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Influenza-associated invasive aspergillosis in the ICU: a prospective, multicentre cohort study

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Abstract

Background Influenza-associated pulmonary aspergillosis (IAPA) can develop in critically ill patients with influenza in the intensive care unit (ICU), even in absence of classical risk factors. Thus far, most studies have been retrospective and the reported incidence has varied. Therefore, we set out to prospectively investigate IAPA incidence, potential risk factors and impact on patient outcomes.

Methods A prospective, multicentre observational cohort study was performed in the Netherlands and Belgium during three influenza seasons (2017–2020). Adult patients with PCR confirmed influenza or non-influenza community-acquired pneumonia (niCAP; control group) admitted to ICU with respiratory distress were included. Diagnosis of niCAP and mycological diagnostic work-up was at the treating physicians' discretion. IAPA was defined according to the 2020 expert opinion case definition.

Results Invasive aspergillosis occurred in 24% (34/140) of patients with influenza, compared to 13% (10/76) of patients with niCAP ($p=0.054$) undergoing mycological diagnostic work-up and radiological imaging. IAPA was diagnosed at a median of 4 days after ICU admission. Patients with IAPA did not differ from those with influenza alone regarding presence of European Organization for Research and Treatment of Cancer/Mycosis Study Group Education and Research Consortium (EORTC/MSGERC) host factors (9/32 [28%] versus 22/85 [26%], respectively; $p=0.82$). They more frequently required invasive ventilatory support (26/29 [90%] versus 50/88 [57%], respectively; $p=0.001$) and renal replacement therapy (15/33 [45%] versus 13/103 [13%], respectively; $p<0.001$) in ICU.

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IAPA ICU mortality was significantly higher with 44% (15/34) compared to 14% (15/106) for influenza without aspergillosis (p < 0.001) and 17% (13/76) for niCAP. Non-survivors with IAPA were predominantly male (13/15 [87%] versus 9/19 [47%] survivors; p = 0.030) and more frequently received systemic corticosteroids in ICU (14/15 [93%] versus 10/19 [53%] survivors; p = 0.020). IAPA was an independent predictor of ICU mortality in patients with influenza (adjusted hazard ratio 1.99 [95% confidence interval 1.05 – 3.76]; p = 0.035).

Conclusions We demonstrate prospectively that IAPA is a frequent complication in critically ill patients with influenza and that it is associated with high mortality and an adverse clinical course, rendering increased awareness among treating physicians imperative.

Keywords Influenza-associated Pulmonary Aspergillosis, Invasive Aspergillosis, Human Influenza, Critical Illness

Background

Invasive pulmonary aspergillosis (IPA) characteristically develops in immunocompromised patients. However, IPA can also develop in critically ill patients with influenza, admitted to the intensive care unit (ICU), then called influenza-associated pulmonary aspergillosis (IAPA), which can occur in the absence of classical risk factors [1]. Although IAPA has been described occasionally during past decades, in recent years increasing numbers of cases and studies have been published. Reported IAPA incidence in ICU cohort studies varies widely from 0 to 45%, with substantial variability in study designs, study populations, and IPA diagnostic criteria [1–20]. Variation between influenza seasons and lack thereof have both been reported [12]. Furthermore, for many studies it is uncertain how many patients underwent mycological testing to diagnose IAPA, complicating interpretation of reported incidence. To date, nearly all studies investigating IAPA have been retrospective. Six studies have reported prospective data on IAPA occurrence; three were not specifically aimed at aspergillosis, but at co-infections in influenza in general, and one had a study population of 17 patients [2, 3, 7].

IPA diagnosis is commonly based on the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) definitions of invasive fungal disease [21, 22]. Based on histopathological findings, predisposing host factors, clinical features and mycological evidence, categories of proven, probable and possible IPA are distinguished. However, patients admitted to ICU lacking host factors can develop IPA as well, frequently without developing characteristic radiological abnormalities, rendering application of the EORTC/MSGERC criteria problematic [23, 24]. An alternative algorithm (the *Asp*ICU criteria) to establish the likelihood of IPA in ICU patients with lower respiratory tract specimen cultures positive for *Aspergillus* was developed, but does not incorporate broncho-alveolar lavage (BAL) fluid and serum galactomannan (GM), potentially affecting its performance [25].

In 2020, an expert opinion-based case definition for IAPA was published to overcome diagnostic criteria variability used in studies [26]. It constitutes classification criteria specifically aimed at patients with influenza in ICU and includes BAL fluid and serum GM as mycological evidence of IAPA.

We performed a prospective cohort study over three consecutive influenza seasons between 2017–2020 in patients admitted to ICU with severe influenza, compared with non-influenza community-acquired pneumonia (niCAP), to determine IAPA/IPA incidence, risk factors and IAPA's impact on outcome, using this case definition. Notably, our prospective study ended when the COVID-19 pandemic arrived. Although we had planned to continue, there were no patients admitted to ICU with influenza and this only started to recur significantly in 2022–2023. Therefore, our prospective cohort is unique in the pre-COVID era and has the advantage to demonstrate the true effect of influenza only on the development of aspergillosis in the ICU.

Methods

Study design and participants

We performed a prospective, multicentre, observational cohort study during three consecutive influenza seasons (2017–2020). During 2017/2018, one Dutch and three Belgian centres participated in the study. The subsequent year, this was expanded to four Dutch and four Belgian sites (with one site substitution). In addition, we included patients from the POSA-FLU trial (ClinicalTrials.gov identifier NCT03378479) from centres participating in both studies [27]. In this randomised, open-label, proof-of-concept trial to assess the efficacy of posaconazole prophylaxis in preventing occurrence of IAPA, adult patients with influenza and respiratory distress were randomised to either posaconazole prophylaxis or standard-of-care (SOC) and underwent a standardised diagnostic mycological work-up at study inclusion. For the current study, we only included patients from the SOC arm of the POSA-FLU trial. For each included patient with influenza, we attempted to include one randomly selected patient with niCAP admitted to ICU in the same

participating centre during the same influenza season (if possible).

All patients aged > 18 years with either polymerase chain reaction (PCR) confirmed influenza or severe community-acquired pneumonia with confirmed absence of influenza (niCAP; control group) admitted to participating ICUs because of respiratory distress were eligible for inclusion. Diagnosis of niCAP and performance of mycological diagnostic work-up (including bronchoscopy and BAL) was at the discretion of the treating physician. We retrospectively excluded patients if influenza was diagnosed > 7 days after ICU admission.

No standardised diagnostic protocol for IAPA/IPA was imposed across the participating centres, in line with the observational nature of this study.

Patients from the POSA-FLU trial underwent BAL for culture and GM (if considered safe) as well as serum GM testing on day one of ICU admission and thereafter at the discretion of the treating physician in case of respiratory deterioration, according to study protocol.

Follow-up continued until 90 days after ICU admission or ICU discharge, whichever occurred last.

Ethical statement

For participating Dutch centres, the study protocol (CMO 2016–3037) was reviewed and approved by the ethics board region Arnhem-Nijmegen before study initiation. Based on the study's observational nature, written informed consent was waived. Patients were not included if they (or their legal representatives) actively objected to participation.

For participating Belgian centres, the study protocol was reviewed and approved by their individual ethical committees (S60757). Written informed consent was obtained for all Belgian participants.

Participants in the POSA-FLU trial all provided written informed consent. This study's protocol was reviewed and approved by the ethics board region Arnhem-Nijmegen for all Dutch participating sites (CMO 2018–4041) and by the ethical committees of all participating centres in Belgium (S60744).

This study was performed in accordance with the latest version of the declaration of Helsinki and local legislation/regulations.

Data collection

Local investigators collected pseudonymised patient data from participants' medical files in an electronic case report form (Castor electronic data capture, Amsterdam, the Netherlands) and Microsoft Excel. We collected data on up to over 800 variables per patient, including information on demographics, past medical history, laboratory and microbiological diagnostic findings, treatment at the time of ICU admission and during ICU stay (including antifungal and corticosteroid treatment), clinical outcome and follow-up at days 30 and 90 after ICU admission and at ICU discharge.

Definitions

IAPA diagnosis was based on the expert opinion case definition published in 2020 and was applied to both the influenza and niCAP cohort (excluding presence of influenza as a criterion in the latter) in retrospect (based on the prospectively collected data). [26].

We defined the following populations (see Supplementary Methods, Additional Files):

1. Total influenza and niCAP cohorts: All included patients with influenza and niCAP.
2. Classifiable influenza and niCAP cohorts: Patients who underwent any of the mycological tests and radiological imaging as specified in the case definition; if one or both criteria (radiological and/or mycological) were negative, patients were classified as "No IAPA/IPA" (and therefore had influenza without IAPA and niCAP without IPA, respectively).
3. Patients with IAPA/IPA: Classifiable patients fulfilling criteria for proven or probable IAPA/IPA.

Incidence of IAPA/IPA was calculated in the classifiable influenza and niCAP cohorts, respectively.

The number of days of illness before hospital and ICU admission refer to the number of days patients had experienced symptoms attributed to their disease episode leading to hospitalization and ICU admission, respectively.

For clinical/laboratory parameters at the time of ICU admission, the worst values at or during the first 24 h of ICU admission were used.

EORTC/MSGERC host factors were defined according to the 2008 guidelines, including a recent history of neutropenia, receipt of an allogeneic stem cell transplant, recent prolonged use of corticosteroids, treatment with other recognized T cell immunosuppressants and inherited severe immunodeficiency, but also receipt of a solid organ transplant and treatment with other recognized B cell immunosuppressants [21].

Primary and secondary outcomes

The primary outcomes were IAPA/IPA incidence in the influenza and niCAP patient group and ICU mortality, age, sex and presence of EORTC/MSGERC host factors in patients who developed IAPA/IPA as compared to those who did not.

Secondary outcomes were defined in patients who developed IAPA/IPA and include rate of disease progression, diagnostic test performance, radiological findings and presence of azole resistance in cultured *Aspergillus*

species. Ultimately, only performance of diagnostic testing could be analysed. Other analyses concern exploratory, descriptive statistics.

Statistical analysis

Data are expressed as n (%) or medians with interquartile range (IQR). Categorical variables were compared by Fisher's or Fisher-Freeman-Halton exact test, continuous variables by Mann–Whitney U test. For survival analyses, the Kaplan–Meier method and log-rank test were used. Cox regression and binary logistic regression analysis was performed to assess independent risk factors for IAPA occurrence and ICU mortality. Variables for regression analysis were selected based upon our primary research questions (e.g., influenza and EORTC/MSGERC host factors for occurrence of IAPA/IPA and occurrence of IAPA for ICU mortality), previously described results (e.g., male sex for occurrence of IAPA/IPA) and associations found in descriptive statistics of our study results. Variables with significant numbers of missing data were not included in the models (e.g., APACHE II scores). P values < 0.05 were considered statistically significant.

Statistical analyses were performed using IBM SPSS Statistics for Windows version 27 (IBM Corp., Armonk, NY, USA) or GraphPad Prism 9 for Windows (GraphPad Software, La Jolla, CA, USA).

Results

Between 28 October 2017 and 7 December 2020, 185 patients with severe influenza and 128 patients with severe niCAP admitted to ICU were screened (Fig. 1). Eleven patients with influenza were excluded: one with a false positive influenza PCR result, eight without informed consent and two testing positive for influenza > 7 days after ICU admission. Ten patients with CAP were excluded for lack of exclusion of influenza. Ultimately, 292 patients were included: 174 patients with severe influenza and 118 patients with severe niCAP (Supplementary Table 1, Additional Files). These included 37 patients from the POSA-FLU trial who received SOC.

Patients with severe influenza or niCAP underwent bronchoscopy in 88/156 (56%) and 43/105 (41%), BAL fluid GM testing in 88/160 (55%) and 44/105 (42%), BAL fluid fungal culture in 80/156 (51%) and 40/105 (38%) and tracheobronchial aspirate fungal culture in 60/147 (41%) and 5/104 (4.8%), respectively.

Influenza versus niCAP

A total of 140 patients with influenza and 76 patients with niCAP underwent mycological investigations and radiological imaging and were therefore classifiable (Fig. 1) [26]. Classifiable patients' characteristics are provided in Table 1 and Supplementary Table 1, Additional Files. Compared to those with niCAP, patients with influenza were younger (65 [IQR 54–72] versus 70 [IQR

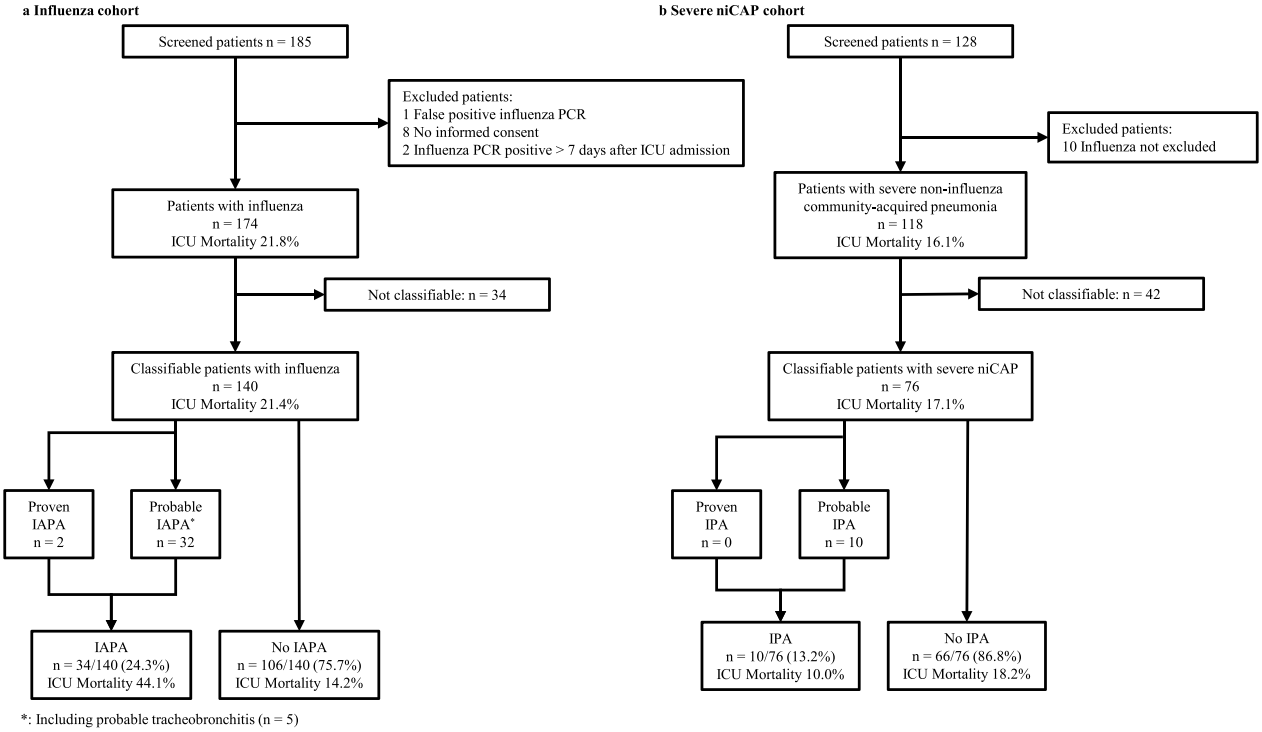


Fig. 1 Study flowchart. IAPA = Influenza-associated pulmonary aspergillosis. IPA = Invasive pulmonary aspergillosis. ICU = Intensive care unit. niCAP = Non-influenza community-acquired pneumonia. PCR = Polymerase chain reaction

Table 1 Patient characteristics of the classifiable influenza and severe non-influenza community-acquired pneumonia (niCAP) cohorts

Baseline Characteristics	Total population (n = 216)	Influenza (n = 140)	Severe niCAP (n = 76)	p value (influenza vs. niCAP)
Median age (years)	66 (55–74)	65 (54–72)	70 (60–76)	**0.015**
Male sex	139/216 (64.4%)	82/140 (58.6%)	57/76 (75.0%)	**0.018**
Caucasian ethnicity	138/151 (91.4%)	96/106 (90.6%)	42/45 (93.3%)	0.76
BMI (kg/m^2)	25.0 (22.0–29.9) (n = 207)	25.5 (22.7–30.4) (n = 131)	24.5 (20.6–29.2)	0.07
Influenza vaccination (season of ICU admission)	54/107 (50.5%)	23/53 (43.4%)	31/54 (57.4%)	0.18
Smoking history	64/175 (36.6%)	48/109 (44.0%)	16/66 (24.2%)	**0.010**
Days between hospital admission and ICU admission	1 (0–1) (n = 215)	1 (0–2) (n = 139)	1 (0–1)	**0.037**
Days of illness before ICU admission	5 (3–8) (n = 200)	6 (3–9) (n = 127)	4 (2–8) (n = 73)	0.30
Any past medical history	198/214 (92.5%)	125/138 (90.6%)	73/76 (96.1%)	0.18
SCT > 90 days before admission	5/214 (2.3%)	2/139 (1.4%)	3/75 (4.0%)	0.35
Other haematological malignancy	14/216 (6.5%)	7/140 (5.0%)	7/76 (9.2%)	0.25
Diabetes mellitus	41/216 (19.0%)	26/140 (18.6%)	15/76 (19.7%)	0.86
COPD	57/216 (26.4%)	41/140 (29.3%)	16/76 (21.1%)	0.20
Solid organ malignancy	39/215 (18.1%)	14/140 (10.0%)	25/75 (33.3%)	**< 0.0001**
CKD requiring RRT	2/216 (0.9%)	1/140 (0.7%)	1/76 (1.3%)	1.00
CKD not requiring RRT	12/216 (5.6%)	5/140 (3.6%)	7/76 (9.2%)	0.12
APACHE II score	18 (14–26) (n = 96)	17 (14–26) (n = 48)	19 (14–27) (n = 48)	0.47
EORTC/MSGERC host factors				
Any EORTC host factor	55/187 (29.4%)	31/117 (26.5%)	24/70 (34.3%)	0.32
Recent neutropenia	1/186 (0.5%)	1/115 (0.9%)	0/71 (0%)	1.00
Allogeneic stem cell transplant recipient	7/216 (3.2%)	3/140 (2.1%)	4/76 (5.3%)	0.24
Solid organ transplant recipient	13/216 (6.0%)	8/140 (5.7%)	5/76 (6.6%)	0.77
Recent prolonged corticosteroid use	22/204 (10.8%)	17/131 (13.0%)	5/73 (6.8%)	0.24
T or B cell immunosuppressants during 90 days prior to ICU admission	34/214 (15.9%)	14/139 (10.1%)	20/75 (26.7%)	**0.003**
Inherited severe immunodeficiency	1/215 (0.5%)	1/139 (0.7%)	0/76 (0%)	1.00
Microbiology before ICU admission				
Aspergillus cultured from respiratory samples during 6 months before ICU admission	3/168 (1.8%)	2/104 (1.9%)	1/64 (1.6%)	1.00
Viruses isolated from respiratory samples during 30 days/4 weeks before ICU admission	28/175 (16.0%)	23/110 (20.9%)	5/65 (7.7%)	**0.031**
Laboratory results at ICU admission				
CRP (mg/L)	170 (68–263) (n = 204)	122 (42–228) (n = 128)	228 (131–287)	**< 0.0001**
WBC (× 10^9/L)	10.36 (5.90–15.14) (n = 210)	8.40 (4.75–14.06) (n = 134)	12.25 (8.14–18.23)	**< 0.001**
Lymphocyte count (× 10^9/L)[a]	0.52 (0.32–1.10) (n = 86)	0.49 (0.32–1.10) (n = 48)	0.63 (0.30–1.25) (n = 38)	0.44
Creatinine (µmol/L)	104 (65–158) (n = 207)	98 (64–142) (n = 133)	128 (69–188) (n = 74)	**0.025**
ALAT (IU/L)	28 (20–58) (n = 143)	28 (20–68) (n = 98)	28 (17–54) (n = 45)	0.51
Microbiology at ICU admission				
Bacterial lung pathogens cultured/PCR positive from respiratory sample < 7 days after influenza or niCAP diagnosis	72/186 (38.7%)	40/113 (35.4%)	32/73 (43.8%)	0.28
Aspergillus cultured from respiratory sample at time of influenza or niCAP diagnosis[b]	8/143 (5.6%)	8/83 (9.6%)	0/60 (0%)	**0.021**
Other viral testing positive on respiratory sample < 7 days after influenza or niCAP diagnosis	68/174 (39.1%)	29/106 (27.4%)	39/68 (57.4%)	**< 0.001**
Diagnostic testing performed in ICU				
BAL performed	133/215 (61.9%)	85/140 (60.7%)	48/75 (64.0%)	0.66

Table 1 (continued)

Baseline Characteristics	Total population (n = 216)	Influenza (n = 140)	Severe niCAP (n = 76)	p value (influenza vs. niCAP)
BALF GM performed	128/215 (59.5%)	84/140 (60.0%)	44/75 (58.7%)	0.88
BALF culture performed	116/211 (55.0%)	76/136 (55.9%)	40/75 (53.3%)	0.77
Serum GM performed	159/214 (74.3%)	100/140 (71.4%)	59/74 (79.7%)	0.25
ICU treatment data				
Ventilatory support during ICU admission	194/211 (91.9%)	125/136 (91.9%)	69/75 (92.0%)	1.00
Invasive ventilatory support during ICU admission	102/168 (60.7%)	76/117 (65.0%)	26/51 (51.0%)	0.12
Prone ventilation during ICU admission	42/211 (19.9%)	35/136 (25.7%)	7/75 (9.3%)	**0.004**
Inhaled NO ventilation	12/155 (7.7%)	6/106 (5.7%)	6/49 (12.2%)	0.20
ECMO/$ECCO_2R$	20/211 (9.5%)	17/136 (12.5%)	3/75 (4.0%)	0.050
Vasopressors/inotropes during ICU admission	126/181 (69.6%)	86/120 (71.7%)	40/61 (65.6%)	0.40
RRT during ICU admission	36/211 (17.1%)	28/136 (20.6%)	8/75 (10.7%)	0.09
Systemic corticosteroids during ICU admission	141/216 (65.3%)	91/140 (65.0%)	50/76 (65.8%)	1.00
Any systemic antifungal treatment during ICU admission	60/216 (27.8%)	49/140 (35.0%)	11/76 (14.5%)	**0.001**
Outcome data				
IAPA/IPA	44/216 (20.4%)	34/140 (24.3%)	10/76 (13.2%)	0.054
ICU Mortality	43/216 (19.9%)	30/140 (21.4%)	13/76 (17.1%)	0.48
LOS ICU (days)	9 (4–20) (n = 206)	9 (4–22) (n = 131)	8 (4–15) (n = 75)	0.39
LOS Hospital (days)	17 (10–34) (n = 183)	20 (10–37) (n = 118)	14 (11–26) (n = 65)	0.13

Data are presented as median (IQR) or as proportions (percentages)

a = Data not available for the 2017/2018 cohort

b = Data not available for patients in the POSA FLU database (n = 26)

AID = Auto-immune disease. ALAT = Alanine aminotransferase. APACHE = Acute physiology and chronic health evaluation. BAL = Bronchoalveolar lavage. BALF = Bronchoalveolar lavage fluid. BMI = Body mass index. CKD = Chronic kidney disease. COPD = Chronic obstructive pulmonary disease. CRP = C reactive protein. $ECCO_2R$ = Extracorporeal carbon dioxide removal. ECMO = Extracorporeal membrane oxygenation. EORTC/MSGERC = European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium. GM = Galactomannan. ICU = Intensive care unit. IAPA = Influenza-associated pulmonary aspergillosis. IPA = Invasive pulmonary aspergillosis. LOS = Length of stay. niCAP = Non-influenza community-acquired pneumonia. NO = Nitric oxide. PCR = Polymerase chain reaction. RRT = Renal replacement therapy. SCT = Stem cell transplantation. WBC = White blood cell count

60–76] years), more frequently female (58/140 [41%] versus 19/76 [25%]), more frequently had a smoking history (48/109 [44%] versus 16/66 [24%]), and had a longer hospital stay before ICU admission (1 [IQR 0–2] versus 0.5 [IQR 0–1] days). Past medical history was comparable, except for solid organ malignancies (more frequent in the niCAP cohort: 25/75 [33%] versus 14/140 [10%]). Presence of any EORTC/MSGERC host factor did not differ significantly (31/117 [26%] in influenza versus 24/70 [34%] in niCAP), although immunosuppressant use was more prevalent in niCAP (20/75 [27%] versus 14/139 [10%] in influenza). Presence of *Aspergillus* in respiratory samples before ICU admission was infrequent and did not differ between the classifiable influenza (2/104 [1.9%]) and niCAP (1/64 [1.6%]) cohorts.

Compared to the niCAP cohort, the classifiable influenza cohort had a lower FiO_2 (70 [IQR 46–80]% versus 75 [IQR 58–100]%), higher peripheral SpO_2 (92 [IQR 88–95]% versus 89 [IQR 84–92]%), higher PaO_2 (9.27 [IQR 7.82–11.84] versus 8.10 [IQR 7.26–9.73] kPa) and less need for ventilatory support (70/102 [69%] versus 39/45 [87%]) at the time of ICU admission. Classifiable influenza patients demonstrated a lower CRP (122 [IQR 42–228] mg/L) and WBC (8.40 [IQR 4.75–14.06] $\times 10^9$/L) than patients with niCAP (228 [IQR 131–287] mg/L and 12.25 [IQR 8.14–18.23] $\times 10^9$/L, respectively), as well as lower creatinine levels (98 [IQR 64–142] versus 128 [IQR 69–188] μmol/L). Influenza A was the most frequent influenza type found (124/140 [89%]). Although need for invasive ventilatory support during ICU stay overall was similar between cohorts, patients with influenza required prone ventilation more frequently (35/136 [26%] versus 7/75 [9.3%]). ICU mortality was 30/140 (21%) for classifiable influenza patients and 13/76 (17%) for classifiable niCAP patients (p = 0.48). Length of stay (LOS) in hospital and ICU was not significantly different between cohorts (p = 0.13 and p = 0.39, respectively). Of investigations performed to assess for IAPA/IPA, only tracheobronchial aspirate fungal cultures differed in frequency between cohorts: 52/130 (40%) in influenza versus 2/74 (2.7%) in niCAP (Supplementary Table 1, Additional Files).

IAPA/IPA in the classifiable influenza and niCAP cohorts

Invasive aspergillosis developed in 34/140 (24%) patients in the classifiable influenza cohort and in 10/76 (13%) in

the classifiable niCAP cohort (p = 0.054, Table 1; Supplementary Table 1, Additional Files; Fig. 1). In the influenza cohort, two patients had proven IAPA and 32 had probable IAPA; in the niCAP cohort, all had probable IPA. Five patients with influenza fulfilled criteria for both probable invasive tracheobronchial aspergillosis (ITBA) and IAPA and one fulfilled criteria for probable ITBA, but was not classifiable for (pulmonary) IAPA. Of the patients with IAPA, 17/31 (54.8%) had a positive BALF culture, 27/34 (79.4%) had a positive BALF GM, 5/29 (17.2%) had a positive serum GM, 8/32 (25.0%) had a positive TBA culture and 4/16 (25.0%) had a positive sputum culture. Within the patient cohort with niCAP who developed IPA, BALF culture was positive in 0/8 (0%), BALF GM was positive in 8/10 (80.0%), serum GM was positive in 4/10 (40.0%), TBA culture was positive in 0/1 (0%) and sputum culture was positive for fungal growth in 0/5 (0%).

Supplementary Table 2, Additional Files, provides detailed information on patients with IAPA/IPA. At the time of influenza or niCAP diagnosis, *Aspergillus* was cultured from respiratory samples in 8/83 [10%] in the influenza cohort versus 0/60 [0%] in the niCAP cohort (p = 0.021; Table 1; Supplementary Table 1, Additional Files). However, IAPA in influenza was diagnosed later than IPA in niCAP (4 [IQR 1–10] days versus 1 [IQR 0–3] day after ICU admission; Supplementary Table 3, Additional Files). Classifiable patients with at least one EORTC/MSGERC host factor (EORTC +) did not develop IAPA/IPA more frequently than those without (influenza: EORTC +: 9/31 [29%]; EORTC–: 23/86 [27%]; p = 0.82; niCAP: EORTC +: 4/24 [17%]; EORTC–: 6/46 [13%]; p = 0.73). Although patients in the niCAP cohort who received corticosteroids during ICU admission developed IPA more frequently (10/50 [20%]) than those who did not (0/26 [0%]; p = 0.013), this was not observed in patients with influenza (24/91 [26%] versus 10/49 [20%]; p = 0.54).

IAPA versus no IAPA

We further analysed data in the classifiable influenza cohort for assessment of factors specific for development of IAPA. Overall, demographic characteristics were comparable between patients with influenza who developed IAPA and those with influenza alone/no IAPA (Table 2; Supplementary Table 1, Additional Files). However, patients with IAPA were less frequently vaccinated against influenza during the season of ICU admission compared to those with influenza only (3/15 [20%] versus 20/38 [53%]). Additionally, they were more frequently (former) smokers (18/26 [69%] versus 30/83 [36%]), had been admitted to hospital (1 [IQR 0–4] versus 0 [IQR 0–2] days) and had been ill (9 [IQR 6–14] versus 4 [IQR 2–7] days) before ICU admission longer. Past medical history and EORTC/MSGERC host factors were comparable between both groups, with EORTC/MSGERC host factors present in 9/32 [28%] of classifiable patients developing IAPA versus 22/85 [26%] of patients who did not. Patients who developed IAPA had higher CRP values at ICU admission (186 [IQR 100–244] versus 89 [IQR 34–223] mg/L) than those who did not. At the time of influenza diagnosis, respiratory sample *Aspergillus* cultures were positive in 6/21 [29%] in patients who developed IAPA versus 2/62 [3.2%] in those with influenza only (p = 0.003; Supplementary Table 1, Additional Files). *Aspergillus fumigatus* was the species most frequently demonstrated (50/58 [86%] of reported isolates in 33 patients).

Clinical impact and predictors of IAPA

ICU mortality was significantly higher in patients with IAPA (15/34 [44%]) than in those without (15/106 [14%]) (Table 2; Supplementary Table 1, Additional Files). Kaplan–Meier survival analysis demonstrated reduced survival in the IAPA group when compared to the no IAPA group (Fig. 2). ICU LOS was significantly longer for IAPA (21 [IQR 9–45] days versus 7 [IQR 3–18] days), also when corrected for ICU mortality (IAPA: 29 [IQR 5–51] days versus no IAPA: 6 [IQR 3–13] days).

A significantly higher ICU mortality was observed in all classifiable patients (influenza and niCAP combined) who received corticosteroid treatment during ICU stay (37/141, 26%, versus 6/75, 8.0%; p = 0.001). This seemed to be entirely driven by the increased mortality in patients with IAPA who received corticosteroids in ICU. Patients with IAPA who did not survive ICU stay had received systemic corticosteroids in ICU more frequently (14/15 [93%]) compared to survivors (10/19 [53%]; Supplementary Table 4, Additional Files).

Furthermore, similar to earlier retrospective data, time between influenza diagnosis and initiation of mould-active antifungal treatment was longer in non-survivors (6 [IQR 4–16] days) compared to survivors (3 [IQR 2–6] days; Supplementary Table 4, Additional Files) [1]. Patients with IAPA more frequently required invasive ventilatory support (26/29 [90%] versus 50/88 [57%]) and renal replacement therapy (15/33 [45%] versus 13/103 [13%]) during ICU stay (Table 2; Supplementary Table 1, Additional Files).

In Cox regression analysis, EORTC host factors were not found to be independent predictors for IAPA occurrence in the classifiable influenza population, but IAPA was found to be an independent predictor for ICU mortality in influenza when correcting for age and sex (adjusted hazard ratio [aHR] 1.99, 95% confidence interval [95%CI] 1.05–3.76, p = 0.035; Supplementary Table 5, Additional Files; Fig. 3a–3b).

Table 2 Patient characteristics of the influenza cohort with and without IAPA

Baseline characteristics	Total classifiable influenza population (n = 140)	IAPA (n = 34)	No IAPA (n = 106)	p value (IAPA versus no IAPA)
Median age (years)	65 (54–72)	65 (56–68)	65 (53–74)	0.43
Male sex	82/140 (58.6%)	22/34 (64.7%)	60/106 (56.6%)	0.43
Caucasian ethnicity	96/106 (90.6%)	24/24 (100%)	72/82 (87.8%)	0.11
BMI (kg/m^2)	25.5 (22.7–30.4) (n = 131)	25.7 (22.7–29.4)	25.0 (22.6–30.7) (n = 97)	0.92
Influenza vaccination (season of ICU admission)	23/53 (43.4%)	3/15 (20.0%)	20/38 (52.6%)	**0.037**
Smoking history	48/109 (44.0%)	18/26 (69.2%)	30/83 (36.1%)	**0.006**
Days between hospital admission and ICU admission	1 (0–2) (n = 139)	1 (0–4)	0 (0–2) (n = 105)	**0.008**
Days of illness before ICU admission	6 (3–9) (n = 127)	9 (6–14) (n = 32)	4 (2–7) (n = 95)	**< 0.001**
Any medical history	125/138 (90.6%)	29/34 (85.3%)	96/104 (92.3%)	0.31
SCT > 90 days before admission	2/139 (1.4%)	1/33 (3.0%)	1/106 (0.9%)	0.42
Other haematological malignancy	7/140 (5.0%)	4/34 (11.8%)	3/106 (2.8%)	0.06
Diabetes mellitus	26/140 (18.6%)	5/34 (14.7%)	21/106 (19.8%)	0.62
COPD	41/140 (29.3%)	9/34 (26.5%)	32/106 (30.2%)	0.83
Solid organ malignancy	14/140 (10.0%)	3/34 (8.8%)	11/106 (10.4%)	1.00
CKD requiring RRT	1/140 (0.7%)	1/34 (2.9%)	0/106 (0%)	0.24
CKD not requiring RRT	5/140 (3.6%)	1/34 (2.9%)	4/106 (3.8%)	1.00
APACHE II score	17 (14–26) (n = 48)	21 (15–29) (n = 17)	16 (13–23) (n = 31)	0.14
EORTC/MSGERC host factors				
Any EORTC host factor	31/117 (26.5%)	9/32 (28.1%)	22/85 (25.9%)	0.82
Recent neutropenia	1/115 (0.9%)	0/31 (0%)	1/84 (1.2%)	1.00
Allogeneic stem cell transplant recipient	3/140 (2.1%)	2/34 (5.9%)	1/106 (0.9%)	0.15
Solid organ transplant recipient	8/140 (5.7%)	2/34 (5.9%)	6/106 (5.7%)	1.00
Recent prolonged corticosteroid use	17/131 (13.0%)	4/34 (11.8%)	13/97 (13.4%)	1.00
T or B cell immunosuppressants during 90 days prior to ICU admission	14/139 (10.1%)	4/34 (11.8%)	10/105 (9.5%)	0.75
Inherited severe immunodeficiency	1/139 (0.7%)	1/34 (2.9%)	0/105 (0%)	0.24
Microbiology before ICU admission				
Aspergillus cultured from respiratory samples during 6 months before ICU admission	2/104 (1.9%)	1/28 (3.6%)	1/76 (1.3%)	0.47
Viruses isolated from respiratory samples during 30 days/4 weeks before ICU admission	23/110 (20.9%)	6/28 (21.4%)	17/82 (20.7%)	1.00
Laboratory results at ICU admission				
CRP (mg/L)	122 (42–228) (n = 128)	186 (100–244) (n = 33)	89 (34–223) (n = 95)	**0.027**
WBC (× 10^9/L)	8.40 (4.75–14.06) (n = 134)	6.42 (3.88–12.02)	10.26 (5.43–14.58) (n = 100)	0.09
Lymphocyte count (× 10^9/L)[a]	0.49 (0.32–1.10) (n = 48)	0.40 (0.26–0.60) (n = 13)	0.56 (0.35–1.18) (n = 35)	0.10
Creatinine (µmol/L)	98 (64–142) (n = 133)	89 (64–133) (n = 33)	102 (64–146) (n = 100)	0.72
ALAT (IU/L)	28 (20–68) (n = 98)	28 (21–67) (n = 22)	29 (20–68) (n = 76)	0.57
Microbiology at ICU admission				
Influenza A	124/140 (88.6%)	32/34 (94.1%)	92/106 (86.8%)	0.36
Influenza B	16/140 (11.4%)	2/34 (5.9%)	14/106 (13.2%)	0.36
Bacterial lung pathogens cultured/PCR positive from respiratory sample < 7 days after influenza diagnosis	40/113 (35.4%)	12/30 (40.0%)	28/83 (33.7%)	0.66
Other viral testing positive on respiratory sample < 7 days after influenza diagnosis	29/106 (27.4%)	15/28 (53.6%)	14/78 (17.9%)	**< 0.001**
Treatment at time of ICU admission				
Oseltamivir	124/140 (88.6%)	29/34 (85.3%)	95/106 (89.6%)	0.54

Table 2 (continued)

Baseline characteristics	Total classifiable influenza population (n = 140)	IAPA (n = 34)	No IAPA (n = 106)	p value (IAPA versus no IAPA)
Aspergillus-active antifungal treatment continued at the time of ICU admission	3/139 (2.2%)	2/34 (5.9%)	1/105 (1.0%)	0.15
ICU treatment data				
Ventilatory support during ICU admission	125/136 (91.9%)	34/34 (100%)	91/102 (89.2%)	0.06
Invasive ventilatory support during ICU admission	76/117 (65.0%)	26/29 (89.7%)	50/88 (56.8%)	**0.001**
Prone ventilation during ICU admission	35/136 (25.7%)	15/34 (44.1%)	20/102 (19.6%)	**0.007**
Inhaled NO ventilation	6/106 (5.7%)	3/24 (12.5%)	3/82 (3.7%)	0.13
ECMO/$ECCO_2R$	17/136 (12.5%)	8/34 (23.5%)	9/102 (8.8%)	**0.036**
Vasopressors/inotropes during ICU admission	86/120 (71.7%)	25/29 (86.2%)	61/91 (67.0%)	0.06
RRT during ICU admission	28/136 (20.6%)	15/33 (45.5%)	13/103 (12.6%)	**< 0.001**
Systemic corticosteroids during ICU admission	91/140 (65.0%)	24/34 (70.6%)	67/106 (63.2%)	0.54
Any systemic antifungal treatment during ICU admission	49/140 (35.0%)	29/34 (85.3%)	20/106 (18.9%)	**< 0.0001**
Outcome data				
ICU Mortality	30/140 (21.4%)	15/34 (44.1%)	15/106 (14.2%)	**< 0.001**
LOS ICU (days)	9 (4–22) (n = 131)	21 (9–45) (n = 33)	7 (3–18) (n = 98)	**< 0.0001**
LOS Hospital (days)	20 (10–37) (n = 118)	27 (16–48) (n = 29)	16 (10–35) (n = 89)	**0.012**
LOS ICU (days) – Alive at ICU discharge	6 (3–19) (n = 101)	29 (5–51) (n = 18)	6 (3–13) (n = 83)	**0.002**
LOS Hospital (days) – Alive at ICU discharge	18 (10–37) (n = 89)	39 (14–69) (n = 14)	15 (8–34) (n = 75)	**0.014**

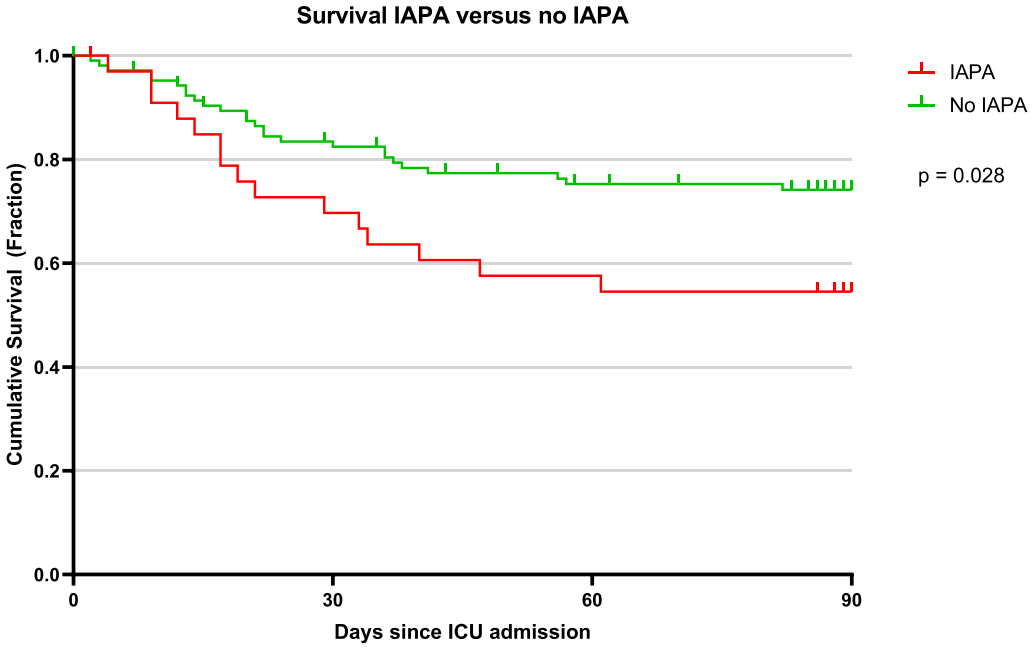
Data are presented as median (IQR) or as proportions (percentages)

a = Data not available for the 2017/2018 cohort

AID = Auto-immune disease. ALAT = Alanine aminotransferase. APACHE = Acute physiology and chronic health evaluation. BAL = Bronchoalveolar lavage. BMI = Body mass index. CKD = Chronic kidney disease. COPD = Chronic obstructive pulmonary disease. CRP = C reactive protein. $ECCO_2R$ = Extracorporeal carbon dioxide removal. ECMO = Extracorporeal membrane oxygenation. EORTC/MSGERC = European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium. GM = Galactomannan. ICU = Intensive care unit. IAPA = Influenza-associated pulmonary aspergillosis. IPA = Invasive pulmonary aspergillosis. LOS = Length of stay. NO = Nitric oxide. PCR = Polymerase chain reaction. RRT = Renal replacement therapy. SCT = Stem cell transplantation. WBC = White blood cell count

Discussion

In this prospective observational cohort study, we found a high incidence of IAPA in critically ill patients admitted to ICU with influenza. Patients with IAPA demonstrated higher ICU mortality, higher frequency of organ supportive therapies and longer ICU and hospital LOS. The incidence of IPA in our classifiable influenza cohort (24%) is in agreement with previous retro- and prospective studies and confirms IAPA as a frequent complication of severe influenza in a prospective study design [1, 5, 6, 9, 11]. However, several studies have found markedly lower incidence rates (ranging from 0.9% to 11%) [2–4, 7, 12, 13, 15, 16, 18–20]. Differences might be due to variation in patient populations, genetic background, IPA case definitions, and/or geographic and environmental differences in exposure to *Aspergillus* [28]. Here, we applied a strict case definition and only performed analyses in patients who had undergone mycological and/or radiological testing. For many studies, it is unclear what proportion of patients underwent mycological investigations, making interpretation of reported IAPA incidence numbers challenging. In our study, we observed that IPA occurred almost twice as frequent in classifiable patients with influenza (24%) than in classifiable patients with niCAP (13%). Although this difference did not reach statistical significance, we emphasize its clinical relevance. Furthermore, IPA occurred more frequently in the classifiable influenza cohort despite this group's younger age and less frequent T or B cell immunosuppressant use compared with the classifiable niCAP cohort. Notably, presence of any EORTC/MSGERC host factors did not seem to contribute to development of IPA in critically ill patients. Over 70% of classifiable patients with influenza who developed IAPA did not have any EORTC/MSGERC host factors, in line with the findings of Schauwvlieghe et al. [9]. This is in keeping with influenza as an independent risk factor for the occurrence of invasive aspergillosis. However, we were not able to demonstrate this in multivariate Cox regression analysis, most likely due to relatively low patient numbers (Fig. 3a; Supplementary Table 5, Additional Files). Within the total classifiable and classifiable influenza population, only invasive ventilatory support (which might be viewed as a marker of disease severity) was an independent predictor of development of IAPA in multivariate Cox regression analysis.



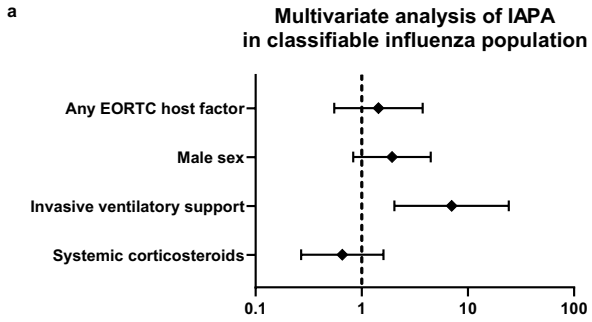
Days since ICU admission	0	30	60	90
Number at risk – IAPA	34	24	20	12
Number at risk – No IAPA	106	83	72	57

Fig. 2 Kaplan–Meier curve of survival over time in influenza-associated pulmonary aspergillosis versus influenza alone. Survival over time is significantly different for classifiable patients with influenza who developed IAPA versus those who did not. Mean estimated survival for the IAPA group is 82 (95% confidence interval [95%CI] 63–100) days; for the no IAPA group it is 136 (95%CI 123–149) days. Survival analysis by log-rank test (Mantel-Cox), for the combined cohorts with study cohort as stratum and with analysis pooled over strata. Data were censored at 90 days after ICU admission for the Figure

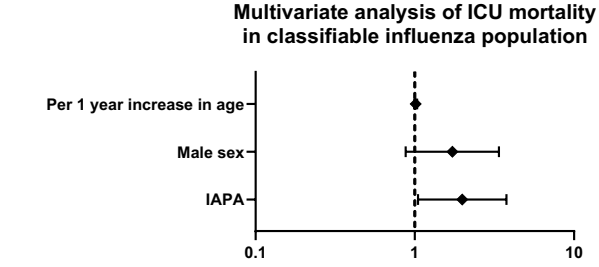
Importantly, our classifiable niCAP cohort's IPA incidence was 13%, higher than that reported for patients admitted to ICU for CAP and acute respiratory distress syndrome due to any cause [9, 29]. Whether this was due to selection bias, low numbers of included patients, IPA being the primary problem on presentation in some patients (the majority of participating centres being university hospitals with potentially complex patient populations) or whether IPA incidence in niCAP is truly higher in the Netherlands and Belgium, remains to be investigated. The finding that the median time between ICU admission and the first positive mycological criterion in our classifiable niCAP population was one day, could suggest that IPA might have been the primary diagnosis leading to hospital admission (and had been present before ICU admission).

The observed IAPA ICU mortality of 44% is in line with other studies reporting ICU mortality rates of 30–67%, and was more than three times higher than in patients with influenza alone [1, 3, 5, 8, 9, 11, 13–16, 18–20]. This increased mortality in patients with mycological findings consistent with aspergillosis supports the concept that *Aspergillus* is not merely colonising the respiratory tract in these patients. Therefore, presence of *Aspergillus* in respiratory samples of patients with influenza in the ICU should not be ignored. Furthermore, IAPA was an independent predictor of ICU mortality in the classifiable influenza population (aHR 1.99; Fig. 3b; Supplementary Table 5, Additional Files). Patients with IAPA were characterised by an adverse clinical course, requiring invasive ventilation and renal replacement therapy more frequently than those with influenza alone, as well as a longer ICU and hospital LOS. Patients with IAPA who died in ICU were more likely to be male and to have received corticosteroids during ICU admission. However, no significant differences in time to positive mycological testing were found between survivors and non-survivors with IAPA, nor an effect of administration of mould-active antifungal treatment in ICU. In line with previous findings, antifungal treatment in survivors was initiated significantly earlier after influenza diagnosis than in non-survivors, highlighting the need for timely clinical suspicion, diagnosis and start of antifungal therapy [1].

The median time between ICU admission and a first positive mycological criterion was 4 days in IAPA, in line with the 3–7 days reported by previous retrospective studies [5, 9, 11, 13–16]. IAPA was diagnosed in 36% of patients within 48 h of ICU admission. In contrast, in our



aHR (95%CI)	p value
1.44 (0.55 – 3.75)	0.46
1.93 (0.83 – 4.47)	0.13
7.03 (2.03 – 24.27)	0.002
0.66 (0.27 – 1.60)	0.35



aHR (95%CI)	p value
1.01 (0.99 – 1.04)	0.25
1.72 (0.88 – 3.38)	0.11
1.99 (1.05 – 3.76)	0.035

Fig. 3 Forest plots of risk factors for IAPA and ICU mortality in classifiable influenza. Risk factors for the development of IAPA (**a**) and for ICU mortality (**b**) in the classifiable influenza population as analysed by Cox regression analysis. All factors shown were used in univariate analysis and subsequently included in a multivariate Cox regression analysis model (see Supplementary Table 5, Additional Files). Invasive ventilatory support refers to any form of invasive ventilation at any time during ICU admission. Systemic corticosteroids refers to any systemic corticosteroids administered at any time during ICU admission. 95%CI = 95% Confidence interval. aHR = Adjusted hazard ratio. EORTC = European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium. IAPA = Influenza-associated pulmonary aspergillosis

recent randomized trial of posaconazole prophylaxis for prevention of IAPA in ICU (POSA-FLU), 71% of IAPA cases were diagnosed within this time period [27]. In addition, a recent Swedish prospective study diagnosed IAPA after 1 day in ICU in 3/5 patients [20]. This difference might be explained by the observational nature of our current study and differences in local diagnostic strategies, whereas all patients underwent early diagnostic work-up in the POSA-FLU study and the Swedish study. Diagnostic work-up early after ICU admission might therefore reduce the time to IAPA diagnosis in high incidence regions. In lower incidence regions, CRP and respiratory sample culture of *Aspergillus* (at the time of influenza diagnosis) might serve as supporting markers for early identification of patients with influenza at risk for IAPA. Similarly, knowledge of vaccination status might be useful since influenza vaccination appeared to be associated with lower IAPA incidence in this study; however, this requires further investigation. Although corticosteroid use before or during ICU admission did not appear to be a risk factor for IAPA development in influenza in our study, corticosteroids during ICU stay could increase the risk of ICU mortality, once IAPA has developed. In this context, use of corticosteroids could potentially have contributed to progressive invasive aspergillosis, thus leading to increased ICU mortality.

Our study has several limitations. First, most patients did not undergo standardised diagnostic work-up for IPA due to the observational nature of our study. Therefore, there is heterogeneity amongst participating centres regarding timing and modality of mycological investigations performed. Results regarding time to positive testing and diagnosis of IPA need to be interpreted with this in mind. In addition, this highlights the need for routine and standardised mycological diagnostic work-up in patients admitted to ICU with influenza; not only will this be essential to optimize patient care, but it will also provide more robust epidemiological data on IAPA (by providing a reliable denominator in calculating IAPA incidence). Subsequent prospective studies assessing whether routine or targeted (based on risk factors) diagnostic testing for IAPA would be most effective could then be considered. Second, the expert opinion IAPA case definition used has not been validated. Our strict application of these criteria might have led to underestimation of IAPA incidence (the FUNDICU criteria were not applied as this case definition was published after study completion) [30]. Third, patients who required invasive ventilatory support at any time during

ICU admission were more likely to undergo bronchoscopy, BAL and tracheobronchial aspirate sampling; this potential diagnostic bias needs to be taken into account when interpreting our results. In addition, calculating IAPA/IPA incidence within the classifiable patient population might have resulted in an overestimation of incidence, as it is possible that patients with low clinical suspicion of IAPA/IPA did not undergo the required diagnostic work-up for classification. On the other hand, incidence has now been calculated in a clearly defined patient population for whom mycological diagnostic results were available. Furthermore, most participating centres are university hospitals with many included patients having co-morbidities, requiring caution when extrapolating our findings to the general population. This holds true for both the influenza and niCAP population, as evidenced by the fact that 70/242 (29%) patients in the total included population in our study had at least one EORTC/MSGERC host factor present. Finally, the COVID-19 pandemic stopped our inclusion. Since then, COVID-19 has been clearly associated with IPA as well, in the form of COVID-19-associated pulmonary aspergillosis (CAPA) [31, 32]. In the near future, the impact of influenza and SARS-CoV-2 co-infection on development of IPA remains to be seen.

Conclusions

In our study population, IAPA affects approximately one in four patients admitted to ICU with influenza in the pre-COVID-19 era. IAPA is characterised by a high ICU mortality of 44%, as well as by a longer ICU and hospital stay and more frequent need for invasive ventilatory support and renal replacement therapy than influenza uncomplicated by IAPA. IAPA was demonstrated to be an independent predictor of ICU mortality within the classifiable influenza population. Our study highlights the need for increased awareness of this serious complication among treating physicians and for implementation of routine and standardised mycological diagnostic work-up early in the course of ICU admission of patients with influenza. Further prospective studies with standardised diagnostic strategies for aspergillosis to elucidate additional risk factors for IAPA and the impact of antifungal treatment or prophylaxis on outcomes are warranted, as well as clinical trials assessing efficacy of antifungal prophylaxis in preventing IAPA, possibly already initiated before ICU admission.

Abbreviations

BAL	Broncho-alveolar lavage
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
EORTC/MSGERC	European organization for research and treatment of cancer/mycosis study group education and research consortium
FiO_2	Fraction of inspired oxygen
GM	Galactomannan
IAPA	Influenza-associated pulmonary aspergillosis
ICU	Intensive care unit
IPA	Invasive pulmonary aspergillosis
ITBA	Invasive tracheobronchial aspergillosis
IQR	Interquartile range
LOS	Length of stay
niCAP	Non-influenza community-acquired pneumonia
PaO_2	Partial pressure of oxygen in arterial blood
PCR	Polymerase chain reaction
SpO_2	Oxygen saturation in peripheral blood
WBC	White blood cell count

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13054-025-05771-3.

Additional file 1

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Author contributions

NAFJ: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, visualization, writing – original draft. LV: Conceptualization, investigation, methodology, project administration, validation, visualization, writing – original draft. CJ: Investigation, methodology, validation, writing – original draft. SF: Investigation, methodology, validation, writing – original draft. KvD: Investigation, project administration, resources, supervision, validation, writing – review and editing. JIvdS: Investigation, project administration, resources, supervision, validation, writing – review and editing. BLtT: Investigation, project administration, resources, supervision, validation, writing – review and editing. NPJ: Investigation, project administration, resources, supervision, validation, writing – review and editing. HA: Investigation, project administration, resources, supervision, validation, writing – review and editing. CHSBvdB: Investigation, project administration, resources, supervision, validation, writing – review and editing. MB: Investigation, project administration, resources, supervision, validation, writing – review and editing. PL: Investigation, project administration, resources, supervision, validation, writing – review and editing. PD: Investigation, project administration, resources, supervision, validation, writing – review and editing. PM: Investigation, project administration, resources, supervision, validation, writing – review and editing. KL: Investigation, supervision, writing – review and editing. EK: Conceptualization, investigation, writing – review and editing. JAS: Investigation, project administration, resources, supervision, writing – review and editing. BJAR: Investigation, project administration, resources, supervision, validation, writing – review and editing. OH: Investigation, project administration, resources, supervision, validation, writing – review and editing. DCJJB: Investigation, project administration, resources, supervision, validation, writing – review and editing. RJMB: Conceptualization, methodology, project administration, resources, supervision, visualization, writing – original draft. PEV: Conceptualization, funding acquisition, methodology, project administration, resources, supervision, visualization, writing – original draft. JW: Conceptualization, investigation, methodology, project administration, resources, supervision, visualization, writing – original draft. FLvdV: Conceptualization, funding acquisition, methodology, project administration,

resources, supervision, visualization, writing – original draft. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
For participating Dutch centres, the study protocol (CMO 2016–3037) was reviewed and approved by the ethics board region Arnhem-Nijmegen before study initiation. Based on the study's observational nature, written informed consent was waived. Patients were not included if they (or their legal representatives) actively objected to participation.
For participating Belgian centres, the study protocol was reviewed and approved by their individual ethical committees (S60757). Written informed consent was obtained for all Belgian participants.
Participants in the POSA-FLU trial all provided written informed consent.
This study's protocol was reviewed and approved by the ethics board region Arnhem-Nijmegen for all Dutch participating sites (CMO 2018–4041) and by the ethical committees of all participating centres in Belgium (S60744).
This study was performed in accordance with the latest version of the declaration of Helsinki and local legislation/regulations.

Consent for publication
Not applicable.

Competing interests
NAFJ reports having been a local (principal) investigator for an industry-sponsored trial for Pulmatrix, Inc. (payments to institution), outside the submitted work. LV reports fees from Pfizer and support for attending meetings and/or travel from Pfizer and Gilead Sciences, outside the submitted work. SF reports fees from HealthBook Company Ltd., Pfizer, Gilead and MundiPharma and support for attending meetings and/or travel from Pfizer and Gilead, outside the submitted work. KvD reports fees from Gilead and Pfizer (paid to institution) and participating on advisory boards for Gilead and Pfizer (payments to institution), outside the submitted work. KL reports grants from TECOmedical (paid to institution), consulting fees from MundiPharma (paid to institution), fees from Gilead, Pfizer, MundiPharma and FUJIFILM Wako Chemicals Europe GmbH (the latter two paid to institution), support for attending meetings and/or travel from Gilead, Pfizer and AstraZeneca and participation on a Data Safety Monitoring Board or Advisory Board for Pfizer (paid to institution), all outside the submitted work. RJMB reports grants from Gilead and Pfizer and consulting fees and payment for expert testimony from Basilea, F2G, Gilead, Pfizer, Astellas, MundiPharma and Pulmocide, all outside the submitted work. PEV reports fees from MundiPharma and Gilead Sciences (paid to institution), outside the submitted work. JW reports investigator-initiated grants from Gilead, Pfizer and MundiPharma, speaker's fees from Gilead, Pfizer, MundiPharma and AstraZeneca and support for attending meetings and/or travel from Gilead, Pfizer and MundiPharma, all outside the submitted work. FLvdV reports grants from the European Union's Horizon 2020 research and innovation programme, outside the submitted work. All other authors declare that they have no competing interests.

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