Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study


Summary

Background Invasive pulmonary aspergillosis 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 invasive pulmonary aspergillosis. We aimed to measure the incidence of invasive pulmonary aspergillosis over several seasons in patients with influenza pneumonia in the intensive care unit (ICU) and to assess whether influenza was an independent risk factor for invasive pulmonary aspergillosis.

Methods We did a retrospective multicentre cohort study. Data were collected from adult patients with severe influenza admitted to seven ICUs across Belgium and The Netherlands during seven influenza seasons. Patients were older than 18 years, were admitted to the ICU for more than 24 h with acute respiratory failure, had pulmonary infiltrates on imaging, and a confirmed influenza infection based on a positive airway PCR test (influenza cohort). We used logistic regression analyses to determine if influenza was independently associated with invasive pulmonary aspergillosis in non-immunocompromised (ie, no 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] host factor) influenza-positive patients (influenza case group) compared with non-immunocompromised patients with severe community-acquired pneumonia who had a negative airway influenza PCR test (control group).

Findings Data were collected from patients admitted to the ICU between Jan 1, 2009, and June 30, 2016. Invasive pulmonary aspergillosis was diagnosed in 83 (19%) of 432 patients admitted with influenza (influenza cohort), a median of 3 days after admission to the ICU. The incidence was similar for influenza A and B. For patients with influenza who were immunocompromised, incidence of invasive pulmonary aspergillosis was as high as 32% (38 of 117 patients), whereas in the non-immunocompromised influenza case group, incidence was 14% (45 of 315 patients). Conversely, only 16 (5%) of 315 patients in the control group developed invasive pulmonary aspergillosis. The 90-day mortality was 51% in patients with influenza and invasive pulmonary aspergillosis and 28% in the influenza cohort without invasive pulmonary aspergillosis (p=0·0001). In this study, influenza was found to be independently associated with invasive pulmonary aspergillosis (adjusted odds ratio 5·19; 95% CI 2·63–10·26; p<0·0001), along with a higher APACHE II score, male sex, and use of corticosteroids.

Interpretation Influenza was identified as an independent risk factor for invasive pulmonary aspergillosis and is associated with high mortality. Future studies should assess whether a faster diagnosis or antifungal prophylaxis could improve the outcome of influenza-associated aspergillosis.

Funding None.

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Introduction

Invasive pulmonary aspergillosis typically occurs in a severely immunocompromised host, and isolation of Aspergillus species in the immunocompetent host is mostly considered colonisation.1,2 The 6-week mortality of invasive pulmonary aspergillosis is 20–30%2,3 but is much higher in patients who are critically ill.4 Influenza is a common viral respiratory tract infection. In a subset of patients with influenza, intensive care admission might be needed because of bacterial superinfection,5,6 but influenza itself can also cause severe acute respiratory distress syndrome (ARDS), which is associated with a mortality of 14–41%.7,8 Influenza-associated aspergillosis was occasionally described decades ago, and several small case series have been reported in the past 5–10 years.8,9,10 65% of the reported cases did not have classic host factors for invasive pulmonary aspergillosis 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).11 These
Evidence before this study
We searched PubMed for articles published between Jan 1, 1963, and Oct 31, 2017, using the search terms “influenza” and “aspergillus” or “aspergillosis”. This search yielded case series which described invasive pulmonary aspergillosis in patients admitted to the intensive care unit (ICU) with influenza. Yet, a systematic evaluation of the risk of invasive pulmonary aspergillosis 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 invasive pulmonary aspergillosis in 432 ICU patients with influenza. 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 invasive pulmonary aspergillosis. Several conclusions could be drawn. First, the incidence of invasive pulmonary aspergillosis was higher than 10% in each of the seven seasons and was almost equal in patients with influenza A and those with influenza B. Therefore, once a patient with influenza needs intensive care support, the risk for invasive pulmonary aspergillosis 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 invasive pulmonary aspergillosis was very substantial at 51%. Finally, we compared 315 non-immunocompromised (ie, no EORTC/MSG host factor) influenza-positive patients with an equal number of non-immunocompromised influenza-negative patients with severe community-acquired pneumonia for the occurrence of invasive pulmonary aspergillosis. We showed that influenza was independently associated with invasive pulmonary aspergillosis (aOR 5·19, 95% CI 2·63–10·26, 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.

EORTC/MSG criteria are used to classify patients with a fungal infection into proven, probable, or possible aspergillosis but are not necessarily applicable to the intensive care unit (ICU) setting. For the ICU setting, an algorithm (AspICU algorithm) was described by Blot and colleagues12 to distinguish invasive pulmonary aspergillosis from Aspergillus colonisation in patients who are critically ill.

In 2012, Wauters and colleagues9 reported an incidence of less than 1% of proven or probable invasive pulmonary aspergillosis. This incidence was based on a retrospective study including 41 patients admitted to the ICU from outside the hospital with respiratory failure not being the primary reason for ICU admission. Exclusion criteria for all patients were respiratory failure not being the primary reason for ICU admission, insufficient available information, and a history of invasive pulmonary aspergillosis. To be on the conservative side, we also excluded all patients in whom the only mycological evidence for invasive pulmonary aspergillosis was a positive culture from the lower respiratory tract (sputum, tracheal aspirate) for Aspergillus species, but who had a negative or unavailable bronchoalveolar lavage (BAL) culture or galactomannan test. These patients were defined as colonised and were excluded from the study.13

The specific inclusion criteria for the influenza cohort was a confirmed influenza infection based on a positive

Methods

Study design and participants
We did a retrospective multicentre cohort study in seven tertiary care ICUs (two in Belgium and five in The Netherlands). We included a cohort of patients with severe influenza, and a control group of patients with severe community-acquired pneumonia without influenza that were not immunocompromised. These patients were selected as a control group, as people with severe community-acquired pneumonia are admitted to the ICU from outside the hospital with respiratory insufficiency due to pneumonia, similar to patients with influenza.

All patients were older than 18 years, were admitted to the ICU for more than 24 h with acute respiratory failure during influenza seasons 2009–16, and had pulmonary infiltrates on imaging. Exclusion criteria for all patients were respiratory failure not being the primary reason for ICU admission, insufficient available information, and a history of invasive pulmonary aspergillosis. To be on the conservative side, we also excluded all patients in whom the only mycological evidence for invasive pulmonary aspergillosis was a positive culture from the lower respiratory tract (sputum, tracheal aspirate) for Aspergillus species, but who had a negative or unavailable bronchoalveolar lavage (BAL) culture or galactomannan test. These patients were defined as colonised and were excluded from the study.13

The specific inclusion criteria for the influenza cohort was a confirmed influenza infection based on a positive
airway PCR test. The strategy for identifying these patients consisted of reviewing all patients with a positive influenza PCR in the registry of the local microbiology department and matching these with ICU admissions (influenza cohort). The influenza cohort was further divided into patients that were non-immunocompromised and those that were immunocompromised according to the EORTC/MSG criteria (appendix). The influenza patients who were non-immunocompromised comprised the influenza case group.

As with the strategy for identifying influenza patients, for the community-acquired pneumonia control group we retrieved a list of patients with a negative influenza PCR from the microbiology departments and we matched these patients for ICU admission. We assessed whether antibiotic therapy was initiated, and whether a diagnosis of community-acquired pneumonia was made at ICU admission. We excluded the patients in whom influenza was diagnosed in the referral centre and we excluded patients being admitted to ICU with hospital-acquired pneumonia. Similar to the influenza case group, the control group had to be non-immunocompromised.

The occurrence of invasive pulmonary aspergillosis was compared between the influenza case group and the influenza-negative control group of patients with community-acquired pneumonia; however, the terms cases and controls do not point towards a case-control study design from a methodological point of view.

The definition used to diagnose invasive pulmonary aspergillosis was modified from the AspICU algorithm and was based on the presence of clinical, radiological, and mycological criteria in all patients with invasive pulmonary aspergillosis (panel). Every patient with influenza was reviewed and consensus was achieved to ascertain whether the modified invasive pulmonary aspergillosis definition was met. Patients in the control group were reviewed in the same way.

The study protocol was approved by the institutional review board (IRB) of both Belgian sites (Ghent University Hospital and University Hospitals of Leuven) and by the IRB of the initiating Dutch centre (Erasmus University Medical Centre, Rotterdam) for the five Dutch sites.

**Statistical analysis**

In univariable analysis, we compared categorical variables by Fisher’s exact test and χ² test, and continuous variables with t test or Mann-Whitney U test where appropriate. For the entire population of the influenza cohort, we did a multivariable analysis by binary logistic regression to detect independent risk factors for the development of invasive pulmonary aspergillosis. The dependent variable was the presence of invasive pulmonary aspergillosis and independent variables were those previously described as a possible risk factors for invasive pulmonary aspergillosis in the ICU or associated with infection in the univariable analysis. The estimate of association was expressed as adjusted odds ratio (aOR) with corresponding confidence intervals of 95%. We did multiple imputations to handle
missing data, using 20 imputations and 1000 iterations following the Markov-Chain Monte Carlo methods. Additionally, we did a binary logistic regression analysis with multiple imputations on the pooled cohort of influenza cases and controls to determine if influenza was independently associated with invasive pulmonary aspergillosis. We analysed data with SPSS version 24 (IBM, Armonk, NY, USA). We did no correction for multiple testing for the univariable analyses and we used a two-tailed significance level of 0·05. These p values should therefore be interpreted with this limitation in mind. A statistician from the department of Biostatistics of Erasmus University Medical Center supervised the analysis.

Role of the funding source
There was no funding source for this study. The corresponding author had full access to all the data and the final responsibility to submit for publication.

Results
Between Jan 1, 2009, and June 30, 2016, 541 patients with influenza were admitted to seven ICUs. 84 patients were excluded for the following reasons: respiratory insufficiency was not the reason for ICU admission (n=67), medical history of invasive pulmonary aspergillosis (n=9), or insufficient clinical data (n=8). Another 25 patients were excluded because they met the criteria for Aspergillus colonisation. In total,
432 patients with influenza were included in the influenza cohort. 315 of whom were included in the influenza case group. The strategy for identifying participants for the control group resulted in the selection of 315 patients with severe community-acquired pneumonia (figure 1).

Patient characteristics of the influenza cohort are summarised in table 1. Mean age was 59 years and 240 (56%) of 432 patients were men. 355 (82%) of 432 patients had influenza A and 77 (18%) of 432 patients had influenza B. 338 (79%) of 428 patients received a neuraminidase inhibitor. 117 (27%) of 432 patients were...
EORTC/MSG host-factor positive. The mean acute physiology and chronic evaluation (APACHE) II score was 22 (8). 326 (75%) of 432 patients required intubation for mechanical ventilation for a median duration of 11 days (IQR 5–21). 52 (12%) of 432 patients received extra-corporal membrane oxygenation (ECMO). 107 (25%) of 432 patients in the influenza cohort died in the ICU.

83 (19%) of 432 patients in the influenza cohort fulfilled the modified invasive pulmonary aspergillosis definition (panel). The proportion of patients with invasive pulmonary aspergillosis varied per centre (6%–26%; appendix). Invasive pulmonary aspergillosis was diagnosed at a median of 3 days (IQR 0–7) after ICU admission. Aspergillus fumigatus was almost exclusively cultured when identification to the species level was available. Susceptibility data were available in 17 patients and four voriconazole-resistant strains were documented. Although the number of patients admitted to the ICU with influenza varied substantially from year to year, the prevalence of invasive pulmonary aspergillosis was greater than 10% in all calendar years (appendix). Invasive pulmonary aspergillosis was found in 71 (20%) of 355 patients with influenza A and 12 (16%) of 77 patients with influenza B. No clear association was shown between the prevalence of invasive pulmonary aspergillosis and the influenza subtypes that circulated in the respective calendar years (appendix).

In 81 (98%) of 83 patients with invasive pulmonary aspergillosis in the influenza cohort a BAL culture was done, yielding a positive Aspergillus culture in 50 (63%) of 80 patients, and a positive galactomannan test (optical density (OD) ≥1-0) in 67 (88%) of 76 patients in whom the BAL was not only cultured but also tested for the presence of galactomannan (table 2). A serum galactomannan test was done in 31 (37%) of 83 patients, yielding a positive Aspergillus species in 20 (65%) patients. A BAL galactomannan test was done in 67 (88%) of 76 patients, while 30 (36%) patients were diagnosed with proven (n=16) or putative (n=32) aspergillosis according to the EORTC/MSG criteria (panel).11 According to the modified invasive pulmonary aspergillosis definition only 36 (43%) of 83 patients had a positive galactomannan test (optical density ≥0·5) in 20 (65%) patients.

Figure 2: Forest plots of risk factors for the development of invasive pulmonary aspergillosis

These have been corrected for centre as well but this is not depicted here as no significant differences were found.

(A) Analysis of risk factors for the influenza cohort to develop invasive pulmonary aspergillosis. (B) Overview of comparison between the influenza case group and the control group. aOR=adjusted odds ratio. APACHE=acute physiology and chronic evaluation score. COPD=chronic obstructive pulmonary disease. *Factors independently associated with the development of invasive pulmonary aspergillosis.
In the influenza cohort (table 1), ICU mortality was higher in patients with invasive pulmonary aspergillosis (37 [45%] of 83 patients) than in patients without it (70 [20%] of 349 patients; p=0.0001) and the ICU stay was longer (19 days [IQR 12–38] vs 9 days [IQR 5–20]; p=0.0001). The mortality 90 days after ICU admission was 51% (42 of 83 patients) in those with invasive pulmonary aspergillosis and 28% (99 of 349 patients) in those without it (p=0.0001). Patients with invasive pulmonary aspergillosis required mechanical ventilation more often (75 [90%] of 83 patients vs 251 [72%] of 349 patients; p=0.0004) and for a longer period (plus 5 days; p=0.001) than did patients without it.

Independent risk factors for the occurrence of invasive pulmonary aspergillosis on the pooled data of all patients in the influenza cohort (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 analysis is presented in table 3A.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>All EORTC/MSG negative (non-immunocompromised) patients (n=650)</th>
<th>Influenza case group (n=315)*</th>
<th>Control group (n=315)*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (SD)</td>
<td>59 (17)</td>
<td>58 (16)</td>
<td>60 (17)</td>
<td>0.15</td>
</tr>
<tr>
<td>Male sex</td>
<td>371 (59%)</td>
<td>169 (54%)</td>
<td>202 (64%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean APACHE II score on admission (SD)</td>
<td>23 (8)</td>
<td>22 (8)</td>
<td>23 (8)</td>
<td>0.29</td>
</tr>
<tr>
<td>Median body mass index, kg/m² (IQR), missing</td>
<td>25 (22–29), 21</td>
<td>27 (23–30), 18</td>
<td>24 (22–28), 3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>114 (19%)</td>
<td>63 (20%)</td>
<td>51 (16%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>44 (7%)</td>
<td>18 (6%)</td>
<td>26 (8%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Chronic kidney disease†</td>
<td>69 (11%)</td>
<td>31 (10%)</td>
<td>38 (12%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>123 (20%)</td>
<td>68 (22%)</td>
<td>55 (17%)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Corticosteroids</th>
<th>All EORTC/MSG negative (non-immunocompromised) patients (n=650)</th>
<th>Influenza case group (n=315)*</th>
<th>Control group (n=315)*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids 28 days before ICU admission</td>
<td>99/619 (16%)</td>
<td>57/304 (19%)</td>
<td>42/315 (13%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Median dose corticosteroids 28 days before ICU admission (mg/kg/day, missing)</td>
<td>0.078 (0.054–0.176), 22</td>
<td>0.070 (0.054–0.171), 10</td>
<td>0.080 (0.055–0.179), 12</td>
<td>0.79</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>ICU data</th>
<th>All EORTC/MSG negative (non-immunocompromised) patients (n=650)</th>
<th>Influenza case group (n=315)*</th>
<th>Control group (n=315)*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical ventilation</td>
<td>475 (75%)</td>
<td>246 (78%)</td>
<td>229 (73%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Median ventilation days (IQR), missing</td>
<td>9 (4–18), 35</td>
<td>11 (5–21), 26</td>
<td>4 (4–14), 9</td>
<td>0.002</td>
</tr>
<tr>
<td>Nitric oxide or high-frequency oscillation ventilation</td>
<td>64 (10%)</td>
<td>37 (12%)</td>
<td>27 (9%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Extracorporeal membrane oxygenation</td>
<td>65 (10%)</td>
<td>45 (14%)</td>
<td>20 (6%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Median extracorporeal membrane oxygenation days (IQR)</td>
<td>10 (6–20)</td>
<td>11 (8–21)</td>
<td>9 (5–18)</td>
<td>0.44</td>
</tr>
<tr>
<td>Vasopressors</td>
<td>415 (66%)</td>
<td>216 (69%)</td>
<td>199 (63%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td>103 (16%)</td>
<td>61/300 (20%)</td>
<td>42 (13%)</td>
<td>0.03</td>
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</table>

<table>
<thead>
<tr>
<th>Outcome data</th>
<th>All EORTC/MSG negative (non-immunocompromised) patients (n=650)</th>
<th>Influenza case group (n=315)*</th>
<th>Control group (n=315)*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU mortality</td>
<td>125 (20%)</td>
<td>58 (18%)</td>
<td>67 (21%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Hospital mortality</td>
<td>164 (26%)</td>
<td>76 (24%)</td>
<td>88 (28%)</td>
<td>0.28</td>
</tr>
<tr>
<td>90-day mortality after ICU admission</td>
<td>177 (28%)</td>
<td>78 (25%)</td>
<td>99 (31%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Median days of ICU stay (IQR), missing</td>
<td>11 (6–21), 19</td>
<td>11 (6–23), 15</td>
<td>10 (6–18), 4</td>
<td>0.15</td>
</tr>
<tr>
<td>Invasive pulmonary aspergillosis</td>
<td>61 (10%)</td>
<td>45 (14%)</td>
<td>16 (5%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnostics</th>
<th>All EORTC/MSG negative (non-immunocompromised) patients (n=650)</th>
<th>Influenza case group (n=315)*</th>
<th>Control group (n=315)*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL sampling performed</td>
<td>318 (50%)</td>
<td>145 (46%)</td>
<td>173 (55%)</td>
<td>0.026</td>
</tr>
<tr>
<td>BAL galactomannan test performed</td>
<td>187 (30%)</td>
<td>81 (26%)</td>
<td>106 (34%)</td>
<td>0.029</td>
</tr>
<tr>
<td>AspICU classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven (of people with IPA)</td>
<td>8 (13%)</td>
<td>6 (13%)</td>
<td>2 (13%)</td>
<td>--</td>
</tr>
<tr>
<td>Putative (of people with IPA)</td>
<td>32 (52%)</td>
<td>27 (60%)</td>
<td>5 (31%)</td>
<td>--</td>
</tr>
<tr>
<td>Coloniser (of people with IPA)</td>
<td>4 (7%)</td>
<td>3 (7%)</td>
<td>1 (6%)</td>
<td>--</td>
</tr>
<tr>
<td>Non-classifiable (of people with IPA)</td>
<td>17 (28%)</td>
<td>9 (20%)</td>
<td>8 (50%)</td>
<td>--</td>
</tr>
</tbody>
</table>

Data are n (%) or n/N (%), unless otherwise specified. Where no denominator is specified, the denominator is the number of participants in the corresponding cell in the first row. 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. APACHE=acute physiology and chronic evaluation score. ICU=intensive care unit. BAL=bronchoalveolar lavage. AspICU=algorithm for invasive aspergillosis in ICU as described by Blot and colleagues. IPA=invasive pulmonary aspergillosis. *The influenza case group included patients with influenza who were non-immunocompromised (ie, without EORTC/MSG host factor). The control group included patients without influenza and without EORTC/MSG host factor, who were admitted to the ICU with community-acquired pneumonia. †Glomerular filtration rate <60 mL/min/1·73 m².

Table 3: Characteristics of patients in the influenza case and control group.
analyses can be found in the appendix. Corticosteroid therapy in the 4 weeks before ICU admission was independently associated with invasive pulmonary aspergillosis (aOR 1.59; 95% CI 1.30–1.99; p<0.0001) per 0.1 mg/kg/day prednisone equivalent). Male sex (aOR 1.84; 95% CI 1.05–3.22; p=0.034) and a higher admission APACHE II score (aOR 1.05; 95% CI 1.01–1.09; p=0.007 per 1.0 point APACHE II increase) were also associated with invasive pulmonary aspergillosis.

45 (14%) of 315 patients in the influenza case group were diagnosed with invasive pulmonary aspergillosis compared with 16 (5%) of 315 patients in the control group. The characteristics of these two groups are summarised in table 3. BAL sampling and galactomannan measurement on BAL was more frequently performed in the control group (BAL in 173 (55%) of 315 patients, galactomannan in 106 (34%) of 315 patients) than in the influenza case group (BAL in 145 (46%) of 315 patients, galactomannan in 81 (26%) of 315 patients). To assess whether influenza was independently associated with invasive pulmonary aspergillosis in the pooled patient population of the influenza case group and control group, a binary logistic regression analysis was done. This analysis confirmed an independent association between influenza and invasive pulmonary aspergillosis (aOR 5.19; 95% CI 2.63–10.26; p<0.0001) (figure 2B). A list of all variables used in the multivariate analyses can be found in the appendix. Other independent risk factors for invasive pulmonary aspergillosis in this analysis were male sex and receipt of corticosteroids in the 4 weeks preceding ICU admission (because all patients in this analysis were EORTC/MSG host-factor negative, the corticosteroids had been used at a median dose <0.3 mg/kg/day in these 4 weeks).

Discussion

To the best of our knowledge, this study is the largest ever done on the incidence, risk factors, and outcome of invasive pulmonary aspergillosis in patients with influenza in the ICU. Furthermore, the data provide evidence that influenza infection is an independent risk factor for invasive pulmonary aspergillosis. Indeed, of 630 non-immunocompromised patients admitted to the ICU with community-acquired pneumonia, 50% infected with influenza, the presence of influenza increased the risk of invasive pulmonary aspergillosis from 5% to 14%. Furthermore, mortality in patients with influenza-associated invasive pulmonary aspergillosis in the ICU was 45% and even in previously healthy individuals, mortality was 33%. These mortality results are in accordance with the 47% mortality described in earlier case series of influenza cases but somewhat lower than described in cohorts over the past 10 years.9,10,11 Of note, 85 patients in this influenza cohort had been included in previous studies.9,11 In the subgroup with an EORTC/MSG host factor, the invasive pulmonary aspergillosis incidence was as high as 32%, and 71% of them had died within 90 days after ICU admission. As the diagnosis of invasive pulmonary aspergillosis is challenging, a systematic approach towards its diagnosis in patients with influenza in the ICU might result in an even higher incidence of
invasive pulmonary aspergillosis and should be the focus of future prospective studies.

The reported overall incidence of invasive pulmonary aspergillosis in patients who are critically ill varied widely from less than 1% to 6·9%\(^{15,17,18}\) and corresponded with the 5% incidence in our control group.\(^{9,13}\) A study\(^{9}\) of 2901 patients with influenza in the ICU showed the presence of a co-infection in 17%, of which \textit{Aspergillus} species accounted for 7%. The lower incidence in this study could be explained by a different diagnostic approach (eg, no use of BAL galactomannan measurement), a lower overall awareness, and the fact that only co-infections diagnosed within 2 days of hospital admission were registered.

As influenza is not considered a host factor for invasive pulmonary aspergillosis, only some of our patients with invasive pulmonary aspergillosis fulfilled one of the diagnostic criteria, as defined by the EORTC/MSG or \textit{AspiICU} algorithm.\(^{11,12}\) Additionally, patients with influenza and invasive pulmonary aspergillosis mostly have non-specific radiology, and classic radiological features only occur in 5% of patients with invasive pulmonary aspergillosis who are critically ill.\(^{11,12,14,22}\) Autopsy series indicated that strict interpretation of the host factors for invasive fungal disease contributes to missed diagnosis of invasive pulmonary aspergillosis.\(^{11,14}\) Therefore, we classified our patients using a modified invasive pulmonary aspergillosis definition in which no specific host factor was required. However, stringent mycological criteria were used, compatible with the case definition of EORTC/MSG, \textit{AspiICU}, and van de Veerdonk and colleagues.\(^{5–11}\) The same classification was used for the control group. Furthermore, to avoid an overestimation of the incidence of invasive pulmonary aspergillosis, we excluded all 25 patients with only a positive lower respiratory tract culture (ie, sputum or tracheal aspirate) but a negative or unavailable BAL culture as the only microbiological evidence for invasive pulmonary aspergillosis.

The OD cutoff above which a BAL galactomannan test should be considered positive is a matter of debate. The sensitivity of BAL galactomannan measurement was 88% when applying an OD of more than 0·5 in patients in the ICU with biopsy or autopsy proven invasive pulmonary aspergillosis.\(^{24}\) However, in an observational study\(^{20}\) the value of BAL galactomannan testing in the ICU was questioned because the specificity, compared with a positive BAL culture, was 38% with a galactomannan OD cutoff of more than 1·0 and 62% with a cutoff of 3·0. However, given the limited sensitivity of BAL culture for the diagnosis of invasive pulmonary aspergillosis, 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 cutoff of more than 1·0. Yet, if an OD of more than 3·0 had been applied, only eight (10%) of 83 patients with invasive pulmonary aspergillosis in the influenza cohort would have been classified differently. Additionally, the median BAL galactomannan OD of all patients with invasive pulmonary aspergillosis in the influenza cohort was as high as 5·8 (IQR 2·8-6·7). Furthermore, when we reviewed all 15 patients with proven invasive pulmonary aspergillosis who also underwent BAL galactomannan testing, 14 patients had a BAL galactomannan OD of more than 1·0. Also, all six patients without invasive pulmonary aspergillosis, as confirmed by lung autopsy, had a BAL galactomannan measurement with a value of less than 1·0. Therefore, the specificity of galactomannan in BAL with a cutoff threshold of 1·0 in our study seems to be excellent. Of 28 patients with a positive BAL galactomannan test, a serum galactomannan test was also available. 17 (61%) of these 28 patients were positive on serum as well. This result suggests that angioinvasion is often present in patients with influenza and invasive pulmonary aspergillosis.

We could not confirm the previous observation that a delayed initiation of antifungal therapy was associated with a fatal outcome.\(^{11}\) A particularly high awareness was present in one of the participating centres because this centre already published on influenza-associated invasive pulmonary aspergillosis in 2012. In this centre, BAL sampling was done in 102 of 149 patients with influenza. 26% of patients in this centre fulfilled the invasive pulmonary aspergillosis diagnosis with an ICU mortality of 38% compared with an ICU mortality of influenza-associated aspergillosis in all other centres of 50%. This difference suggests that increased awareness might 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 four of them.

Why patients with influenza are at risk for invasive pulmonary aspergillosis is not yet clear.\(^{27,28}\) Respiratory epithelial damage and mucociliary clearance dysfunction might facilitate the invasion of \textit{Aspergillus} (figure 3).\(^{3,29}\) Moreover, influenza-induced ARDS and hypoxia might cause immune paralysis.\(^{30–32}\) Almost all cases to date have been associated with the pandemic influenza A H1N1 infection but influenza B could also trigger an \textit{Aspergillus} superinfection.\(^{31,35}\) This observation was confirmed in this study because an almost equal proportion of patients with influenza A or influenza B developed invasive pulmonary aspergillosis. We were unable to look at the influenza subtype as a possible risk factor for invasive pulmonary aspergillosis because subtyping was only available in a small number of patients. However, no association could be found between invasive pulmonary aspergillosis and the influenza subtypes that circulated in our countries during the respective calendar years. Furthermore, the fact that the incidence of invasive pulmonary aspergillosis was more than 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 also applies to patients with pneumonia admitted to the ICU with a respiratory virus other than influenza remains to be seen. The observation that the use of corticosteroids before ICU admission was independently associated with invasive pulmonary aspergillosis is in accordance with a Cochrane review showing an association between corticosteroid use and increased influenza mortality. Conversely, corticosteroid 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 corticosteroids in patients with influenza argues against its use, as long as data from prospective randomised clinical trials are scarce.

Given the high incidence of invasive pulmonary aspergillosis we observed, antifungal prophylaxis might be a valid approach. Whether antifungal prophylaxis will be superior to a standardised diagnostic approach combined with prompt initiation of antifungal therapy as soon as invasive pulmonary aspergillosis is diagnosed, remains to be shown.

Our study had some limitations. First, given the retrospective design of this study, confounding cannot be ruled out and a standardised diagnostic approach towards invasive pulmonary aspergillosis was not used. However, the time needed to collect a similar amount of data prospectively clearly argues for the added value of this retrospective study. Also, because we did not correct for multiple testing, all univariate p values should be interpreted with this in mind. Second, as only 60% of patients with invasive pulmonary aspergillosis had a positive BAL culture, the diagnosis of invasive pulmonary aspergillosis was based on a positive BAL galactomannan test in a substantial number of patients. Given the observation that BAL sampling was done in 98% of patients with influenza and invasive pulmonary aspergillosis, but only in 44% of patients with influenza but without invasive pulmonary aspergillosis, we cannot exclude that the actual incidence of invasive pulmonary aspergillosis might be even higher. We have no reason to believe that compared with the influenza cohort, a risk of underdiagnosis of invasive pulmonary aspergillosis in the control group was present. Actually, BAL galactomannan sampling was more often done in our control group. Third, in a subset of the patients, invasive pulmonary aspergillosis might have developed before ICU admission and might 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, invasive pulmonary aspergillosis is highly prevalent and associated with a high mortality. A fourth limitation is that all but one of the seven centres were tertiary care academic ICUs. Therefore, extrapolation to small primary care ICUs should be done with caution. However, the incidence of invasive pulmonary aspergillosis in the single primary care ICU of this study was comparable at 15%. The use of ECMO support was somewhat higher in the influenza cohort (14%) than in the control group (6%), and therefore ECMO might be a confounder in the analysis. However, only four of 83 patients in the influenza cohort were diagnosed with invasive pulmonary aspergillosis more than 72 h after the start of ECMO support. Also, in a 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 patients with influenza on ECMO, the incidence of invasive pulmonary aspergillosis was 14%. A final limitation is the choice of our comparison group. By choosing patients with severe community-acquired pneumonia as controls, we can only conclude that the presence of influenza is a risk factor for invasive pulmonary aspergillosis compared with this control group. Several other patient groups (eg, non-infectious ARDS) could also have been chosen, but we preferred a control group that was most similar to the influenza cohort. Therefore, we considered patients with community-acquired pneumonia the most appropriate controls because, similar to patients with influenza, they present with acute respiratory failure and are admitted to the ICU from the community.

In conclusion, in patients with influenza admitted to the ICU, the incidence of invasive pulmonary aspergillosis was high, as was the mortality. Influenza was independently associated with invasive pulmonary aspergillosis. An aggressive diagnostic approach should be pursued and the value of antifungal prophylaxis should be studied.

**Contributors**

AFADS, JW, and BJAR designed the study. NP, RV, and AFADS coordinated the information technology and database. All authors prepared the data. NP, RV, LV, and AFADS collected the data. NP, JW, RV, BJAR, and AFADS analysed the data. E-RA, RV, and AFADS completed the statistical analysis. AFADS, JW, and BJAR wrote the first draft. All authors revised the manuscript.

**Declaration of interests**

BJAR received research grants from Gilead and MSD outside of the context of this study, travel grants from MSD, Gilead, BMS, Jansen-Cilag, ViV, and Abbvie, personal fees from Gilead, ViV, and Great-Lake pharmaceuticals, and served as an adviser to Gilead, ViV, BMS, Jansen-Cilag, and Merck Sharp and Dohme (MSD). AFADS received travel grants to attend international conferences from Gilead, Pfizer, and Roche outside of the context of this study. JW received research grants from Pfizer and MSD outside of the context of this study; received travel grants from MSD, Gilead, and Pfizer. KL received research grants from Pfizer, Gilead, and MSD outside of the context of this study. RV received travel grants from MSD, Gilead, and Pfizer, and served as an adviser for Pfizer and MSD. PEV reports research grants from F2G, MSD, and Gilead Sciences, and non-financial support from OLM Diagnostics and IMMY Diagnostics outside of the context of this study. All other authors declare no competing interests.

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