival distribution between putative and proven IA was p = 0.156.

Table 4 Mortality risk of putative and proven invasive aspergillosis relative to *Aspergillus* colonization

	Unadjusted				**Adjusted**			
		95% CI for HR				**95% CI for HR**		
	HR	**Lower**	**Upper**	**p value**	**HR**	**Lower**	**Upper**	**p value**
Colonization	1.00				1.00			
Putative IA	2.18	1.68	2.83	< 0.001	1.18	0.93	1.81	0.16
Proven IA	3.12	2.30	4.24	< 0.001	1.51	1.00	2.26	0.05

HR = hazard ratio; CI = confidence interval.

Table 5 Risk factors for mortality among patients with proven or putative invasive aspergillosis (IA)

Variable	Univariable analysis		Multivariable analysis	
	P value	OR (95% CI)	*P* value	OR (95% CI)
Age, years	0.008	1.023 [1.006-1.040]	0.001	1.034 [1.014-1.055]
Male	0.264	0.751 [0.455-1.241]		
BMI	0.024	1.069 [1.009-1.133]		
Septic shock	0.689	1.158 [0.565-2.374]		
Pneumonia	0.880	0.948 [0.476-1.888]		
Primary brain injury	0.216	0.218 [0.020-2.437]		
Acute cardiac failure	0.225	3.655 [0.450-29.660]		
Sepsis on admission	0.110	1.503 [0.912-2.478]		
APACHE on admission	0.003	1.049 [1.017-1.083]		
Diabetes	0.576	1.211 [0.619-2.371]		
Chronic heart disease	0.030	3.890 [1.140-13.266]		
COPD	0.605	0.872 [0.520-1.463]		
Liver failure	0.070	2.749 [0.922-8.192]		
HIV	0.564	0.441 [0.027-7.132]		
Smoking	0.937	1.029 [0.509-2.078]		
Alcohol	0.298	0.639 [0.276-1.483]		
Chronic dialysis	0.148	4.615 [0.582-36.603]		
Bone marrow transplantation	0.013	3.875 [1.326-11.327]	0.039	3.352 [1.060-10.598]
Solid tumor	0.085	2.241 [0.894-5.617]		
Cancer	0.769	1.101 [0.579-2.094]		
Neutropenia	0.646	0.827 [0.368-1.858]		
Chemotherapy/radiotherapy	0.525	0.802 [0.405-1.586]		
Solid organ transplantation	0.877	1.055 [0.534-2.084]		
Corticosteroids	0.248	1.376 [0.801-2.366]		
Immune deficit	0.117	0.342 [0.090-1.306]		
Aspergillus species	0.040	2.183 [1.038-4.592]		
Lung involvement	0.998	1.001 [0.300-3.340]		
SOFA score at diagnosis	<0.001	1.180 [1.107-1.256]	<0.001	1.140 [1.062-1.224]
Vasopressor therapy at diagnosis	<0.001	4.309 [2.299-8.078]		
Mechanical ventilation at diagnosis	<0.001	6.498 [2.590-16.303]	0.009	3.916 [1.408-10.891]
Renal replacement therapy at diagnosis	<0.001	3.293 [1.895-5.722]	0.011	2.339 [1.212-4.516]

APACHE = Acute Physiology and Chronic Health Evaluation; SOFA = Sequential organ failure assessment; RRT = renal replacement therapy; COPD = chronic obstructive pulmonary disease; HIV = human immunodeficiency virus; BMI = body mass index; IA = Invasive aspergillosis.

Discussion

In this cohort of 563 patients with an *Aspergillus*-positive culture, 53% was diagnosed with putative or proven IA. Compared to *Aspergillus*-colonized patients, IA patients had more cancer, organ transplantation, sepsis/ARDS on admission, needed more supportive therapy, and had higher mortality. Patients with proven IA had more frequently medical admissions and higher SOFA scores than patients with putative IA, but outcomes were similar. Older age, bone marrow transplantation, higher SOFA scores and mechanical ventilation or RRT were independently associated with a poor outcome.

The true epidemiology of IA remains uncertain and depends on case-mix, environmental factors, and diagnostic strategy. In our cohort, clinical and radiological manifestations were non-specific and possibly masked by the underlying acute process. Although Barberan et al.

showed that radiological worsening or cavitation on chest X-ray/CT-scan was associated with IA [30], in another series up to 60% of cases of IA were exclusively diagnosed by autopsy because of lack of reliable clinical and radiological tests [7]. Moreover, in an ICU cohort, only 12 on 67 ICU patients with proven IA had a halo-sign or cavitation on chest CT-scan [14]. To overcome these limitations, BAL GM has been suggested, which, with a cut-off of 0.5, had 88% sensitivity and 87% specificity to detect IA, whereas sensitivity of serum GM was only 42% [5]. In our study, we found a higher proportion of proven and putative IA cases with positive GM levels (both 84%) when compared to only 18% in colonized patients. However, with GM in ICU patients being limited to suspected cases [6,30], use of a clinical algorithm to discriminate colonization from IA [23] remains a valid option for identifying patients for a more extensive work-up.

Few studies report on the epidemiology of IA in ICUs. In a large US cohort, ICU patients with aspergillosis had several comorbid diseases, high mortality, prolonged hospitalization, and increased costs [18]. Specifically, over 70% of patients needed ventilation and received high-dose corticosteroids; over 35% had acute renal failure, COPD or septic shock. In a multicenter Italian study, aspergillosis represented 35 of 384 invasive mycoses in ICU patients [31]. Previous corticosteroid administration for autoimmune disease or COPD was the major host factor associated with IA. IA occurred more frequently in medical patients and mortality attributed to aspergillosis was higher compared to candidiasis (63% vs. 46%, $p = 0.01$). These studies, however, only considered possible and proven IA. Petri et al. isolated *Aspergillus* in only 4% of 435 non-neutropenic ICU patients while none had IA [32]. Contrastingly, in the present study, *Aspergillus* isolation appeared to represent IA in over 50% of patients. Immunosuppression and sepsis/ARDS on ICU admission were more frequent in proven/putative IA than in colonization. A particular feature of this study was the finding that an alternative diagnostic algorithm in patients with *Aspergillus*-positive respiratory tract cultures encompassed a larger segment of the burden of IA than the stricter EORTC/MSG criteria [9]. Hence, identification of risk factors for IA may help determine which ICU patients would benefit from specific screening when an *Aspergillus*-positive culture is found. Another new finding is that we could describe the epidemiology of non-pulmonary *Aspergillus* cultures in ICU patients, which were predominantly associated with proven IA as affected organs were frequently considered sterile body sites such as the brain [33].

Pulmonary IA has been documented in 15% of bone marrow transplant patients [34]. Prolonged neutropenia or solid organ transplantation also have been identified as high-risk factors [34-37]. These conditions are rare in ICU patients. In one study, only 14% of ICU patients with IA were neutropenic [7]. Risk factors for IA in ICU patients include COPD, liver cirrhosis and severe sepsis [38,39]. In COPD patients, the incidence of *Aspergillus* isolates from lower respiratory tract samples has increased progressively [40]. In a study of 118 COPD patients, nearly 60% was colonized and 48 patients had IA [41]; IA patients were more likely to have advanced respiratory disease, as suggested by the Global initiative for Obstructive Lung Disease (GOLD) classification. IA patients had higher severity scores and had worse prognoses than *Aspergillus*-colonized patients. The emergence of IA in patients with COPD is mainly attributed to prolonged administration of corticosteroids [42]. Corticosteroids also represent a risk for IA in ICU patients receiving immunosuppression following solid organ transplant or autoimmune disease [14]. Corticosteroids predispose to opportunistic infections through quantitative and qualitative functional impairment of macrophages and neutrophil function [42]. Liver cirrhosis also unfavorably alters humoral and cellular immune response or complement activity, thereby increasing the risk for

infections [43]. Finally, sepsis-associated immune-regulatory disturbances, such as macrophage deactivation and altered cellular immune response, can induce a state of immunoparalysis hampering adequate host response to fungal disease [44]. Recently, IA was also associated with H1N1 infection, suggesting that viral infections may play a causal role [45]. Unfortunately, we did not collect data on H1N1 co-infection. Finally, we also found that higher SOFA scores and RRT requirement, was associated with IA.

We reported a 72% 12-week mortality in IA patients. In a study on medical ICU patients, mortality was 92% [46]; however, most of these patients experienced hematological disease or neutropenia, which may have influenced survival. In our study, a minority of patients had cancer and/or immunosuppressive therapies and our findings provide interesting data also on general ICU population. Mortality rates have improved in ICU patients developing IA. A recent study focusing on hospitalized patients with IA reported a crude mortality rate of 37% [47]. In another study, mortality for IA was 50% [6]. However, because IA often develops in patients with greater disease severity, it remains difficult to determine whether this fungal disease contributes *per se* to the poor outcome or if it is just a marker of disease severity. Khasawneh et al. reported a 15% greater mortality among ICU patients with IA than that predicted by APACHE II [6]; these authors also showed similar mortality rates in patients with colonization and those with invasive disease. Another study estimated the attributable mortality for IA to be 19% [48]. Both studies [6,48] were biased by the lack of a diagnostic approach to differentiate colonization from IA; also, the availability of more effective antifungal agents in the later study may have explained the differences. In our study, patients with putative and proven IA had significantly higher mortality rates than colonized patients; however, after adjustment for several confounders, this difference remained significant only for proven IA. Older age, bone marrow transplantation, higher SOFA scores, need for mechanical ventilation or RRT, predicted poor outcome in IA. These findings highlight the need for a more timely diagnostic approach to evaluate the effect of earlier initiation of therapy.

Our study has some limitations. First, we could not analyze a specific multimodal diagnostic approach as only a minority of patients had CT-scans and GM measurements. Moreover, we did not specifically collect whether GM measurement was performed in blood, BAL or other biological fluids sampling. The rare use of GM in the diagnostic algorithm could be related to the cost of the analysis and/or to its delayed implementation, as the study initiated before the publication of the most important papers dealing with GM assessment in critically ill patients [5,22]. Additionally, we only included patients with *Aspergillus*-positive culture, thereby excluding patients with suspected disease based on radiological imaging or biomarkers. Second, we did not assess reasons for death and cannot exclude that some patients died because of concomitant conditions. Third, we did not collect any data on the incidence of positive *Aspergillus* cultures on the total number of ICU admission to the participating centers over the study period; thus, we could not evaluate the burden of this disease among critically ill patients. Importantly, IA remains a rare disease and it was reported in 1-2% of ICU patients in a large international database [10]. Fourth, we did not record laboratory data in detail, which may have been useful to better characterize the cohort. We also did not collect data on daily corticosteroid dose and could not fully explore the impact of dose or duration on mortality. Importantly, we did not collect the amount of antibacterial drugs that patients received before IA diagnosis and whether this may have influenced on the development of the disease. Fifth, routine autopsy would have increased the number of cases of IA diagnosis; autopsy studies have shown that IA is the most frequently missed infectious

diagnosis in patients requiring ICU admission [15,49,50]. Sixth, we did not consider environmental sources of IA, such as construction works contaminating hospital air [51,52].

Seventh, a part of these data has already been included a previous publication; however, we focused on epidemiology findings while the previous study aimed to validate the diagnostic accuracy of a clinical algorithm. Finally, we did not report data on the differences among the drugs used to treat *Aspergillus* spp. in this cohort, although the drug of choice in non-neutropenic patients needs to be further studied [53].

Conclusions

Compared to *Aspergillus*-colonized patients, those with IA more frequently had sepsis or respiratory failure on admission, had more underlying conditions including immunocompromized status. IA patients had higher disease severity and needed more organ support. IA in the ICU is associated with an unacceptably high mortality rate.

Key messages

- In this multicentric cohort of ICU patients, half of patients with a positive *Aspergillus* culture had either putative or proven IA
- Patients with IA were more frequently immunosuppressed than those with colonization; however, sepsis and higher severity of illness were also more frequent
- Mortality in patients with IA was significantly higher than in those with *Aspergillus* colonization, even after adjustment for several risk factors.

Competing interests

This study received an unrestricted educational grant from Pfizer Belgium and a Research grant from Ghent University. SB holds a research mandate from Ghent University. The *Asp*ICU project was endorsed by the European Critical Care Research Network of the European Society of Intensive Care Medicine. There are no other conflicts of interest.

Authors' contributions

FST contributed to acquisition and analyses of data, interpretation of the results, drafted the manuscript and approved the final version. AMVDA contributed to acquisition of the data, interpretation of the results, revised the paper for important intellectual content, and approved the final version. PB contributed to acquisition of the data, interpretation of the results, revision the paper for important intellectual content, and approval of the final version. BM contributed to conception and design of the study, acquisition of the data, interpretation of the results, revision of the paper for important intellectual content and approval of the final version. WM contributed to acquisition of the data, interpretation of the results, revision the paper for important intellectual content, and approval of the final version. TC contributed to acquisition of the data, interpretation of the results, revision the paper for important intellectual content, and approval of the final version. MBN contributed to acquisition of the data, interpretation of the results, revision the paper for important intellectual content, and approval of the final version. JAP contributed to conception and design of the study, acquisition of the data, interpretation of the results, revision of the paper for important

intellectual content and approval of the final version. EDL contributed to acquisition of the data, interpretation of the results, revision the paper for important intellectual content, and approval of the final version. GD contributed to conception and design of the study, acquisition of the data, interpretation of the results, revision of the paper for important intellectual content and approval of the final version. JR contributed to conception and design of the study, acquisition of the data, interpretation of the results, revision of the paper for important intellectual content and approval of the final version. DV contributed to conception and design of the study, acquisition of the data, interpretation of the results, revision of the paper for important intellectual content and approval of the final version. SB contributed to conception and design of the study, analyses and interpretation of the data, drafted the manuscript and approved the final version.

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Appendix 1

List of ethics committees / institutional review board of the collaborating centers.

- Comité Ético de Investigación Clínica, Hospital Universitario Severo Ochoa (Madrid, Spain)
- Comité d'Ethique Médicale, Cliniques Universitaires U.C.L. de Mont-Godinne (Yvoir, Belgium)
- Comissão de Ética, Hospital de Santo Antonio (Porto, Portugal)
- Commission d'Ethique de la Société de Réanimation de Langue Francaise (France)
- Comité d'Ethique, Cliniques de l'Europe (Brussels, Belgium)
- Comité d'Ethique, Cliniques Universitaires Saint Luc (Brussels, Belgium)
- Medisch Ethische Commissie, H.-Hartziekenhuis Roeselaere-Menen (Roeselaere, Belgium),
- Ethics Committee of University Hospital Attikon (Athens, Greece)
- Comité d'Ethique, Centre Hôspitalier Régional Mons-Warquignies (Mons, Belgium),
- Research Ethics Committee, Shanghai Public Health Clinical Center, Fudan University (Shangai, China)
- Comité d'Ethique, Centre Hospitalier du Grand Hornu (Hornu, Belgium)
- Commissie Medische Ethiek, UZ Leuven (Leuven, Belgium)
- Comissão de Ética, Hospital de Sao Joao, (Porto, Portugal)
- Comissão de Ética do Santa Casa-Complexo Hospitalar (Porto Allegre, Brazil)
- Comité Ético de Investigación Clínica, Vall d'Hebron University Hospital (Barcelona, Spain)
- Ethics Committee, Apollo Hospital (Jubilee Hills Hyderabad, India)
- Comitè Ètic d'Investigació Clínica, Hospital Universitari de Tarragona Joan XXIII (Tarragona, Spain)
- Commissie Medische Ethiek, Reflectiegroep Biomedische Ethiek, Universitair Ziekenhuis Brussel (Brussels, Belgium)
- Comité d'Ethique, Hôpital Erasme (Brussels, Belgium)
- Commissie Medische Ethiek, AZ St. Lucas (Ghent, Belgium)
- Ethisch Comité, UZ Gent (Ghent, Belgium).

Appendix 2

The clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients [23].

Proven invasive pulmonary aspergillosis

Microscopic analysis on sterile material: histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or sterile biopsy in which hyphae are seen accompanied by evidence of associated tissue damage. Culture on sterile material: recovery of *Aspergillus* by culture of a specimen obtained by lung biopsy

Putative invasive pulmonary aspergillosis (all four criteria must be met)

1. *Aspergillus*-positive lower respiratory tract specimen culture (= entry criterion)

2. Compatible signs and symptoms (one of the following)
   - Fever refractory to at least 3 d of appropriate antibiotic therapy
   - Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause
   - Pleuritic chest pain
   - Pleuritic rub
   - Dyspnea
   - Hemoptysis
   - Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support
3. Abnormal medical imaging by portable chest X-ray or CT scan of the lungs
4. Either 4a or 4b

   4a Host risk factors (one of the following conditions)

   Neutropenia (absolute neutrophil count <500/mm^3) preceding or at the time of ICU admission

   Underlying hematological or oncological malignancy treated with cytotoxic agents

   Glucocorticoid treatment (prednisone equivalent > 20 mg/d)

   Congenital or acquired immunodeficiency

   4b Semi-quantitative *Aspergillus*-positive culture of BAL fluid (+ or ++), without bacterial growth together with a positive

   cytological smear showing branching hyphae

Aspergillus respiratory tract colonization

When >1 criterion necessary for a diagnosis of putative IPA is not met, the case is classified as *Aspergillus* colonization.

BAL = broncho-alveolar lavage; CT = computed tomography; ICU = intensive care unit.

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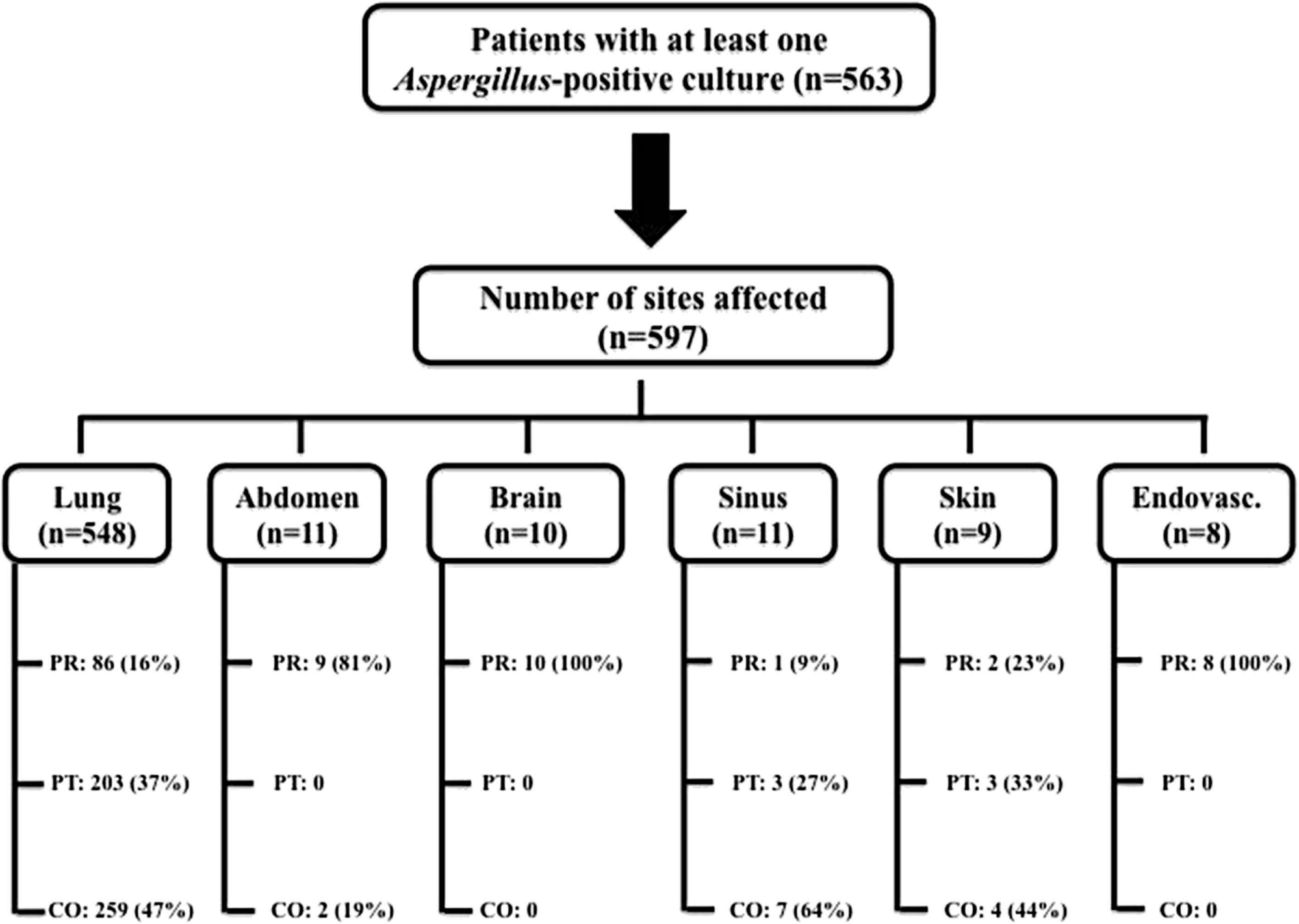
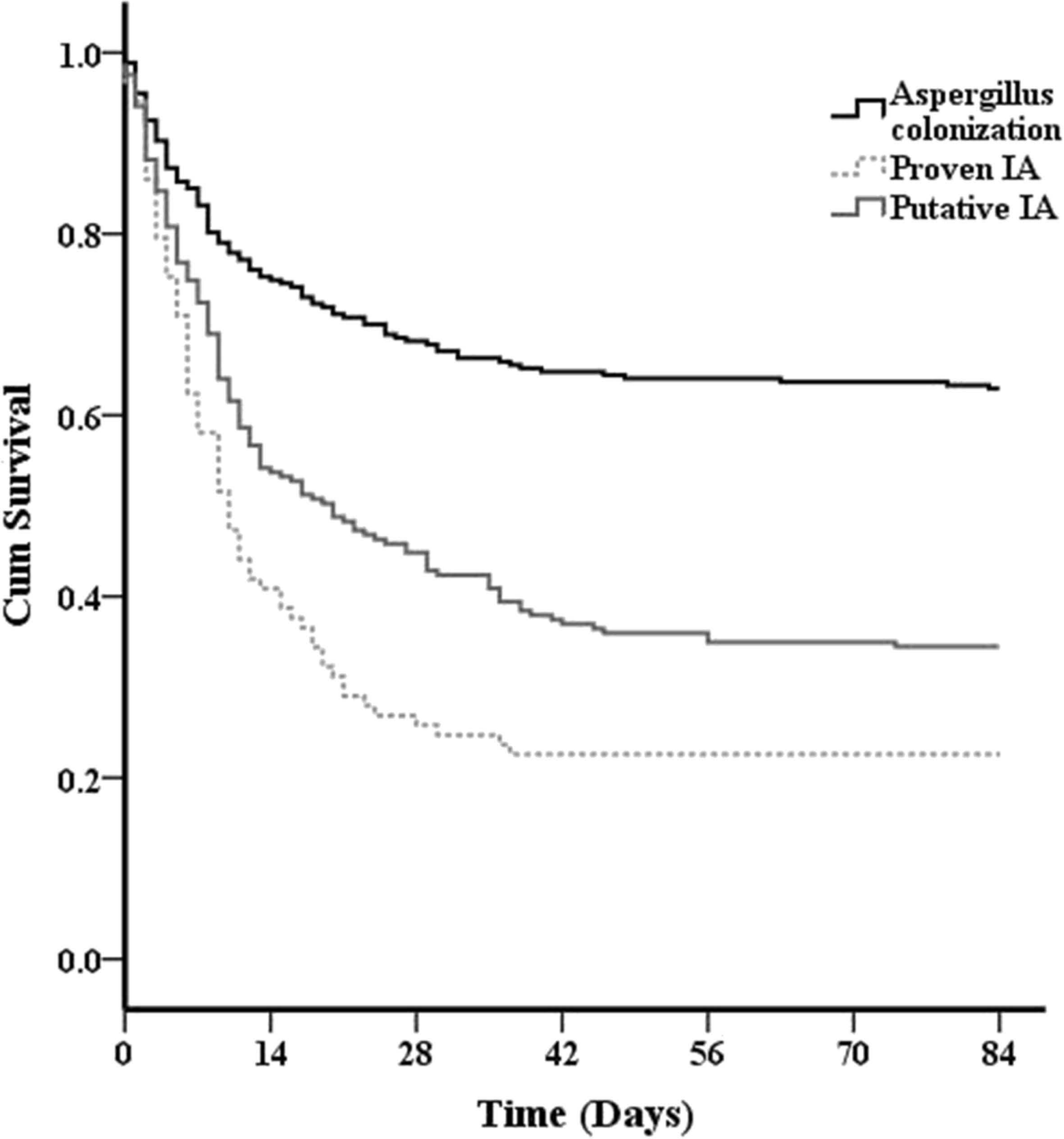

Patients with at least one *Aspergillus*-positive culture (n=563)
Number of sites affected (n=597)
Lung (n=548)
Abdomen (n=11)
Brain (n=10)
Sinus (n=11)
Skin (n=9)
Endovasc. (n=8)
PR: 86 (16%)
PT: 203 (37%)
CO: 259 (47%)
PR: 9 (81%)
PT: 0
CO: 2 (19%)
PR: 10 (100%)
PT: 0
CO: 0
PR: 1 (9%)
PT: 3 (27%)
CO: 7 (64%)
PR: 2 (23%)
PT: 3 (33%)
CO: 4 (44%)
PR: 8 (100%)
PT: 0
CO: 0


1.0
0.8
0.6
0.4
0.2
0.0
Cum Survival
0
14
28
42
56
70
84
Time (Days)
Aspergillus colonization
Proven IA
Putative IA