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

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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 in the influenza cohort with 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.

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%[3,4] but is much higher in patients who are critically ill.[4,5] 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,[1,6,7] but influenza itself can also cause severe acute respiratory distress syndrome (ARDS), which is associated with a mortality of 14–41%.[8,9]

Influenza-associated aspergillosis was occasionally described decades ago, and several small case series have been reported in the past 5–10 years.[1,9,10] 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).[1,11] These EORTC/

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Research in context

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.

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 colleagues[12] to distinguish invasive pulmonary aspergillosis from *Aspergillus* colonisation in patients who are critically ill.

In 2012, Wauters and colleagues[9] reported an incidence of 23% of proven or probable invasive pulmonary aspergillosis in 44 patients with H1N1 influenza in two consecutive influenza seasons (2009–11). Remarkably, 44% of patients with invasive pulmonary aspergillosis did not have any of the classical EORTC/MSG host factors. A Dutch study[13] described 23 (16%) of 144 patients with invasive pulmonary aspergillosis who were admitted to the ICU with influenza during the 2015–16 influenza season. These observations suggest that influenza infection that requires ICU admission is a risk factor for invasive pulmonary aspergillosis and that incorporation of influenza as a host factor in the current diagnostic criteria might be appropriate. However, whether influenza is independently associated with the occurrence of invasive pulmonary aspergillosis and whether the risk varies from season to season remains unclear. This study aimed to describe the epidemiology and outcome of invasive pulmonary aspergillosis in patients admitted to the ICU over seven consecutive influenza seasons and to assess whether influenza was independently associated with invasive pulmonary aspergillosis.

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.[14]

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).[12] 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.

See Online for appendix

Statistical analysis

In univariable analysis, we compared categorical variables by Fisher's exact test and χ^2 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.[4,15] The estimate of association was expressed as adjusted odds ratio (aOR) with corresponding confidence intervals of 95%. We did multiple imputations to handle

***Panel*: The modified definition of invasive pulmonary aspergillosis**

The definition of invasive pulmonary aspergillosis was modified from the *AspICU* algorithm and was based on the presence of clinical, radiological, and mycological criteria in all invasive pulmonary aspergillosis cases.

This modified invasive pulmonary aspergillosis definition did not require a European Organisation for Research and Treatment of Cancer (EORTC)-defined host factor because otherwise patients with influenza but without an EORTC host factor could never fulfil the definition, as long as influenza is not part of the EORTC host factor definition.

Clinical criteria

One of the following signs or symptoms had to be present:

- Fever refractory to at least 3 days of appropriate antibiotic therapy.
- Recrudescent fever after a period of defeverescence of at least 48 h while still on antibiotics and without other apparent cause.
- Dyspnoea.
- Haemoptysis.
- Pleural friction rub or chest pain.
- Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support.

Radiological criteria

Any infiltrate on pulmonary imaging by portable chest x-ray or CT scan of the lungs. This radiological definition was different from the EORTC-defined radiological criteria (eg, halo sign or air-crescent sign) because these EORTC criteria apply to patients with prolonged neutropenia but are of little use for ICU patients.

Mycological criteria

One or more of the following had to be present:

- Histopathology or direct microscopic evidence of dichotomous septate hyphae with positive culture for *Aspergillus* from tissue.
- A positive *Aspergillus* culture from a bronchoalveolar lavage (BAL).
- A galactomannan optical index on BAL of ≥1.
- A galactomannan optical index on serum of ≥0·5.

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

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,

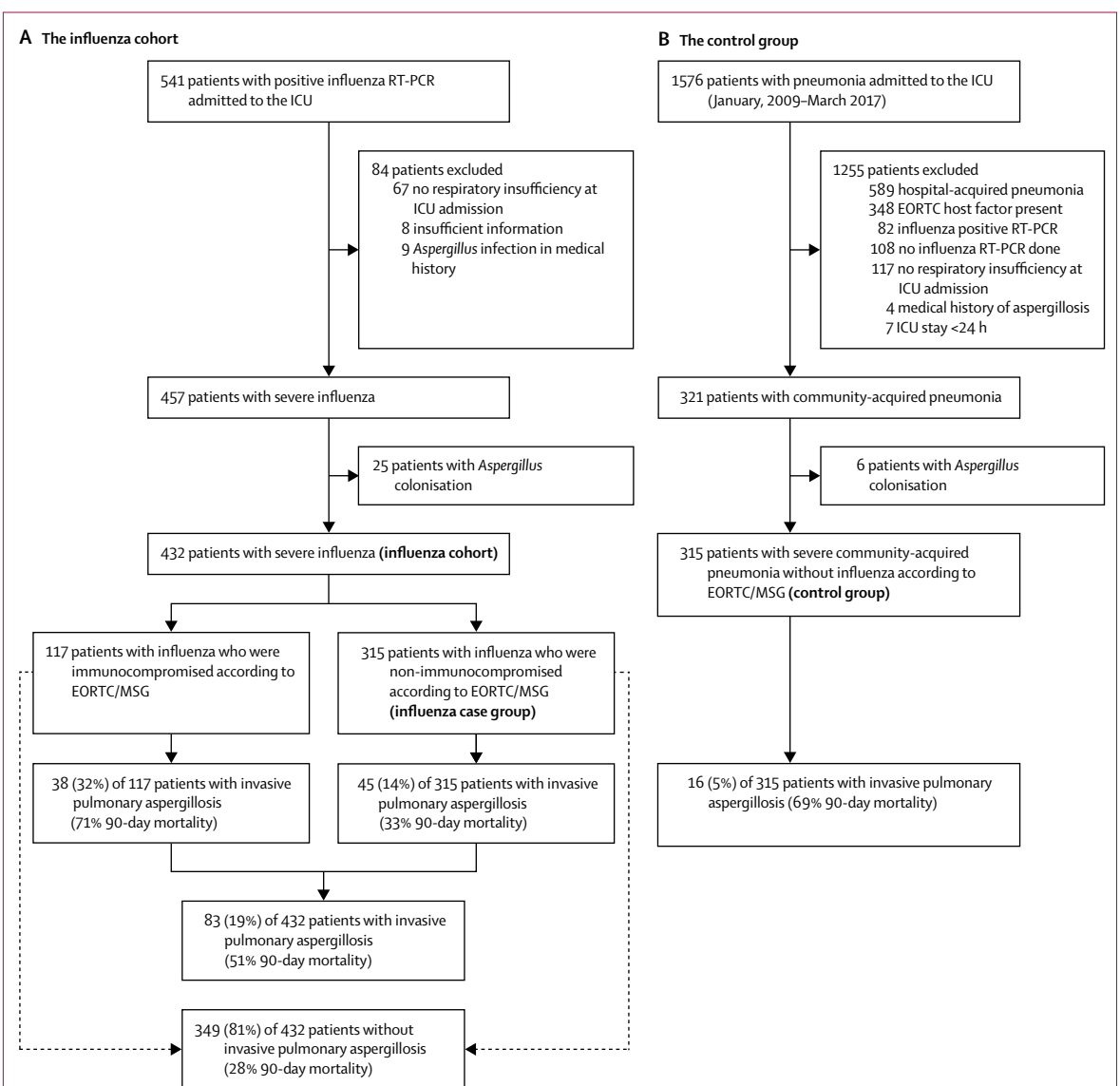

Figure 1: **Overview of inclusion process for cases and controls, and the corresponding number with invasive pulmonary aspergillosis and associated deaths**
EORTC/MSG=European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group. ICU=intensive care unit.

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

	Influenza cohort (n=432)	With invasive pulmonary aspergillosis (n=83)	Without invasive pulmonary aspergillosis (n=349)	p value
Baseline characteristics				
Mean age, years (SD)	59 (15)	60 (12)	59 (16)	0·35
Male sex	240 (56%)	56 (67%)	184 (53%)	0·015
Mean APACHE II score on admission (SD)	22 (8)	25 (9)	22 (7)	0·005
Body-mass index >30 kg/m²	93/410 (23%)	17/83 (20%)	76/327 (23%)	0·59
Diabetes	88 (20%)	10 (12%)	78 (22%)	0·036
Liver cirrhosis	25 (6%)	5 (6%)	20 (6%)	1·0
Chronic kidney disease*	71 (16%)	16 (19%)	55 (16%)	0·44
Known risk factors				
EORTC/MSG host factor	117 (27%)	38 (46%)	79 (23%)	<0·0001
Haematological malignancy	66 (15%)	22 (27%)	44 (13%)	0·002
Solid organ transplant	32 (7%)	11 (13%)	21 (6%)	0·024
Solid organ malignancy	21 (5%)	4 (5%)	17 (5%)	1·0
Neutropenia	22 (5%)	11 (13%)	11 (3%)	0·001
Chronic obstructive pulmonary disease	79 (18%)	13 (16%)	66 (19%)	0·49
Studied risk factors				
Corticosteroids 28 days before ICU	145/426 (34%)	46/82 (56%)	99/344 (29%)	<0·0001
Median dose corticosteroids 28 days before ICU admission (IQR), mg/kg/day	0·14 (0·06–0·28)	0·22 (0·10–0·33)	0·10 (0·06–0·24)	0·003
Smoking in the past year	114/332 (34%)	26/61 (43%)	88/271 (32%)	0·13
ICU data				
Mechanical ventilation	326 (75%)	75 (90%)	251 (72%)	0·0004
Mechanical ventilation days (IQR)	11 (5–21)	14 (9–31)	9 (4–17)	0·001
Nitric oxide or high-frequency oscillation ventilation	42 (10%)	13 (16%)	29 (8%)	0·04
Extracorporeal membrane oxygenation	52 (12%)	16 (19%)	36 (10%)	0·024
Vasopressors	287/423 (67%)	67/82 (81%)	220/341 (65%)	0·002
Renal replacement therapy	100/423 (24%)	35/83 (42%)	65/340 (19%)	<0·0001
Outcome data				
Median days of ICU stay (IQR)	11 (6–23)	19 (12–38)	9 (5–20)	<0·0001
ICU mortality	107 (25%)	37 (45%)	70 (20%)	<0·0001
Hospital mortality	133 (31%)	41 (49%)	92 (26%)	<0·0001
90-days mortality after ICU admission	141 (33%)	42 (51%)	99 (28%)	0·0001
Influenza				
Influenza A	355 (82%)	71 (86%)	284 (81%)	0·37
Influenza B	77 (18%)	12 (14%)	65 (19%)	0·37
Influenza treatment with a neuraminidase inhibitor	338/428 (79%)	70/83 (84%)	268/345 (78%)	0·25
Diagnostics				
BAL sampling performed	233 (54%)	81 (98%)	152 (44%)	<0·0001
BAL galactomannan test performed	137 (32%)	76 (92%)	61 (17%)	<0·0001
Serum galactomannan test performed	47 (11%)	31/83 (37%)	16 (5%)	<0·0001

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. APACHE=acute physiology and chronic evaluation score. EORTC=European Organization for Research and Treatment of Cancer. ICU=intensive care unit. BAL=bronchoalveolar lavage. *Glomerular filtration rate <60 mL/min/1·73 m².

Table 1: **Characteristics of patients in the influenza cohort**

	Number of patients in the influenza cohort with invasive pulmonary aspergillosis (n=83)
BAL culture positive	50/80 (63%)*
BAL galactomannan test positive	67/76 (88%)
Serum galactomannan test positive	20/31 (65%)
EORTC/MSG criteria	
Proven	16 (19%)
Probable	20 (24%)
Not classifiable	47 (57%)
AspICU criteria	
Proven	16 (19%)
Putative	32 (39%)
Coloniser	5 (6%)
Not classifiable	30 (36%)
Initial treatment	
Voriconazole	61 (73%)
Echinocandins	2 (2%)
Combination (triazole plus echinocandins)	9 (11%)
Liposomal amfotericin B	4 (5%)
No treatment	7 (8%)

Data are n (%) or n/N (%). BAL=bronchoalveolar lavage. 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. *Procedure was not adequate in one sample.

***Table 2*: Invasive pulmonary aspergillosis characteristics of patients in the influenza cohort**

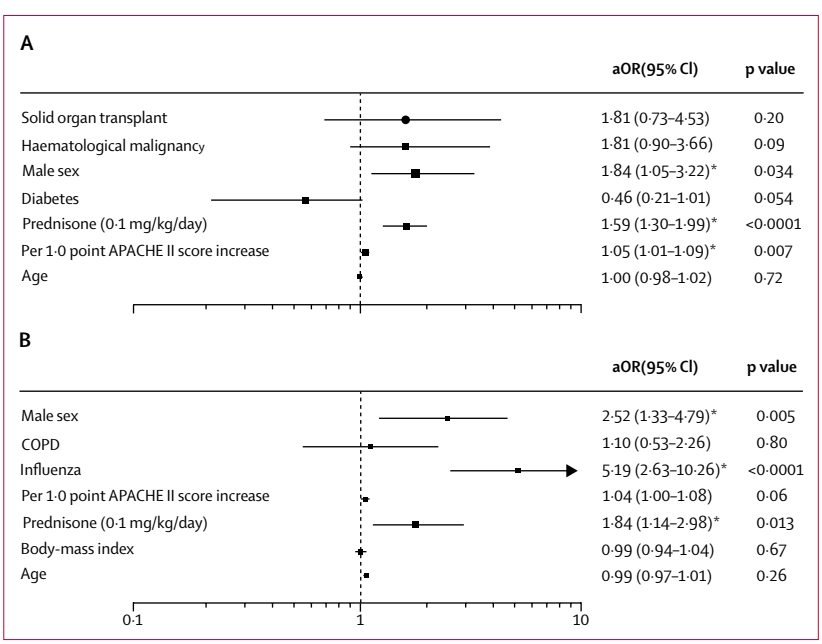

***Figure 2*: Forest plots of risk factors for the development of invasive pulmonary aspergillosis**
These have been corrected for centre as well but this is not depicted here as no significant differences were found. (A) Analysis of risk factors for the influenza cohort to develop invasive pulmonary 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.

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 extracorporeal 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 and was positive (ie, ≥0·5) in 20 (65%) patients. 21 (25%) of 83 patients had no previous comorbidities and seven (33%) patients died. Given the fact that by definition, patients with influenza who are non-immunocompromised do not fulfil the EORTC/MSG host-factor definition, only 36 (43%) of 83 patients had a proven (n=16) or probable (n=20) invasive pulmonary aspergillosis according to the EORTC/MSG classification.[11] According to the *AspICU* algorithm, specifically designed for patients in the ICU, 48 (58%) of 83 patients were diagnosed with proven (n=16) or putative (n=32) invasive pulmonary aspergillosis, while 30 (36%) patients were not classifiable because they had a positive galactomannan test on BAL but a negative lower respiratory tract culture, which is the entry criterion in the *AspICU* algorithm.[12] 76 (92%) of 83 patients received mould-active antifungal therapy. In these patients, no significant difference in the number of days from influenza diagnosis to antifungal therapy initiation was observed between survivors and non-survivors 90 days after ICU admission (4 days [IQR 1–10] *vs* 5 days [IQR 1–7] days; p=0·64).

	All EORTC/MSG negative (non-immunocompromised) patients (n=630)	Influenza case group (n=315)*	Control group (n=315)*	p value
Baseline characteristics				
Mean age, years (SD)	59 (17)	58 (16)	60 (17)	0·15
Male sex	371 (59%)	169 (54%)	202 (64%)	0·008
Mean APACHE II score on admission (SD)	23 (8)	22 (8)	23 (8)	0·29
Median body-mass index, kg/m² (IQR), missing	25 (22–29), 21	27 (23–30), 18	24 (22–28), 3	<0·0001
Diabetes	114 (19%)	63 (20%)	51 (16%)	0·21
Liver cirrhosis	44 (7%)	18 (6%)	26 (8%)	0·21
Chronic kidney disease†	69 (11%)	31 (10%)	38 (12%)	0·37
Chronic obstructive pulmonary disease	123 (20%)	68 (22%)	55 (17%)	0·19
Corticosteroids				
Corticosteroids 28 days before ICU	99/619 (16%)	57/304 (19%)	42/315 (13%)	0·005
Median dose corticosteroids 28 days before ICU admission (IQR), mg/kg/day, missing	0·078 (0·054–0·176), 22	0·070 (0·054–0·171), 10	0·080 (0·053–0·179), 12	0·79
ICU data				
Mechanical ventilation	475 (75%)	246 (78%)	229 (73%)	0·12
Median ventilation days (IQR), missing	9 (4–18), 35	11 (5–21), 26	4 (4–14), 9	0·002
Nitric oxide or high-frequency oscillation ventilation	64 (10%)	37 (12%)	27 (9%)	0·17
Extracorporeal membrane oxygenation	65 (10%)	45 (14%)	20 (6%)	0·04
Median extracorporeal membrane oxygenation days (IQR)	10 (6–20)	11 (8–21)	9 (5–18)	0·44
Vasopressors	415 (66%)	216 (69%)	199 (63%)	0·17
Renal replacement therapy	103 (16%)	61/307 (20%)	42 (13%)	0·03
Outcome data				
ICU mortality	125 (20%)	58 (18%)	67 (21%)	0·37
Hospital mortality	164 (26%)	76 (24%)	88 (28%)	0·28
90-day mortality after ICU admission	177 (28%)	78 (25%)	99 (31%)	0·70
Median days of ICU stay (IQR), missing	11 (6–21), 19	11 (6–23), 15	10 (6–18), 4	0·15
Invasive pulmonary aspergillosis	61 (10%)	45 (14%)	16 (5%)	<0·0001
Diagnostics				
BAL sampling performed	318 (50%)	145 (46%)	173 (55%)	0·026
BAL galactomannan test performed	187 (30%)	81 (26%)	106 (34%)	0·029
AspICU classification				
Proven (of people with IPA)	8 (13%)	6 (13%)	2 (13%)	..
Putative (of people with IPA)	32 (52%)	27 (60%)	5 (31%)	..
Coloniser (of people with IPA)	4 (7%)	3 (7%)	1 (6%)	..
Non-classifiable (of people with IPA)	17 (28%)	9 (20%)	8 (50%)	..

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.[12] 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**

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

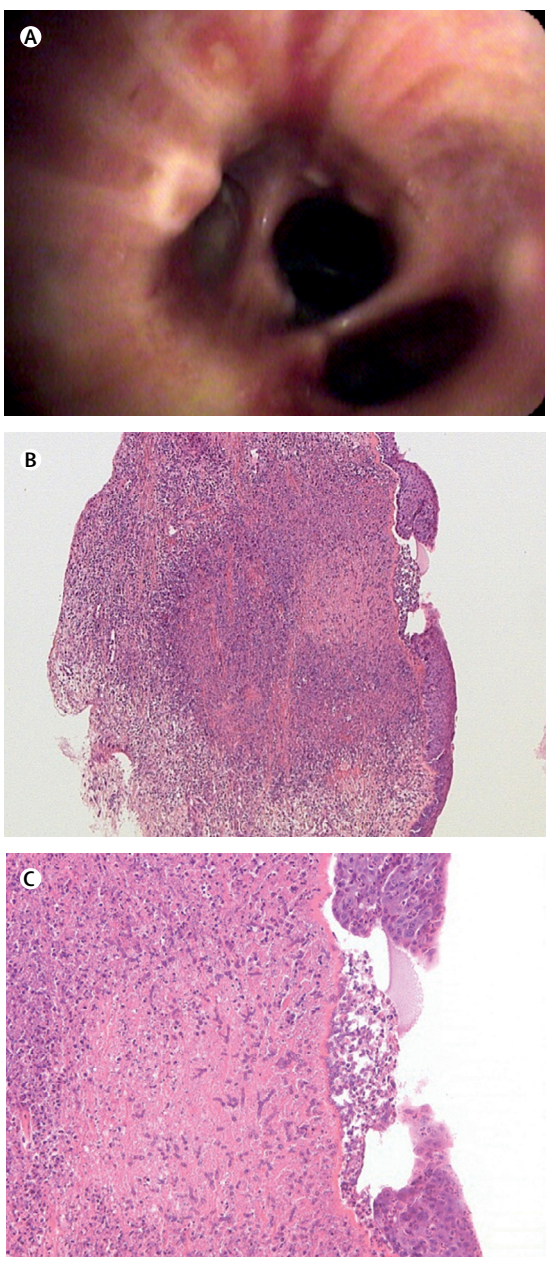

Figure 3: **Bronchoscopy and histopathology of a representative case of influenza-associated *Aspergillus* tracheobronchitis**
(A) Bronchoscopic examination reveals diffusedly inflamed mucosal tissue, with multiple whitish nodules, dispersed from the trachea into main bronchial structures. Some nodules show central necrosis. (B) Haematoxylin and eosin staining of a biopsied specimen from the trachea at 50 × magnification, showing focal ulceration with submucosal necrosis and squamous metaplasia. (C) Haematoxylin and eosin staining of a biopsied specimen at 100 × magnification, revealing invasion of submucosa by fungal hyphae, type *Aspergillus*, and dense infiltration with neutrophils.

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[1] but somewhat lower than described in cohorts over the past 10 years.[13,16] Of note, 85 patients in this influenza cohort had been included in previous studies.[9,13] 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.[9,13,19] A study[20] of 2901 patients with influenza in the ICU showed the presence of a co-infection in 17%, of which *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 *AspICU* 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.[1,12,14,21,22] Autopsy series indicated that strict interpretation of the host factors for invasive fungal disease contributes to missed diagnosis of invasive pulmonary aspergillosis.[5,23] 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, *AspICU*, and van de Veerdonk and colleagues.[11–13] 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[25] 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.[13] 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 epithelium damage and mucociliary clearance dysfunction might facilitate the invasion of *Aspergillus* (figure 3).[7,9,29] 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 *Aspergillus* superinfection.[13,33] 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[34] 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[35] 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, ViiV, and Abbvie, personal fees from Gilead, Viiv, and Great-Lake pharmaceuticals, and served as an adviser to Gilead, ViiV, 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, and received travel grants from MSD, Gilead, and Pfizer. KL received research grants from Pfizer, Gilead, and MSD outside of the context of this study, 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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