Invasive aspergillosis in patients admitted to ICU with severe influenza: A retrospective cohort study

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Teachers open the door, but you must enter by yourself. (Chinese wijsheid)

ABSTRACT

Background

Invasive pulmonary aspergillosis (IPA) typically occurs in an immunocompromised host. For almost a century, influenza has been known to set up for bacterial superinfections, but recently patients with severe influenza were also reported to develop IPA. We conducted a retrospective multicentre cohort study to measure the incidence of IPA over several seasons in ICU patients with influenza pneumonia and to evaluate whether influenza was an independent risk factor for IPA.

Methods

Data were collected from patients admitted to 7 ICUs with severe influenza during seven influenza seasons. To determine if influenza was independently associated with IPA, a subgroup of non-immunocompromised influenza-positive patients (cases) were compared with influenza-negative patients (controls) admitted to the ICU with community-acquired pneumonia (CAP) using logistic regression analyses.

Findings

Of the 432 patients admitted to the ICU with influenza, IPA was diagnosed in 19% (83/432) a median of 3 days after ICU admission. The incidence was comparable for influenza A and B. The incidence in the 117 immunocompromised influenza patients was as high as 32% (38/117), while 14% (45/315) of the non-immunocompromised influenza patients developed IPA. In contrast, only 5% (16/315) of the non-immunocompromised influenza-negative controls developed IPA ($p<0{\cdot}0001$). The 90-day mortality in influenza patients with and without IPA was 51% and 28%, respectively ($p<0{\cdot}0001$). In the retrospective cohort study, influenza was found to be independently associated with IPA (aOR 5·2, 95% CI 2·6-10·3, $p<0{\cdot}0001$), besides a higher APACHE II score, male sex and use of corticosteroids.

Interpretation

Influenza was identified as an independent risk factor for IPA and associated with a high mortality. Future studies should evaluate whether a faster diagnosis and/or antifungal prophylaxis could improve outcome of influenza-associated aspergillosis.

Funding

None

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed for articles published between January 1963 and October 2017, using the search terms “influenza” and “aspergillus” or “aspergillosis”. This search yielded case series which described invasive pulmonary aspergillosis (IPA) in patients admitted to the ICU with influenza. Yet, a systematic evaluation of the risk of IPA in a large population of ICU patients with influenza over several consecutive influenza seasons was missing. Also, it remained to be demonstrated if influenza was independently associated with aspergillosis.

Added value of this study

This study is, to our knowledge, the largest study ever performed on the risk for IPA in this patient population with 432 ICU patients with influenza included. It is also the first to evaluate this complication over several consecutive seasons in a large number of ICUs. Furthermore, by comparing non-immunocompromised influenza-positive and influenza-negative patients, we aimed to show that influenza was an independent risk factor for IPA. The following conclusions could be drawn: First, the incidence of IPA was >10% in each of the 7 seasons and was almost equal in influenza A and influenza B patients. Therefore, once a patient with influenza needs intensive care support, the risk for IPA does not depend on the influenza season and influenza subtype. Second, the overall incidence of aspergillosis was 19% and was as high as 32% in the subgroup of patients who were also immunocompromised at the time of their influenza infection. The overall mortality in the patients with IPA was very substantial at 51%. Last but not least, we compared 315 non-immunocompromised (i.e. no EORTC/MSG host factor) influenza-positive patients with an equal number of non-immunocompromised influenza-negative patients with severe community-acquired pneumonia (CAP) for the occurrence of IPA. We showed that influenza was independently associated with IPA (aOR 5·2, 95% CI 2·6-10·3, p<0·0001).

Implications of all the available evidence

The independent association between influenza and IPA and the high mortality calls for increased awareness and a more aggressive diagnostic approach. Future studies should evaluate if prophylaxis is useful.

INTRODUCTION

Invasive pulmonary aspergillosis (IPA) typically occurs in a severely immunocompromised host and isolation of *Aspergillus* species in the immunocompetent host is mostly considered colonization.[1,2] The six-week mortality of IPA is 20-30%[3,4] but is much higher in critically-ill patients.[4,5] Influenza is a common viral respiratory tract infection. In a subset of patients with influenza, intensive care admission is needed. This may be due to bacterial superinfection[1,6,7] but also influenza in itself can cause severe acute respiratory distress syndrome (ARDS),which is associated with a mortality of 14% to 41%.[8,9]

Influenza-associated aspergillosis was occasionally described decades ago and several small case series were reported recently.[1,9,10] 65% of the reported cases did not have classic host factors for IPA as defined by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG).[1,11] These EORTC/MSG criteria are used to classify patients with a fungal infection into proven, probable or possible aspergillosis but are not applicable to the ICU setting. For the ICU setting, an algorithm (AspICU algorithm) was described by Blot and colleagues to distinguish IPA from *Aspergillus* colonization in critically-ill patients.[12]

In 2012, Wauters and colleagues reported an incidence of 23% of proven or probable IPA in 44 H1N1 influenza patients in two consecutive influenza seasons (2009-2011). Remarkably, 44% of the IPA cases lacked any of the classical EORTC/MSG host factors.[9] Recently, a Dutch study described 23 (16%) cases of IPA among 144 patients admitted to the ICU with influenza during the 2015-16 H1N1 influenza season.[13] These observations suggest that influenza infection requiring ICU admission is a risk factor for IPA and that the incorporation of influenza as a host factor in the current diagnostic criteria may be appropriate. However, it remains unclear if influenza is independently associated with the occurrence of IPA and if the risk varies from season to season. This study aims to describe the epidemiology and outcome of IPA in ICU patients over seven consecutive influenza seasons and to evaluate whether influenza is independently associated with IPA.

METHODS

Study design and data collection

We performed a retrospective cohort study in seven tertiary care ICUs (2 in Belgium and 5 in The Netherlands). The search strategy for influenza-positive patients admitted to the ICU during influenza seasons 2009-2016 consisted of reviewing all patients with a positive influenza polymerase chain reaction (PCR) in the registry of the local

microbiology department and matching these with ICU admissions. We selected a group of patients admitted to the ICU with severe community-acquired pneumonia (CAP) and with a documented negative influenza PCR test as the comparison group because these patients are equally admitted to the ICU from outside the hospital with respiratory insufficiency due to pneumonia as well (figure 1). A list of patients with a negative influenza PCR was retrieved from the microbiology departments and these patients were matched for ICU admission. All patients were evaluated whether an infiltrate was present on chest imaging, antibiotic therapy was initiated and if a diagnosis of CAP was made at ICU admission. The patient files were also reviewed to exclude that an influenza infection was diagnosed elsewhere and to confirm that the pneumonia was not hospital acquired. Figure 1 describes the inclusion process in detail. The study protocol was approved by the institutional review board (IRB) of both Belgian sites and by the IRB of the initiating Dutch centre (Erasmus University Medical Centre, Rotterdam) for the 5 Dutch sites.

Study population (figure 1)

Patients were ≥18 years, admitted to the ICU for >24 hours with acute respiratory failure, had pulmonary infiltrates on imaging and a confirmed influenza infection based on a positive airway PCR test. A subgroup of the influenza-positive cohort (cases) was compared with an influenza-negative comparison group (control group) for the occurrence of IPA. Cases were the subgroup of influenza-positive patients that did not have an EORTC/MSG host factor (table S1, appendix p3), already posing them at risk for IPA. Controls were patients admitted to the ICU for severe CAP with a negative influenza PCR. Like the cases, controls were EORTC/MSG host factor negative. Exclusion criteria for all patients were respiratory failure not being the primary reason for ICU admission and a history of IPA. Please note that the terms cases and controls do not point towards a case-control study design from a methodological point of view. They describe two patient groups where cases should be interpreted as “influenza-positive non-immunocompromised patients with respiratory insufficiency” and controls as “influenza-negative non-immunocompromised patients with CAP”.

Definition of Invasive Pulmonary Aspergillosis (IPA)

The definition of IPA is modified from the AspICU algorithm and is based on the presence of clinical, radiological and mycological criteria in all IPA cases.[12] Details on the definitions can be found in table 1.

This modified IPA definition does not require an EORTC defined host factor because otherwise patients with influenza but without an EORTC host factor could never fulfil the IPA definition as long as influenza is not part of the EORTC host factor definition. To be on the conservative side, we excluded all patients in whom the only mycological

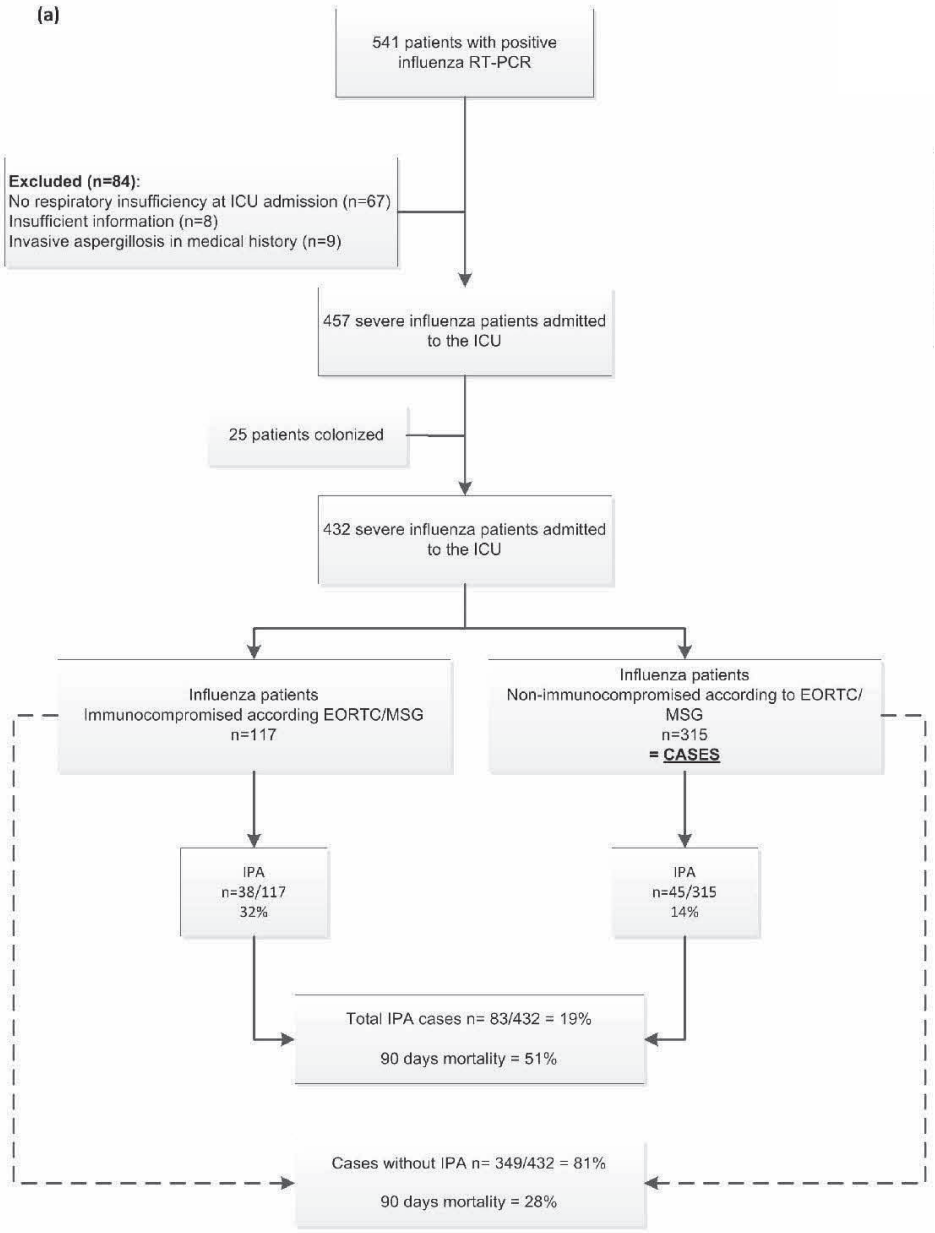
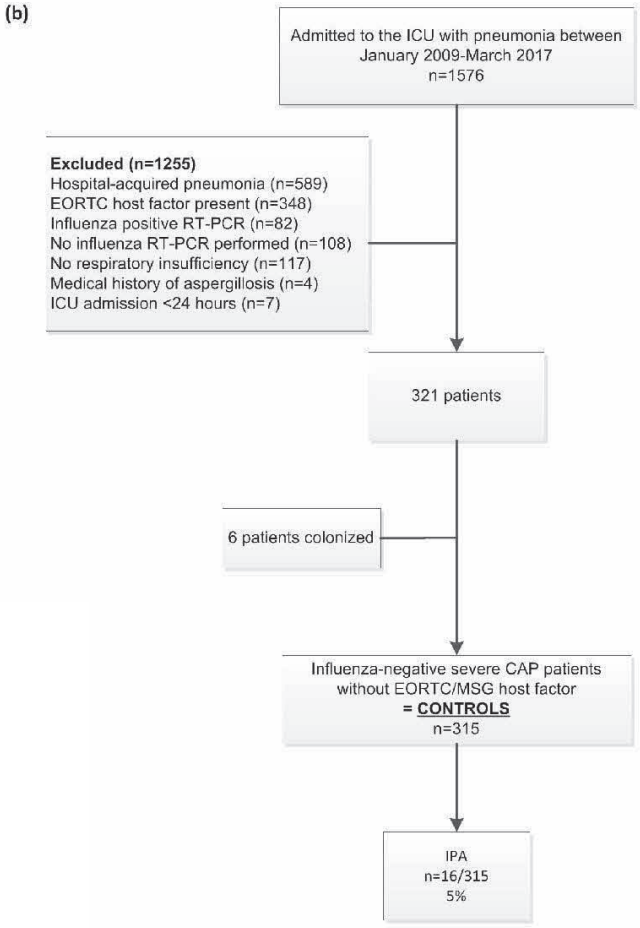


Figure 1: Overview of inclusion process and IPA cases with corresponding mortality: (a) inclusion process influenza patients and cases; (b) inclusion process control group.
Abbreviations: CAP= Community acquired pneumonia; EORTC/MSG= European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group; ICU= intensive care unit; IPA= Invsasive pulmonary aspergillosis; RT-PCR= real-time polymerase chain reaction



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evidence for IPA was a lower positive respiratory tract culture (sputum, bronchial aspirate) for *Aspergillus* species but who had a negative or unavailable broncho-alveolar lavage (BAL) culture and galactomannan test. These patients were defined as colonized and were excluded from the final analysis.[14] Every influenza patient was reviewed and consensus was achieved (NP,RV,AS,LV,JW,BR) to ascertain whether the modified IPA definition was met.

MODIFIED IPA DEFINITION: The definition of IPA is modified from the *AspICU* algorithm and is based on the presence of clinical, radiological and mycological criteria in all IPA-cases.
CLINICAL CRITERIA: *one of the following signs/symptoms has to be present:* • **Fever refractory to at least 3 days of appropriate antibiotic therapy** • **Recrudescent fever after a period of defeverescence of at least 48 hours while still on antibiotics and without other apparent cause** • **Dyspnea** • **Hemoptysis** • **Pleural friction rub/chest pain** • **Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support**
RADIOLOGICAL CRITERIA: any infiltrate on pulmonary imaging by portable chest X-ray or CT-scan of the lungs. This radiological definition is different from the EORTC defined radiological criteria (e.g. halo sign or air-crescent sign) because these EORTC criteria apply to patients with prolonged neutropenia but are of little use for ICU patients.
MYCOLOGICAL CRITERIA: *≥1 of the following has to be present* • **Histopathology or direct microscopic evidence of dichotomous septate hyphae with positive culture for *Aspergillus* from tissue** • **A positive *Aspergillus* culture from a broncho-alveolar lavage (BAL)** • **A galactomannan optical index on BAL of ≥1** • **A galactomannan optical index on serum of ≥ 0.5.**

Table 1: Modified IPA definition
The Platelia Aspergillus test was used for galactomannan detection in all centers (Bio-Rad Laboratories, Marnes-la-Coquette, France). *Aspergillus* species were identified by their culture characteristics and microscopic morphology.

STATISTICAL ANALYSIS

In univariable analysis, categorical variables were compared by Fisher's exact test and Chi-square test, and continuous variables with t-test or Mann-Whitney U test where appropriate. On the entire population of the influenza-positive cohort, a multivariable analysis was done by binary logistic regression to detect independent risk factors for the development of IPA in influenza patients. The dependent variable was the presence of IPA and independent variables were those previously described as a possible risk factor for IPA in the ICU or associated with IPA in the univariable analysis.[4,15] The estimate of association was expressed as adjusted odds ratio (aOR) with corresponding

95% confidence interval (CI). Multiple imputations were performed to handle missing data, using 20 imputations and 1000 iterations following the Markov-Chain Monte Carlo methods, and the pooled results are presented (Table S4, appendix p5). In addition, a binary logistic regression analysis with multiple imputations was performed on the entire cohort of cases and controls to determine if influenza was independently associated with IPA. Data were analysed with SPSS Version 24 (Armonk, NY:IBM corp). No correction for multiple testing was performed for the univariable analyses and a two-tailed significance level of 0·05 was used. These p-values should therefore be interpreted with this limitation in mind. A statistician from the department of Biostatistics of Erasmus University Medical Center (ERA) supervised the analysis.

Role of funding source

This study was part as part of our routine work. No funding was provided. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

RESULTS

Inclusion/exclusion process (figure 1)

Between 01/2009 and 06/2016, 541 influenza-positive patients were admitted to 7 ICUs. 84 patients were excluded for the following reasons: respiratory insufficiency was not the reason for ICU admission (n=67), medical history of IPA (n=9) or insufficient clinical data (n=8). Another 25 patients were excluded because they met the criteria for *Aspergillus* colonization. In total, 432 patients with influenza were included in the influenza cohort. 315 of them were EORTC/MSG host factor negative and were defined as cases. The search strategy for the comparison group with severe CAP patients resulted in the selection of 315 patients (figure 1).

Influenza patient characteristics (table 2)

Patient characteristics are summarized in table 2. Mean age was 59 years and 56% (240/432) were male. Influenza A and B was found in 82% (355/432) and 18% (77/432), respectively. 79% (338/432) of the patients received a neuraminidase inhibitor. 27% (117/432) were EORTC/MSG host factor positive. The mean APACHE II score was 22 SD±8. 75% (326/432) of the patients required intubation for mechanical ventilation for a median duration of 11 [IQR 5,21] days. 52 patients received extracorporeal membrane oxygenation (ECMO). The overall ICU mortality in the influenza-positive patients was 25% (107/432).

	Influenza cohort (*n* = 432)	IPA (*n* = 83)	No IPA (*n*=349)	*p*
BASELINE FACTORS				
Age (years)	59 ± 15	60 ± 12	59 ± 16	0·35
Sex (men), *n (%)*	240 (56)	56 (67)	184 (53)	0·015*
APACHE II score on admission	22 ± 8	25 ± 9	22 ± 7	0·005*
BMI >30 kg/m^2, *n (%)*	93/410 (23)	17/83 (20)	76/327 (23)	0·59
Diabetes, *n (%)*	88 (20)	10 (12)	78 (22)	0·036*
Liver cirrhosis	25 (6)	5 (6)	20 (6)	1·0
Chronic kidney disease	71 (16)	16 (19)	55 (16)	0·44
KNOWN RISK FACTORS				
EORTC host factor, *n (%)*	117 (27)	38 (46)	79 (23)	<0·0001*
Haematological malignancy, *n (%)*	66 (15)	22 (27)	44 (13)	0·002*
Solid organ transplant, *n (%)*	32 (7)	11 (13)	21 (6)	0·024*
Solid organ malignancy, *n (%)*	21 (5)	4 (5)	17 (5)	1·0
Neutropenia, *n (%)*	22 (5%)	11 (13)	11 (3)	0·001*
COPD, *n (%)*	79 (18)	13 (16)	66 (19)	0·49
STUDIED RISK FACTORS				
CS 28 days before ICU, *n (%)*[a]	145/426 (34)	46/82 (56)	99/344 (29)	<0·0001*
Median dose CS 28days before ICUadm (IQR)	0·14 (0·06;0·28)	0·22 (0·10;0·33)	0·10(0·06;0·24)	0·003*
Smoking in the past year	114/332 (34)	26/61 (43)	88/271 (32)	0·13
ICU DATA				
Mechanical ventilation, *n (%)*	326 (75)	75 (90)	251 (72)	0·0004*
Mechanical ventilation days (IQR)	11 (5,21)	14 (9,31)	9 (4,17)	0·001*
NO/HFOV, *n (%)*	42 (10)	13 (16)	29 (8)	0·04*
ECMO or ECCOR, *n (%)*	52 (12)	16 (19)	36 (10)	0·024*
Vasopressors, *n (%)*	287/423 (67)	67/82 (81)	220/341 (65)	0·002*
RRT, *n (%)*	100/423 (24)	35/83 (42)	65/340 (19)	<0·0001*
Median days of ICU stay [IQR]	11 [6,23]	19 [12,38]	9 [5,20]	<0·0001*
OUTCOME DATA				
ICU mortality, *n (%)*	107 (25)	37 (45)	70 (20)	<0·0001*
Hospital mortality, *n (%)*	133 (31)	41 (49)	92 (26)	<0·0001*
90 days mortality after ICU admission, *n (%)*	141 (33)	42 (51)	99 (28)	0·0001*
INFLUENZA				
Influenza A, *n (%)*	355 (82)	71 (86)	284 (81)	0·37
Influenza B, *n (%)*	77 (18)	12 (14)	65 (19)	0·37
Influenza treatment, *n (%)*	338/428 (79)	70/83 (84)	268/345 (78)	0·25
DIAGNOSTICS				
BAL sampling performed, *n (%)*	233 (54)	81 (98)	152 (44)	<0·0001*
BAL culture positive, *n (%)*		50/80 (62·5)		
BAL Galactomannan performed, *n(%)*	137 (32)	76 (92)	61 (17)	<0·0001*
BAL Galactomannan positive, *n (%)*		67/76 (88)		
Serum GM serum performed, *n (%)*	47 (11)	31/83 (37)	16 (5)	<0·0001*

Table 2: Overview of the characteristics of influenza-positive cohort
APACHE= acute physiology and chronic evaluation score, BAL= broncho-alveolar lavage, BMI= body mass index, COPD= chronic obstructive pulmonary disease, CS= corticosteroids, ECMO= extracorporeal membrane oxygenation, EORTC= European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group, GM= galactomannan; ICU= intensive care unit, IPA= invasive pulmonary aspergillosis, IQR= interquartile range, NO/HFOV= nitric oxide/high-frequency oscillation ventilation, RRT= renal replacement therapy, SD= standard deviation.

IPA characteristics in patients with Influenza (table 3)

83 (19%) of the severe influenza patients admitted to the ICU fulfilled the IPA definition. The proportion of patients with IPA among the influenza cases varied per centre (6% to 26%, table S2, appendix p3). IPA was diagnosed at a median of 3 (IQR 0,7) days after ICU admission. *A. fumigatus* was almost exclusively cultured when identification to the species level was available. Susceptibility data were available in 17 patients and 4 voriconazole-resistant strains were documented. While the number of patients admitted to the ICU with influenza varied substantially from year to year, the prevalence of IPA was >10% in all calendar years (figure S4, see appendix p5). IPA was found in 20% (71/355) and 16% (12/77) from the patients with influenza A and influenza B pneumonia, respectively. No clear association could be demonstrated between the prevalence of IPA and the influenza subtypes that circulated in the respective calendar years (table S6, appendix p6).

In 98% of the IPA cases (81/83) a BAL was done, yielding a positive Aspergillus culture in 50 (60%) and a positive galactomannan test (optical density (OD) ≥1·0) in 67 (88%) of the 76 patients of whom the BAL was not only cultured but also tested for the presence of galactomannan (table 3). Serum galactomannan test was done in 31/83 (37%) of the IPA cases and was positive (i.e.≥0·5) in 65% (20/31). 21 of the 83 patients (25%) with IPA were previously healthy and 7 (33%) of them died. Given the fact that by definition patients with influenza who are not immunocompromised do not fulfil the EORTC/MSG host factor definition, only 36 of 83 (43%) had a proven (n=16) or probable (n=20) IPA according to the EORTC/MSG classification.[11] According to the AspICU algorithm, specifically designed for ICU patients, 48 patients (58%) were diagnosed with IPA while 30 were not classifiable as they had a positive galactomannan test on BAL but a negative lower respiratory tract culture which is the entry criterion in the AspICU algorithm.[12] 92% of the IPA cases (76/83) received mould-active antifungal therapy. In these patients, no difference in the number of days from influenza diagnosis to antifungal therapy initiation was observed between survivors and non-survivors 90 days after ICU admission (4 [IQR 1,10] versus 5 [IQR 1,7] days, p=0·64).

IPA characteristics in patients with Influenza	
BAL culture positive, *n (%)*	50/80 (62·5)
BAL Galactomannan positive, *n (%)*	67/76 (88)
Serum Galactomannan positive, *n (%)*	20/31 (65)
EORTC/MSG criteria	
• **Proven, *n (%)***	16 (19)
• **Probable, *n (%)***	20 (24)
• **Not classifiable, *n (%)***	47 (57)
AspICU criteria	
• **Proven, *n (%)***	16 (19)
• **Putative, *n (%)***	32 (39)
• **Colonizer, *n (%)***	5 (6)
• **Not classifiable, *n (%)***	30 (36)
Initial Treatment:	
• **Voriconazole, *n (%)***	61 (73)
• **Echinocandine, *n (%)***	2 (2)
• **Combination (Triazole + Echinocandine), *n (%)***	9 (11)
• **Liposomal-Amfotericine B, *n (%)***	4 (5)
• **No treatment, *n (%)***	7 (8)

Table 3: IPA characteristics in patients with Influenza
BAL= broncho-alveolar lavage, EORTC/MSG= European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group, IPA= invasive pulmonary aspergillosis.

Comparison of influenza patients with and without IPA (Table 2)

ICU mortality in the IPA cases was higher than in patients without IPA (37/83 or 45% versus 70/349 or 20%, p<0·0001) and the ICU stay was longer (19 days [IQR 12,38] versus 9 days [IQR 5,20], p<0·0001). The mortality 90 days after ICU admission was 51% (42/83) in patients with IPA and 28% (99/349) in those without IPA (p<0·0001).

Patients with IPA required mechanical ventilation more often (90% (75/83) versus 72% (251/349), p=0·0004) and for a longer period (+5 days; p=0·001).

Independent risk factors for the occurrence of IPA on the pooled data of all influenza-positive patients (regardless of the presence or absence of EORTC/MSG host factor) are presented in figure 2a. A list of all variables used in the multivariate analyses can be found in the appendix (table S4, appendix p5). Corticosteroid (CS) therapy in the 4 weeks before ICU admission was independently associated with IPA (aOR 1·59, 95% CI 1·30 to 1·99;p<0·0001) per 0·1mg/kg/day prednisone equivalent). Finally, male sex (aOR 1·84, 95% CI 1·05 to 3·22;p=0·034) and a higher admission APACHE II were associated with IPA as well (aOR 1·05,95% CI 1·01 to 1·09;p=0·007 per 1·0 point APACHE II increase).

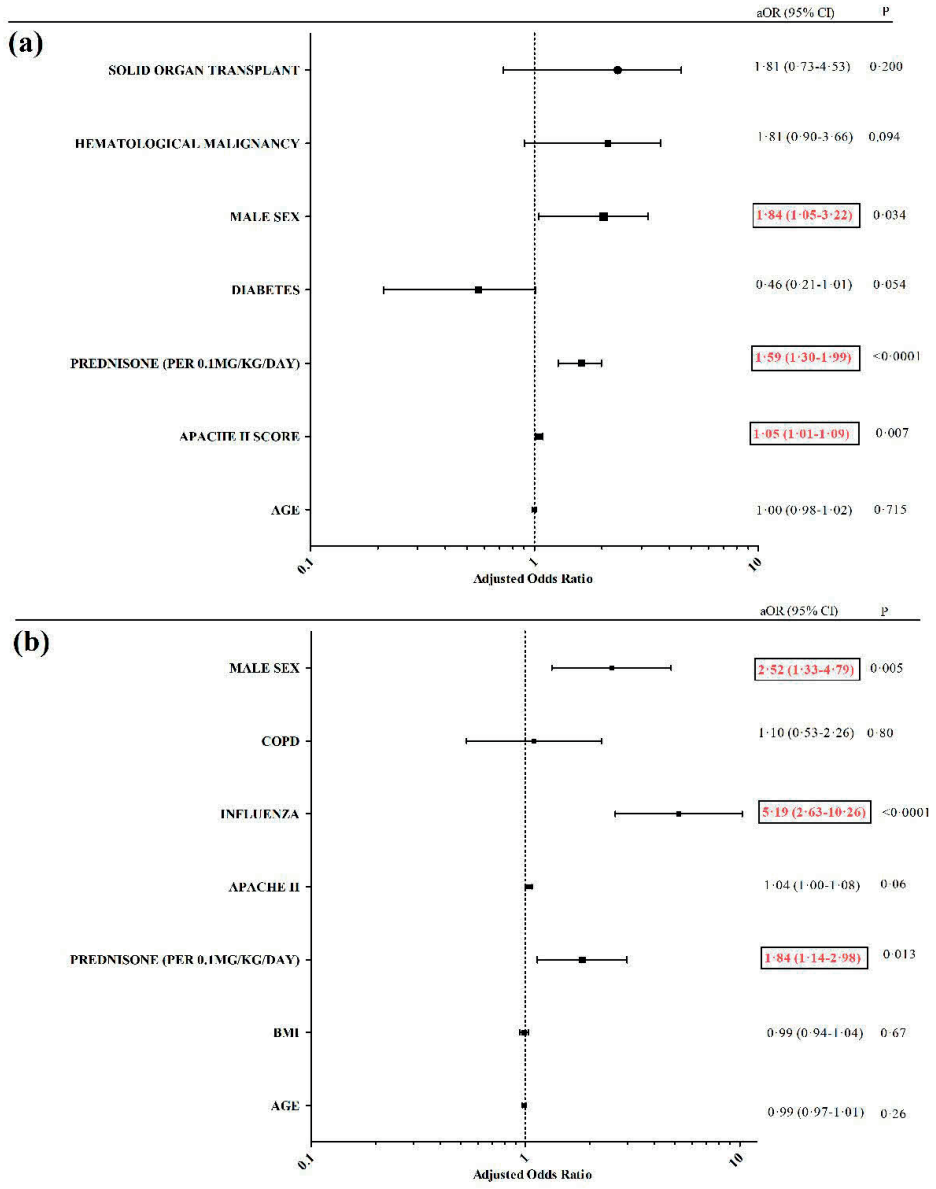

Figure 2: Forest plots of risk factors for the development of IPA
The solid lines represent the 95% confidence intervals (CIs). There has been corrected for centre as well but this is not depicted here as no significant differences were found. (a) Analysis of risk factors for patients with influenza in the ICU to develop IPA. (b) Overview of case-control comparison. Factors independently associated with the development of IPA are highlighted in red.
Abbreviations: APACHE= acute physiology and chronic evaluation score; BMI= body mass index; CI= confidence interval; COPD= chronic obstructive pulmonary disease.

	All EORTC neg patients (n=630)	Influenza + CASES (n=315)	Influenza - CONTROLS (n=315)	*p*
BASELINE CHARACTERISTICS				
Age (years)	59 ± 17	58 ± 16	60 ± 17	0·15
Sex (men), ***n (%)***	371 (59)	169 (54)	202 (64)	0·008*
APACHE II admission	23 ± 8	22 ± 8	23 ± 8	0·29
BMI (kg/m^2), median (IQR) Missing, n	25 (22,29) 21	27 (23,30) 18	24 (22,28) 3	<0·0001*
Diabetes, ***n (%)***	114 (19)	63 (20)	51 (16)	0·21
Liver cirrhosis, ***n (%)***	44 (7)	18 (6)	26 (8)	0·21
Chronic Kidney Disease^x,***n (%)***	69 (11)	31 (10)	38 (12)	0·37
COPD, ***n (%)***	123 (20)	68 (22)	55 (17)	0·19
CORTICOSTEROIDS				
CS 28 days before ICU, ***n (%)***	99/619 (16)	57/304 (19)	42/315 (13)	0·005
Median dose CS 28days before ICUadm (IQR) when receiving CS	·078 [·054, ·176]	·070 [·054, ·171]	·080 [·053, ·179]	0·79
Missing, n	22	10	12	
ICU PARAMETERS				
Mechanical ventilation, ***n (%)***	475 (75)	246 (78)	229 (73)	0·12
Median ventilation days, days [IQR] Missing, n	9 [4, 18] 35	11 [5, 21] 26	4 [4, 14] 9	0·002*
NO/HFOV, ***n (%)***	64 (10)	37 (12)	27 (9)	0·17
ECMO or ECCOR, ***n (%)***	65 (10)	45 (14)	20 (6)	0·04*
ECMO days, median [IQR]	10 [6, 20]	11 [8, 21]	9 [5, 18]	0·44
Vasopressors, ***n (%)***	415 (66)	216 (69)	199 (63)	0·17
Renal replacement therapy, n(%)	103 (16)	61/307 (20)	42 (13)	0·03*
OUTCOME DATA				
ICU mortality, ***n (%)***	125 (20)	58 (18)	67 (21)	0·37
Hospital mortality, ***n (%)***	164 (26)	76 (24)	88 (28)	0·28
Mortality< 90 days after ICU admission	177 (28)	78 (25)	99 (31)	0·70
Median ICU stay, days [IQR] Missing	11 [6, 21] 19	11 [6, 23] 15	10 [6, 18] 4	0·15
IPA, ***n (%)***	61 (10)	45 (14)	16 (5)	<0·0001*
DIAGNOSTICS				
BAL sampling, ***n (%)***	318 (50)	145 (46)	173 (55)	0·026*
BAL Galactomannan performed, ***n(%)***	187 (30)	81 (26)	106 (34)	0·029*
IPA AspICU CLASSIFICATION				
IPA-proven, *n (% of IPA cases)*	8 (13)	6 (13)	2 (13)	
IPA-putative, *n (% of IPA cases)*	32 (52)	27 (60)	5 (31)	
IPA-colonizer, *n (% of IPA cases)*	4 (7)	3 (7)	1 (6)	
IPA non classifiable, *n (% of IPA cases)*	17 (28)	9 (20)	8 (50)	

Table 4: Overview of characteristics of the cases and controls

APACHE= acute physiology and chronic evaluation score, AspICU: algorithm for invasive aspergillosis in ICU as described by Blot and colleagues[12], BAL= broncho-alveolar lavage, BMI= body mass index, COPD= chronic obstructive pulmonary disease, CS= corticosteroids, ECCOR= extracorporeal CO2 removal, ECMO= extracorporeal membrane oxygenation, adm= admission, EORTC= European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group, ICU= intensive care unit, IPA= invasive pulmonary aspergillosis, IQR=Interquartile range, NO/HFOV= nitric oxide/high-frequency oscillation ventilation, RRT= renal replacement therapy, SD= standard deviation. X=Glomerular filtration rate < 60mg /1.73m2

Comparison of influenza-positive cases and influenza-negative CAP controls (Table 4 and association analysis figure 2b)

315 of the influenza patients had no underlying EORTC/MSG risk factor and were defined as cases and 45 (14%) of them were diagnosed with IPA. In comparison, 16 of the 315 CAP controls (5%) were diagnosed with IPA. Baseline characteristics of the cases and controls are summarized in table 4. BAL sampling and galactomannan measurement on BAL was more frequently performed in CAP controls (BAL in 55% (173/315), galactomannan in 34% (106/315)) than in influenza-positive cases (46% (145/315) and 26%(81/315)). To evaluate if in the pooled patient population of influenza cases and CAP controls, influenza was associated with IPA, a binary logistic regression analysis was performed. This analysis confirmed the independent association between influenza and IPA (aOR 5·19 95% CI, 2·63 to 10·26; p<0·0001) (figure 2b). A list of all variables used in the multivariate analyses can be found in the appendix (table S5, appendix p5). In the case-control analyses, other independent risk factors for IPA were male sex and receipt of corticosteroids in the 4 weeks preceding ICU admission at a dose below the corticosteroid dose that is included in the EORTC/MSG host factor definition (figure 2b).

DISCUSSION

To the best of our knowledge, this study is the largest ever performed on the incidence, risk factors and outcome of IPA in ICU patients with influenza. Furthermore, the data provide evidence that influenza infection is an independent risk factor for IPA. Indeed, in a total of 630 non-immunocompromised patients admitted to the ICU with CAP of which 50% were infected with influenza, the presence of influenza increased the risk of IPA from 5 to 14%. Furthermore, the ICU mortality in the IPA cases was 45% and even in previously healthy individuals the mortality was 33%. This is in accordance with the 47% mortality described in earlier case series[1] but somewhat lower than described in recent cohorts.[13,16] Of note, 85 patients of this cohort had been included in previous studies.[9,13] In the subgroup with an EORTC/MSG host factor, the IPA incidence was as high as 32% and 61% of them had died 90 days after ICU admission. As the diagnosis of

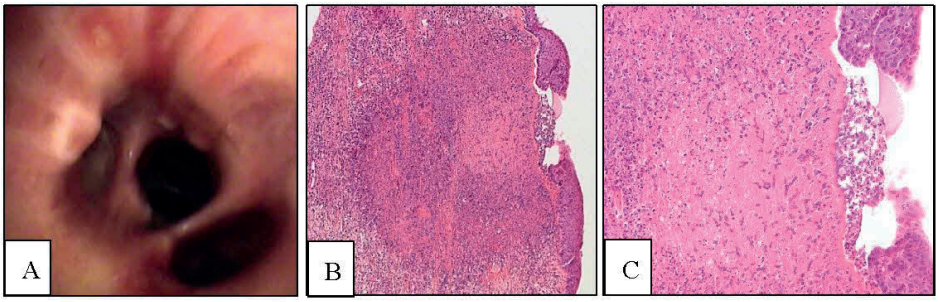

Figure 3: Bronchoscopy and histopathology of a representative case of influenza-associated ***Aspergillus*** tracheobronchitis.

A. Bronchoscopic examination reveals diffusedly inflamed mucosal tissue, with multiple whitish nodules, dispersed from trachea into main bronchial structures. Some nodules show central necrosis.

B. Haematoxylin-Eosin staining of biopsied specimen from trachea at 50x magnification, showing focal ulceration with submucosal necrosis and squamous metaplasia.

C. Haematoxylin-Eosin staining of biopsied specimen at 100x magnification, revealing invasion of submucosa by fungal hyphae, type *Aspergillus*, and dense infiltration with neutrophils.

IPA is challenging, a systematic approach towards the diagnosis of IPA in ICU patients with influenza may result in an even higher incidence of IPA and should be the focus of future prospective studies.

The reported overall incidence of IPA in critically ill patients varies widely from <1 to 6·9%[15,17,18] and corresponds with the 5% incidence in our CAP control group.[9,13,19] A recent study of 2901 ICU patients with influenza showed the presence of a co-infection in 17% and *Aspergillus* spp. in 7% of these patients with a co-infection.[20] The lower incidence in this study could be explained by a different diagnostic approach (e.g. no use of BAL galactomannan measurement), a lower overall awareness as well as the fact that only co-infections diagnosed within two days of hospital admission were registered.

As influenza is not considered a host factor for IPA, only part of our patients with IPA fulfilled one of the diagnostic criteria for IPA as defined by the EORTC/MSG or AspICU algorithm.[11,12] In addition, influenza patients with IPA mostly have non-specific radiology and classic radiological features only occur in 5% of critically-ill patients with IPA.[1,12,14,21,22] Autopsy series indicated that strict interpretation of the host factors for invasive fungal disease contributes to missed diagnosis of IPA.[5,23] Therefore, we classified our patients using a modified IPA definition in which no specific host factor was required. However, stringent mycological criteria were used, compatible with the case definition of EORTC/MSG, AspICU and van de Veerdonk and colleagues.[11-13] Of course, the same classification was used for the control group. Furthermore, to avoid an overestimation of the incidence of IPA, we excluded all 25 patients with only a positive

respiratory tract (i.e. sputum or tracheal aspirate) but a negative or unavailable BAL culture as the only microbiological evidence for IPA.

The optical density (OD) cut-off above which a galactomannan test in BAL should be considered positive is a matter of debate. The sensitivity of BAL galactomannan measurement was 88% when applying an OD≥0·5 on biopsy proven IPA cases in the ICU.[24] However, in a recent observational study the value of BAL galactomannan testing in the ICU was questioned because the specificity compared with a positive BAL culture was low at 38% and 62% with a galactomannan OD cut-off of ≥1·0 and 3.0 respectively.[25] However, given the limited sensitivity of BAL culture for the diagnosis of IPA, the use of a positive culture as the gold standard makes the interpretation of their results difficult. In our case definition we used a galactomannan OD cut-off of ≥1·0. Yet, when an OD≥3·0 would be applied only 8 (10%) of the 83 IPA-influenza cases would have been classified differently. In addition, the median galactomannan OD of all influenza-IPA cases was as high as 5·8 IQR [2·8-6·7]. Furthermore, when we reviewed all 15 patients with proven IPA that also underwent BAL galactomannan testing, in 14 of these 15 patients with biopsy proven IPA, the BAL GM optical density was >1.0. Also, 6 patients without IPA had a galactomannan BAL measurement with a value <1.0 and were autopsied. In none of them, an IA was found at autopsy. Therefore, the specificity of galactomannan in BAL with a cut-off threshold of 1.0 in our study seems to be excellent. Remarkably, the observation that 17 of the 28 patients (61%) with a positive BAL galactomannan test, also had a positive serum GM was unexpected as in non-neutropenic haematology patients the sensitivity of serum galactomannan is low. This suggests that angio-invasion is often present in influenza patients with IPA.

We could not confirm the previous observation that a delayed initiation of anti-fungal therapy was associated with a fatal outcome.[13] A particularly high awareness was present in one of the participating centres as this centre already published on influenza-associated IPA in 2012. In this centre, BAL sampling was performed in 102 of 149 influenza patients. 26% of the patients in this centre fulfilled the IPA diagnosis with an ICU mortality of 38% compared to an ICU mortality of influenza-associated aspergillosis in all other centres of 50%. This suggests that increased awareness may improve outcome.

Azole resistance is an emerging problem and has been particularly reported in The Netherlands with a prevalence of 13% in 2016.[26] As azole resistance testing has only recently become a standard procedure in ICU, data on azole resistance were available for 17 patients only and resistance was documented in 4 of them.

Why patients with influenza are at risk for IPA is not yet clear.[27,28] Respiratory epithelium damage and mucociliary clearance dysfunction may facilitate the invasion of *Aspergillus* (figure 3).[7,9,29] Moreover, influenza-induced ARDS and hypoxia may cause immune-paralysis.[30-32] Almost all cases to date have been associated with the pandemic

influenza A H1N1 infection but influenza B may trigger an *Aspergillus* superinfection as well.[13,33] This observation was confirmed in this study as an almost equal proportion of both influenza A and influenza B patients developed IPA. We were unable to look at the influenza subtype as a possible risk factor for IPA as subtyping was only available in a small number of patients. However, no association could be found between the of IPA and the influenza subtypes that circulated in the respective calendar years. Furthermore, the fact that the of IPA was >10% in all calendar years suggests that the severity of illness rather than influenza subtype is more important. Whether our observation is specific for influenza or if it may also apply to pneumonia patients admitted to the ICU with a respiratory virus other than influenza remains to be seen. The observation that the use of CS prior to the ICU admission was independently associated with IPA is in accordance with a Cochrane review showing an association between CS use and increased influenza mortality. On the other hand, CS use before ICU admission could be a marker of the severity of the influenza infection, making it a possible confounder by indication. However, the available evidence on the value of CS in patients with influenza argues against its use as long as data from a prospective randomized clinical trial are lacking.[34]

Given the high incidence of IPA we observed, antifungal prophylaxis might be a valid approach. Whether antifungal prophylaxis will be superior to a standardized diagnostic approach combined with prompt initiation of antifungal therapy as soon as IPA is diagnosed remains to be demonstrated.

Limitations

First, given the retrospective design of this study, confounding cannot be ruled out and a standardized diagnostic approach towards IPA was lacking. However, the time needed to collect a similar amount of data prospectively clearly argues for the added value of this retrospective study. Also, as we did not correct for multiple testing, all univariate p-values should be interpreted with this in mind. Second, as only 60% of the patients with IPA had a positive BAL culture, the diagnosis of IPA was based on a positive galactomannan BAL test in a substantial number of patients. Given the observation that BAL sampling was performed in 98% of the influenza patients diagnosed with IPA but only in 44% of the patients not diagnosed with IPA we cannot exclude that the actual incidence of IPA may be even higher. We have no reasons to believe that compared with the influenza patients, a risk of underdiagnosis of IPA in the influenza-negative controls was present. Actually, BAL galactomannan sampling was more often performed in our control group. Third, one may argue that in a subset of the patients the IPA will have developed before ICU admission and may have resulted in clinical deterioration and ultimately ICU admission. However, this does not change the conclusion that in patients with influenza that need ICU support, IPA is highly prevalent and associated with a high mortality. Another limitation is that all but 1 of the 7 centres were tertiary

care academic ICUs. Therefore, extrapolation to small primary care ICUs should be done with caution. However, the incidence of IPA in the single primary care ICU of this study was comparable at 15%. The use of ECMO support was somewhat higher in the influenza-positive cases (14%) than in the influenza-negative CAP controls (6%) and therefore one may argue that ECMO may be a confounder in the analysis. However, only 4 of 83 IPA infections in our study were diagnosed with IPA >72h after the start of ECMO support. Also, in a recent study on fungal infections in 2129 patients on ECMO the incidence of *Aspergillus* superinfections was similar to the general intensive care population. Importantly, this study confirmed that in the subgroup of influenza patients on ECMO, the incidence of IPA was 14%.[35] A final limitation is the choice of our comparison group. By choosing severe CAP patients as controls we can only conclude that compared with influenza-negative patients with CAP, the presence of influenza is a risk factor for IPA. Several other patient groups could have been chosen as controls as well (e.g. non-infectious ARDS) but we preferred a control group that was comparable to the influenza cases as much as possible. Therefore, we considered patients with CAP the most appropriate controls as, like influenza patients, they present with acute respiratory failure and are admitted to the ICU from the community.

CONCLUSION

In ICU patients with influenza, the incidence of IPA was high as was the mortality. Influenza was independently associated with IPA. An aggressive diagnostic approach should be pursued and the value of antifungal prophylaxis studied.

Contributors

Study design: A.F.A.D.S., J.W., B.J.A.R.; information technology and database: N.P., R.V., A.F.A.D.S.; data preparation: all authors; data collection: N.P., R.V., L.V., A.F.A.D.S; data analysis: N.P., J.W. R.V., B.J.A.R., A.F.A.D.S.; statistical analysis: E.R.A., R.V., A.F.A.D.S.; writing of first draft: A.F.A.D.S, J.W., B.J.A.R; revision of manuscript: all authors.

Declaration of interests

BJAR received research grants from Gilead and MSD outside the context of this study. He also received travel grants from MSD, Gilead, BMS, Jansen-Cilag, ViiV and Abbvie and received personal fees from Gilead, Viiv and Great-Lake pharmaceuticals. He served as an advisor to Gilead, ViiV, BMS, Abbvie, Jansen-Cilag and MSD. AFADS received travel grants to attend international conferences from Gilead, Pfizer and Roche outside the context of this study. JW received research grants from Pfizer and MSD outside the

context of this study. He also received travel grants from MSD, Gilead and Pfizer. KL received research grants from Pfizer, Gilead and MSD outside the context of this study. She also received travel grants from MSD, Gilead and Pfizer and served as an advisor for Pfizer and MSD. PEV reports research grants from F2G, MSD, Gilead Sciences and non-financial support from OLM diagnostics and IMMY diagnostics outside the context of this study. All other authors: none to declare.

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