Clinical Infectious Diseases

MAJOR ARTICLE

A National Strategy to Diagnose Coronavirus Disease 2019–Associated Invasive Fungal Disease in the Intensive Care Unit

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(See the Editorial Commentary by Hoenigl on pages e1645–8.)

Background. Fungal coinfection is a recognized complication of respiratory virus infections, increasing morbidity and mortality, but can be readily treated if diagnosed early. An increasing number of small studies describing aspergillosis in coronavirus disease 2019 (COVID-19) patients with severe respiratory distress are being reported, but comprehensive data are lacking. The aim of this study was to determine the incidence, risk factors, and impact of invasive fungal disease in adult COVID-19 patients with severe respiratory distress.

Methods. An evaluation of a national, multicenter, prospective cohort evaluation of an enhanced testing strategy to diagnose invasive fungal disease in COVID-19 intensive care patients. Results were used to generate a mechanism to define aspergillosis in future COVID-19 patients.

Results. One-hundred and thirty-five adults (median age: 57, M/F: 2.2/1) were screened. The incidence was 26.7% (14.1% aspergillosis, 12.6% yeast infections). The overall mortality rate was 38%; 53% and 31% in patients with and without fungal disease, respectively ($P = .0387$). The mortality rate was reduced by the use of antifungal therapy (mortality: 38.5% in patients receiving therapy vs 90% in patients not receiving therapy ($P = .008$). The use of corticosteroids ($P = .007$) and history of chronic respiratory disease ($P = .05$) increased the likelihood of aspergillosis.

Conclusions. Fungal disease occurs frequently in critically ill, mechanically ventilated COVID-19 patients. The survival benefit observed in patients receiving antifungal therapy implies that the proposed diagnostic and defining criteria are appropriate. Screening using a strategic diagnostic approach and antifungal prophylaxis of patients with risk factors will likely enhance the management of COVID-19 patients.

Keywords. *Aspergillus*; COVID-19; critical care; incidence; risk factors and diagnosis.

The emergence of the novel coronavirus disease 2019 (COVID-19) has placed a major strain on healthcare services globally, and efforts are focused on the management of this disease. Secondary infections, including invasive pulmonary aspergillosis (IPA), are a recognized complication of respiratory virus infections [1]. A strong association has been observed in patients presenting with acute respiratory failure resulting from influenza (Influenza Associated pulmonary aspergillosis [IAPA] incidence: 19%), possibly a result of damage to epithelial cells and/or immune dysregulation [2, 3].

An increased incidence of IPA in those suffering with severe respiratory virus infection has led to concerns that this may also occur in patients with acute respiratory failure resulting from COVID-19 infection, particularly because this infection causes pulmonary damage and an inflammatory environment permissive for fungal infection [4–8]. However, data on COVID-19-associated IPA (CAPA) are currently limited to anecdotal reports or small case studies. Larger studies are needed to determine an accurate incidence, optimize diagnostics, and improve patient management [9–15].

The Public Health Wales Mycology Reference Centre has 20 years' experience of using nonculture fungal diagnostics to assist in the management of patients at risk of invasive fungal disease (IFD) [16]. Given the urgent need for evidence to guide diagnostic and antimicrobial prescribing policy, a testing strategy to diagnose IFD in critically ill COVID-19 patients across Wales was recommended with the aim of determining the incidence, impact, and risk factors (Figure 1). This manuscript describes

Received 22 July 2020; editorial decision 24 August 2020; accepted 28 August 2020; published online August 29, 2020.

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Clinical Infectious Diseases® 2021;73(7):e1634–44

DOI: 10.1093/cid/ciaa1298

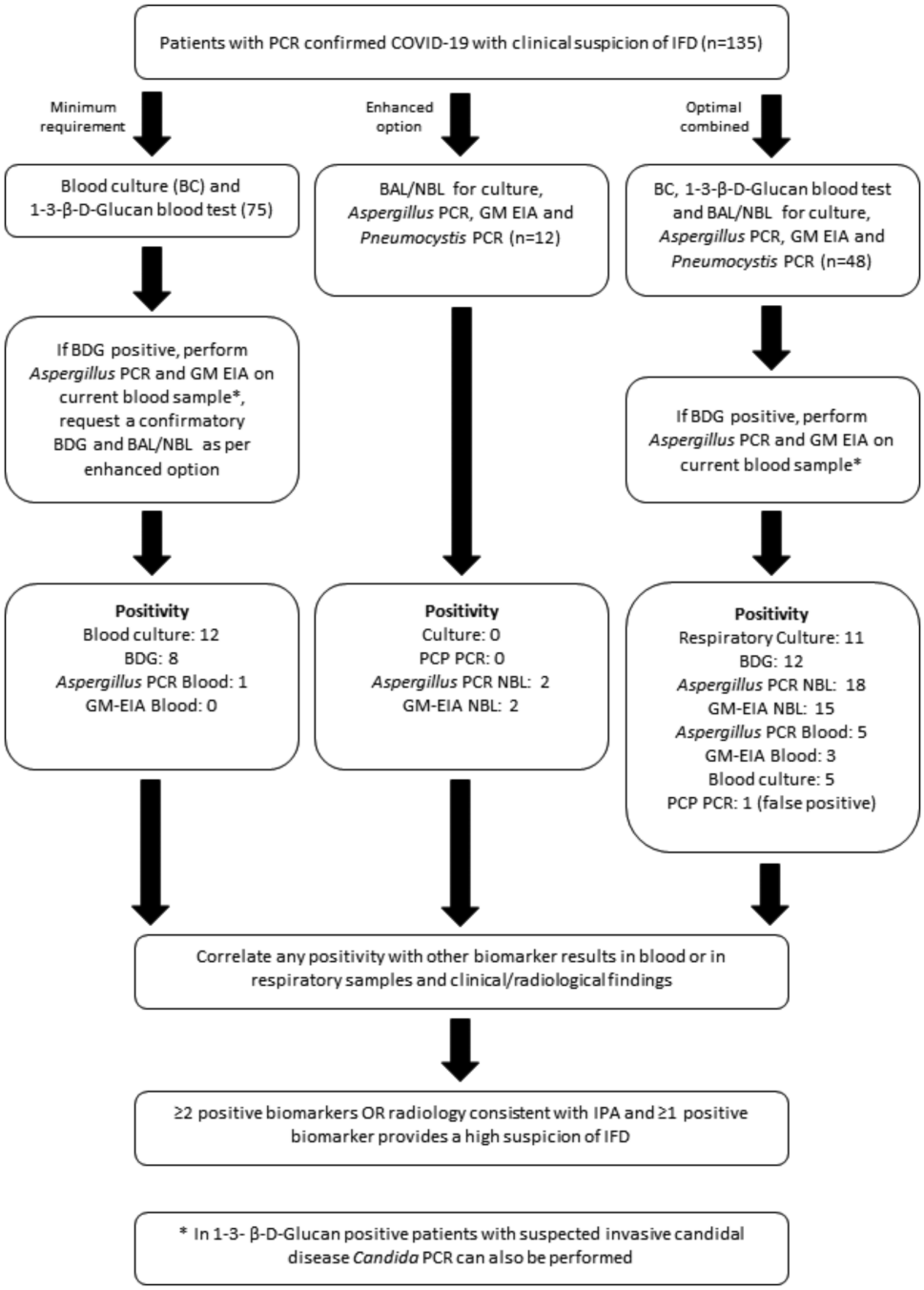

Figure 1. Diagnostic screening algorithm when managing COVID-19 patients at risk of invasive fungal disease (n = patient numbers). Samples were sent to the Public Health Wales Mycology reference laboratory at the discretion of clinicians from PCR-confirmed COVID-19 adult (≥18 years) patients requiring critical care management for prolonged (>7 days) or worsening severe respiratory dysfunction despite clinical intervention. As part of the diagnostic workup, *Aspergillus* PCR/GM-EIA and *Pneumocystis* PCR testing on NBL/BAL fluid was recommended. In addition, BDG testing of serum was advised, and, if positive, led to further fungal investigations (eg, *Aspergillus* PCR/GM-EIA). For optimal diagnosis, both respiratory and blood testing was recommended, but in the absence of a respiratory sample, BDG testing serum was a minimum requirement. Blood culture was performed according to national guidelines on the investigation of sepsis [17]. Once-weekly testing was recommended while the patient was in a critical state. In 16 patients with ≥2 *Aspergillus*-positive tests, 15 had an NBL tested, 86.7% (95% CI, 62.1–96.3) were GM-EIA positive, 80% (95% CI, 54.8–93.0) were *Aspergillus* PCR positive, and 10 patients were positive by both tests. All 16 had BDG testing of serum and 68.8% (95% CI, 44.4–85.8) were positive, being positive in 1 patient where NBL testing was not available, but *Aspergillus* PCR was also positive in blood (Table 4). In the 5 patients in whom BDG was negative, both *Aspergillus* PCR and GM-EIA were positive in NBL from 4 patients, with GM-EIA on NBL being positive on multiple occasions in 1 patient. *Aspergillus fumigatus* was cultured from NBL from a total of 11 patients (8.2%; 95% CI, 4.6–14.0) and in 56.3% (95% CI, 33.2–76.9) of patients with multiple *Aspergillus*-positive results. In 2 of the 4 patients, potentially with unspecified IFD, the BDG assay was serially positive in 2 patients in whom NBL was negative by both GM-EIA and *Aspergillus* PCR, despite radiological evidence of IFD, in the remaining patients' NBL was not available for testing but BDG was serially positive. Abbreviations: BAL, bronchoalveolar lavage; BDG, (1-3)-β-D-Glucan; COVID-19, coronavirus disease 2019; CI, confidence interval; GM-EIA, galactomannan enzyme immunoassay; IFD, invasive fungal disease; NBL, nondirected bronchial lavage; PCR, polymerase chain reaction.

the findings and is, to our knowledge, the first national, prospective screening of polymerase chain reaction (PCR)-confirmed COVID-19 patients for IFD, incorporating novel diagnostics.

MATERIALS AND METHODS

Testing Strategy and Patient Population

Enhanced mycological testing of intensive care unit (ICU) patients with refractory severe respiratory illness or deterioration of respiratory function 1 week post-COVID diagnosis was recommended. The optimum strategy, in line with recent international opinion, involved obtaining both blood and deep respiratory samples for mycological investigation of both yeast and mold infections (Figure 1) [6]. To enhance the detection of yeast infection, blood culture was combined with (1-3)-β-D-Glucan (BDG) testing, the latter also of benefit for the diagnosis of IPA, when combined with molecular, antigen, and culture investigation of respiratory samples. The service was available to all ICUs across Wales with samples sent as part of the routine investigations for COVID-19-associated secondary infections. Antifungal therapy (AFT) was administered at the clinicians' discretion. The appropriateness of AFT was determined by considering the type of IFD diagnosed (yeast or mold infection), the degree of identification when the first AFT was administered and how this related to international therapy guidelines. All data generated and interpreted were part of routine patient management, forming a prospective, consecutive cohort study covering the first 7 weeks of service, with 1-month follow-up, not requiring ethical approval.

Novel definitions, their justification for classifying CAPA, and comparison with previous definitions used to classify IPA in the ICU are described in Table 1. Novel definitions are in line with recent opinion, stratifying the confidence of IPA diagnosis according to the degree of clinical/mycological evidence [6].

Routine Investigations for Invasive Fungal Disease

Samples were tested by the BioRad *Aspergillus* antigen assay (GM-EIA) (BioRad, Hemel Hempstead, UK) following the manufacturer's instructions, using a positivity threshold of ≥0.5 in serum and ≥1.0 in deep respiratory samples (nondirected bronchial lavage [NBL] or bronchoalveolar lavage [BAL]). *Aspergillus* PCR testing was performed on 0.5 mL of serum/plasma and NBL/BAL, following international recommendations, using the BioMerieux Emag extractor, with a well-validated "in-house" quantitative-PCR assay performed on the Qiagen Rotorgene Q-HRM [22, 24]. Serum BDG was detected using the Fungitell assay (Associates of Cape Cod, Liverpool, UK) following the manufacturer's instructions, with a positivity threshold of 80 pg/mL. Samples were tested in duplicate and the mean value used for interpretation.

Blood and central venous catheter cultures were performed following national guidance for investigating sepsis, with 5–10 mL of blood incubated up to 10 days on the BD Bactec FX Automated Blood Culture Analyser [17]. Yeast were identified using the Bruker Matrix-Assisted Laser Desorption/Ionization Time-of-Flight system.

Radiological investigations were performed at the clinicians' discretion. The investigations included computed tomography (CT) of the thorax, with or without high-resolution enhancement, and CT pulmonary angiogram. Data were retrieved from prospective reports generated by the consultant radiologist, no independent analysis was performed. Radiological evidence such as nodules, halos, cavities, wedge-shaped, lobar, or segmental consolidation and tree in bud presentation were recorded as evidence typical of IPA, given these findings are not usually associated with COVID-19 infection and following well-established international definitions for IFD [19, 25]. All other evidence of chest infection was considered nonspecific. Alternative reasons for the chest radiology considered typical of IPA was documented. Given sinusitis is a frequent presentation of aspergillosis, evidence of sinusitis on CT scan of the head/sinus was recorded but not deemed typical of CAPA because of its presence in ventilated and/or COVID-19 patients. The lack of bronchoscopic investigation meant it was not possible identify mucosal plaques suggestive of *Aspergillus* tracheobronchitis, evident in IAPA [2].

Statistical Analysis

The positivity rate for each test was determined for both specimens and patients. For proportionate value, 95% confidence interval (CI) and, where required, *P* value (Fisher's exact test; $P = .05$) were generated to determine significance. Median values were compared using a Mann-Whitney U test for pairwise analysis when comparing multiple median values. Associations between clinical factors were determined for combined IFD, and IPA and candidosis individually.

RESULTS

Over the period, 257 patients were admitted to Welsh ICUs with COVID-19 infection. Fifty-three percent (135 patients) were screened for IFD, 123 patients had blood cultures and BDG testing performed, 60 patients had an NBL tested, and 48 of these patients had all tests (Figure 1). Patient demographics, clinical information, and associated mycology are shown in Table 2.

Positivity Rates of Mycological Testing

Fifty-one of the 135 patients (37.8%; 95% CI, 30.0–46.2) had ≥1 positive mycological test (culture, BDG, GM-EIA, or PCR) (Table 2). Seventeen patients (12.6%; 95% CI, 8.0–19.2) had evidence of invasive yeast infection, mainly (93.8%) *Candida* (Table 3). There was 1 case of *Rhodotorula* fungaemia. Thirty

Table 1. Strategies for Defining Invasive Pulmonary Aspergillosis (IPA) in Intensive Care Unit (ICU) Patients With COVID-19 Infection

Strategy (Abbreviation/Reference)	Requirement		
	Clinical	Radiological	Mycological
Aspergillosis in the ICU (AspICU) (18)	One of: refractory fever despite at least 3 days antibiotics. Recrudescent fever of at least 48 hours despite antibiotics. Dyspnea, Hemoptysis, Pleural rub or chest pain. Worsening respiratory function despite antibiotics and ventilatory support	Abnormal imaging on chest x-ray or chest CT	Proven: Histology/microscopy demonstrating dichotomous septate hyphae in tissue; Positive culture from tissue Putative: Positive culture from lower respiratory tract specimen in a patient with host risk factors (neutropenia, underling hematological/oncological malignancy, corticosteroids (20 mg/d), congenital/acquired immunodeficiency, COPD, decompensated cirrhosis). Semiquantitative positive culture from BAL with a positive cytological smear in the absence of bacterial growth in patient without host factors
Dutch/Belgian Influenza Associated pulmonary (IAPA) aspergillosis (2)	One of: refractory fever despite at least 3 days antibiotics. Recrudescent fever of at least 48 hours despite antibiotics Dyspnea, Hemoptysis, Pleural rub or chest pain. Worsening respiratory function despite antibiotics and ventilatory support	Any infiltrate on chest x-ray or chest CT	At least one of: Proven: Histology/microscopy demonstrating dichotomous septate hyphae in tissue; Positive culture from tissue Putative: Positive culture from BAL; positive GM-EIA in BAL (I ≥1.0). Positive GM-EIA in serum (I ≥0.5)
COVID-19 Associated pulmonary aspergillosis (CAPA)	PCR confirmed COVID-19 infection and one of: Refractory fever despite at least 3 days antibiotics. Recrudescent fever of at least 48 hours despite antibiotics Dyspnea, Hemoptysis, Pleural rub or chest pain. Worsening respiratory function despite antibiotics and ventilatory support	New infiltrates on chest x-ray or chest CT when compared with admission, including progression of signs attributed to viral infection. Radiological signs typical of invasive pulmonary aspergillosis (nodules, halos, cavities, wedge-shaped and segmental or lobar consolidation) or evidence of sinusitis should be associated with heightened suspicion of fungal disease [19, 20].	Proven: Histology/Microscopy demonstrating dichotomous septate hyphae in tissue; Positive culture from tissue Putative: Nonspecific radiology: 2 or more positives across different test types, or multiple positives within 1 test type, from the following: positive culture from NBL/BAL-positive GM-EIA in NBL/BAL (I ≥1.0) Positive GM-EIA in serum (I ≥0.5) Positive *Aspergillus* PCR in NBL BAL or blood; Positive 1-3-β-D-Glucan in serum/plasma Radiology typical of IA: One positive mycological test as listed, unless the typical radiological signs can be attributed to a different underlying infection (eg, lung cancer, alternative infection). In this scenario multiple positive results would be required to attain a diagnosis of putative IPA. Note: Given the etiological diversity associated with sinusitis, multiple positive tests from this list are required to attain a diagnosis of putative IPA.

Given the limitations of previous definitions for classifying fungal disease in ICU patients, it was decided to develop novel definitions to enhance both sensitivity and specificity. The format of the definitions, using clinical, radiological, and mycological criteria was maintained. The EORTC/MSGERC definitions are, generally, not applicable to the ICU setting because of the lack of host factors in ICU patients, and have not been included [19]. The ASPICU definitions are based on the recovery of *Aspergillus* from a respiratory tract specimen, an investigation that lacks sensitivity and is slow [18, 21]. The recently proposed definitions for classifying influenza-associated pulmonary aspergillosis (IAPA) enhanced the mycology criterion by incorporating GM-EIA of BAL and serum, but the radiological criterion, based on nonspecific chest radiology is difficult to interpret when evaluating a secondary chest infection in a patient with an underlying chest infection [2]. In the novel COVID-19 Associated Pulmonary Aspergillosis (CAPA), the radiological criterion was enhanced to reflect a progression of respiratory illness due to a secondary chest infection, but also the possible presence of chest radiology typical of IPA. The mycological criteria were extended to reflect the availability of further diagnostic investigations, including the testing of blood samples where sensitivity may be compromised but specificity is high [22]. *Aspergillus* PCR testing was included to reflect the recent acceptance of this testing format due to methodological standardization [19, 22]. 1-3-β-D-Glucan testing of serum was included, despite not being specific for *Aspergillus* because of the documented improved sensitivity over GM-EIA when testing serum for the diagnosis of aspergillosis in ICU patients [23]. The reliance on mycology for completing a classification was dependent on the presence of radiology typical of IPA. Outside of the neutropenic population typical IPA radiology is usually absent, and signs of IPA are nonspecific. It was predicted that the presence of typical IPA radiology would be highly specific, and if present would only require the support of a single positive mycological result [20]. This still represents a likely increased specificity over the IAPA definitions that combine nonspecific radiology with a single positive GM-EIA result. In the presence of nonspecific chest radiology, the novel CAPA definitions are designed to maintain increased specificity by combining the radiology with ≥2 positive mycology results. The definitions were retrospectively applied to the current patient cohort to determine respective incidences.

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; GM-EIA, *Aspergillus* antigen assay; NBL, nondirected bronchial lavage; PCR, polymerase chain reaction.

patients (22.2%; 95% CI, 16.0–30.0) had ≥1 *Aspergillus*-positive results, 14 having just a single positive result and 16 having ≥2 *Aspergillus*-positive results (Table 4). In addition, 4 patients, potentially with unspecified IFD, were BDG positive on multiple occasions. There were no documented cases of *Pneumocystis* pneumonia.

Sample and patient positivity rates for the primary diagnostic investigations are shown in Table 2 and Figure 1. Testing more samples and the optimal approach of combining NBL with BDG testing were associated with an increased likelihood of mycological positivity.

Associations Between Clinical/Pharmaceutical Factors and IFD

There was a significant association between patients with positive mycology and patients diagnosed with or treated for a solid malignancy (Table 1). Among the 57 patients in which corticosteroids data were available, there was a strong association between patients with multiple *Aspergillus*/BDG (≥2)-positive results and the use of high-dose systemic corticosteroids (13/15 patients; odds ratio [OR], 7.9; 95% CI, 1.6–39.3; $P = .007$), compared with 19 of 42 with ≤1 positive result. There was a significant association for patients with an underlying chronic respiratory condition to have multiple positive *Aspergillus*/

Table 2. Basic Demographics, Comorbidities, Risk Factors, and Test Performance According to Population

	Population			
	All ICU Patients (n = 135)	Mycology Positive (n = 51)	Mycology Negative (n = 84)	*P* Value
Median age (25th/75th percentile)	57 (48/64)	58 (50/69)	57 (47/63)	.2305
Male/female	2.2/1	2/1	2.36/1	.7038
Comorbidities (n/N)	112/131 (4 not available)	41/51	71/80 (4 not available)	.2097
Comorbidities listed	Diabetes mellitus: 38; hypertension: 35; chronic respiratory illness: 30; obesity/hyperlipidemia: 27; cardiac/vascular disease: 18; autoimmune/inflammatory conditions: 18; solid cancer: 10; kidney disease: 8; hematology malignancy: 4; other infection: 4; other: 8	Diabetes mellitus: 13; hypertension: 16; chronic respiratory illness: 14; obesity/hyperlipidemia: 10; cardiac/vascular disease: 6; autoimmune/inflammatory conditions: 8; solid cancer: 7; kidney disease: 4; hematology malignancy: 2; other infection: 0; other: 4	Diabetes mellitus: 25; hypertension: 19; chronic respiratory illness: 16; obesity/hyperlipidemia: 17; cardiac/vascular disease: 12; autoimmune/inflammatory conditions: 10; solid cancer: 3; kidney disease: 4; hematology malignancy: 2; other infection: 4; other: 4	.5559; .4185 .3947; 1.0000 .7954; .6083 **.0466**; .7106 .6424; .1563 .7106
Antibacterials administered (n/N, %)	115/122 (94.3%) (13 unavailable)	50/51 (98.0%)	65/71 (91.5%) (13 unavailable)	.2369
Antifungals administered (n/N, %)	54/121, 44.6% (14 unavailable)	35/50, 70.0% (1 unavailable)	19/71, 36.5% (13 unavailable)	**< .0001**
Invasive ventilatory support (n/N, %)	122/134, 91.0% (1 unavailable)	44/51, 86.3%	78/83, 94.0% (1 unavailable)	.2108
Corticosteroids[a] (n/N, %)	32/57, 56.1% (2 unavailable)	20/35 (57.1%)	12/22 (54.5%)	1.0
ICU LOS (days median, 25th/75th percentile))	17.5 (5.3/27.8)	19.5 (12.3/33.3)	12.0 (2.8/22.3)	.0504
Total leukocytes (median, (25th/75th percentile))	12.95 (8.95/19.30)	13.20 (9.00/20.80)	12.70 (8.80/18.70)	.4475
Neutrophils (median, (25th/75th percentile))	9.90 (6.60/16.10)	9.95 (6.40/16.15)	9.50 (6.70/16.10)	.8376
Lymphocytes (median, (25th/75th percentile))	1.20 (0.70/1.60)	1.20 (0.80/1.825)	1.10 (0.70/1.60)	.2545
PCT (median, (25th/75th percentile))	0.85 (0.29/2.20)	0.77 (0.18/2.22)	1.02 (0.35/2.20)	.4509
CRP (median, (25th/75th percentile))	139 (82.5/249)	136 (79/243)	141 (95/260)	.5300
Mortality rate (%, 95% CI)	38.3 (30.3–46.9)	47.1 (34.1–60.5)	31.3 (22.2–42.1)	.0952
Mycology				
Significant yeast culture, n/N (%, 95% CI)	17/135 (12.6, 8.0–19.2)	17/51 (33.3, 22.0–47.0)	…	
Aspergillus respiratory culture, n/N (%, 95% CI)	11/135 (8.2, 4.6–14.0)	11/51 (21.6, 12.5–34.6)	…	
Combined NBL/BDG testing strategy, n/N (%, 95% CI)	48/135 (35.6, 28.0–43.9)	30/51 (58.8, 45.2–71.3)	18/84 (21.4, 14.0–31.4)	**<.0001**
(1-3)-β-D-Glucan mean concentration, pg/mL, (95% CI)	85.6 (67.7–103.4)	151.1 (114.6–187.7)	33.5 (31.9–35.1)	**<.0001**
(1-3)-β-D-Glucan, median tests per patient, (25th/75th percentile))	2.0 (1.0/2.0)	2.0 (1.0/3.0)	1.0 (1.0/2.0)	**.0006**

Table 2. Continued

	Population			
	All ICU Patients (n = 135)	Mycology Positive (n = 51)	Mycology Negative (n = 84)	*P* Value
(1-3)-β-D-Glucan sample positivity, n/N (%, 95% CI)	38/217 (17.5, 13.0–23.1)	38/96 (39.6, 30.4–49.6)	…	
(1-3)-β-D-Glucan patient positivity, n/N (%, 95% CI)	19/122 (15.6, 10.2–23.1)	19/45 (42.2, 29.0–56.7)	…	
GM-EIA-NBL, mean GMI (95% CI)	1.2 (.7–1.7)	1.7 (1.0–2.4)	0.1 (.09–.14)	**.0024**
GM-EIA-NBL, median tests per patient, (25th/75th percentile)	2.0 (1.0/3.0)	2.0 (1.0/4.0)	1.0 (1.0/2.0)	**.0205**
GM-EIA-NBL sample positivity rate, n/N (%, 95% CI)	27/135 (20.0, 14.1–27.5)	27/93 (29.0, 20.8–38.9)	…	
GM-EIA-NBL patient positivity rate, n/N (%, 95% CI)	17/60 (28.3, 18.5–40.8)	17/35 (48.6, 33.0–64.4)	…	
Aspergillus PCR-NBL median tests per patient (25th/75th percentile)	2.0 (1.0/3.0)	2.0 (1.0/4.0)	1.0 (1.0/2.0)	**.0205**
Aspergillus PCR-NBL sample positivity rate, n/N (%, 95% CI)	31/131 (23.7, 17.2–31.6)	31/91 (34.1, 25.2–44.3)	…	
Aspergillus PCR-NBL patient positivity rate, n/N (%, 95% CI)	20/60 (33.3, 22.7–45.9)	20/35 (57.1, 40.9–72.0)	…	

P values compared data from the mycology positive and negative populations. Significant differences highlighted in bold text.

[a]Data are only available for patients admitted to the ICU of the University Hospital of Wales, Cardiff, UK.

Abbreviations: BDG, (1-3)-β-D-Glucan; CI, 95% confidence interval; CRP, C-reactive protein; GM-EIA, Galactomannan enzyme immunoassay; GMI, Galactomannan index value 95%; ICU, intensive care unit; LOS, length of stay; NBL, nondirected bronchial lavage; PCT, procalcitonin.

Table 3. Cases of Culture Confirmed Invasive Yeast Disease

Case No.	Comorbidities	Corticosteroids	Ventilatory Support	Radiological Evidence	Mycological Evidence	Antifungal Therapy[a]	Type of Infection		Died (Day 30)
							Line	Deep	
1	HTN, obesity	N/A	Yes	Nonspecific	Yeast (no ID) (CVC) tip	Fluconazole	Yes		Yes
2	HTN	None	Yes	Nonspecific	*Rhodotorula* (BC)	Caspofungin, L-Amb		Yes	Yes
3	Esophagectomy, cancer	Hydrocortisone	Yes	Nonspecific	Yeast (no ID) sterile fluid (chest drain)	Fluconazole		Yes	Yes
4	Ulcerative colitis	None	Yes	Nonspecific	*Candida albicans* (CVC)	None	Yes		No
5	DM, HTN, obesity, asthma	None	Yes	Nonspecific	*C albicans* (CVC)	Fluconazole	Yes		No
6	HTN, asthma	None	Yes	Nonspecific	*C albicans* (BC), *Candida* PCR positive, BDG 156, 95,86	Caspofungin, fluconazole		Yes	No
7	Hematological malignancy, cardiac	None	Yes	Nonspecific	*C albicans* (BC)	None		Yes	Yes
8	None	N/A	Yes	Nonspecific	*C albicans* (CVC)	Fluconazole	Yes		No
9	Cardiac, CKD, cancer (bowel)	N/A	Yes	Nonspecific	*C albicans* (CVC)	Caspofungin	Yes		Yes
10	Inflammatory, asthma, IBS	N/A	Yes	Nonspecific	*Candida* sp (CVC) BDG: (60)	Voriconazole	Yes		Yes
11	None	N/A	Yes	Nonspecific	*Candida parapsilosis* (CVC)	Caspofungin	Yes		No
12	None	N/A	Yes	Nonspecific	*C albicans* (BC, CVC)	Fluconazole	Yes	Yes	Yes
13	None	N/A	Yes	Nonspecific	*C albicans* (BC), BDG: >500, Candida PCR positive	Fluconazole, caspofungin		Yes	No
14	DM, HTN, obesity	N/A	Yes	Nonspecific	*C albicans* (CVC)	Fluconazole	Yes		No
15	Hepatitis, IVDU, neutropenia, cellulitis	N/A	Yes	Nonspecific	*C albicans* and *C parapsilosis* (BC), BDG: 386	Fluconazole, L-Amb		Yes	No
16	DM, inflammatory, alcoholic	Yes, not specified	Yes	Nonspecific	*C albicans* (ascites)	Caspofungin, voriconazole		Yes	No
17	DM, HTN	N/A	Yes	Nonspecific	*C albicans* (CVC), BDG: >500	Fluconazole, voriconazole	Yes		Yes

[a]Antifungal therapy was deemed appropriate if it were in line with international guidelines. For instance, if a yeast was recovered from blood or a CVC, but not identified to species level and the patient was commenced on fluconazole, then this would be considered inappropriate in the absence of an antifungal with a broader spectrum of activity.

Abbreviations: BC, blood culture; BDG, (1-3)-β-D-Glucan; CKD, chronic kidney disease; CVC, central venous catheter; DM, diabetes mellitus; HTN, hypertension; IBS, irritable bowel syndrome; IVDU, intravenous drug user; L-Amb, liposomal amphotericin B; no ID, no species identification available.

Table 4. Cases of COVID-19-Associated Invasive Aspergillosis (CAPA) Classified According to the Various Definitions (Described in Table 1)

Case No.	Comorbidities	Corticosteroids	Ventilatory Support	Radiological Evidence	Mycological Evidence	Antifungal Therapy[a]	Case Definition			Died (Day 30)
							AspICUV	IAPA[b]	CAPA	
1	Vasculitis, essential thrombocythemia	Hydrocortisone	Yes	Cavities, sinusitis	BDG: >500 (×3) Asp PCR NBL Positive (×2) Asp PCR plasma: Positive GM-EIA NBL: 8.3, 7.6 GM-EIA plasma: 4.9 *Aspergillus fumigatus* from NBL	Voriconazole	Yes	Yes	Yes	Yes
2	None specified	Dexamethasone	Yes	Nonspecific	BDG: 251, 237, 164 Asp PCR NBL Positive (×2) Asp PCR plasma: Positive GM-EIA NBL: 8.2, 8.4 GM-EIA plasma: (0.4) *A fumigatus* from NBL	Voriconazole	Yes	Yes	Yes	No
3	Solid cancer, CR	None	Yes	Nonspecific	*A fumigatus* from NBL	None	Yes	Yes	No	Yes
4	DM, CR	Prednisolone	Yes	Nodule	Asp PCR NBL Positive GM-EIA NBL: 12.8, (0.7) *A fumigatus* from NBL	L-Amb	Yes	Yes	Yes	Yes
5	Solid cancer	Hydrocortisone, prednisolone, dexamethasone	Yes	Tree-in-bud, nodule	BDG: 85, 105, 154 Asp PCR NBL Positive (×7) Asp PCR serum Positive (×2) GM-EIA NBL: 16.6, 3.8, 3.6, 3.2, (0.9) *A fumigatus* from NBL	Voriconazole, L-Amb	Yes	Yes	Yes	No

Table 4. Continued

Case No.	Comorbidities	Corticosteroids	Ventilatory Support	Radiological Evidence	Mycological Evidence	Antifungal Therapy[a]	Case Definition			Died (Day 30)
							AspICUV	IAPA[b]	CAPA	
6	DM, CR	IV hydrocortisone	Yes	Nodule, sinusitis	Asp PCR NBL Positive (×2) GM-EIA NBL: 5.6, 3.7 *A fumigatus* from NBL	Voriconazole	Yes	Yes	Yes	No
7	CR, autoimmune	Prednisolone (methotrexate before COVID-19)	No	Nonspecific, but not typical of COVID-19	Asp PCR NBL Positive GM-EIA NBL: 16.4, 5.2 *A fumigatus* from NBL	Voriconazole	Yes	Yes	Yes	Yes
8	HM, liver dysfunction	Methylprednisolone, IV hydrocortisone	Yes	Nodules, cavities, sinusitis	BDG: 292, 445 Asp PCR NBL Positive (×2) Asp PCR serum Positive (×2) GM-EIA NBL: 16.4, 5.2 GM-EIA serum: 0.9 *A fumigatus* from NBL	Anidulafungin, L-Amb	Yes	Yes	Yes	Yes
9	CR (asthma), obesity	Not systemic, but did received inhaled beclomethasone dipropionate and formoterol	Yes	Nonspecific, sinusitis	*A fumigatus* from NBL GM-EIA NBL: (0.5)	None	No[c]	Yes	No	No
10	DM	N/A	Yes	Cavitation, sinusitis	BDG: >500 (×2), 485 Asp PCR NBL Positive *A fumigatus* from NBL	Caspofungin, voriconazole	No[c]	Yes	Yes	No
11	CR, obesity	N/A	Yes	Nonspecific	BDG: >500 GM-EIA NBL: 1.8	None	No	Yes	Yes	Yes
12	CR	Low-dose hydrocortisone and Inhaled beclomethasone dipropionate and formoterol	Yes	Nonspecific	GM-EIA NBL: 6.8	Caspofungin, voriconazole	No	Yes	No	No
13	CR, HTN	Dexamethasone	No	Nodule	BDG: >500, 489, 367 Asp PCR NBL Positive (×2) GM-EIA NBL: 6.8, 1.2	Voriconazole	No	Yes	Yes	No
14	CR, DM, HTN	Prednisolone	No	Cavitation	BDG: 142 Asp PCR NBL Positive GM-EIA NBL: 1.5	Voriconazole	No	Yes	Yes	Yes
15	None specified	N/A	Yes	Nonspecific	GM-EIA NBL: 1.1 BDG: 109	None	No	Yes	Yes	Yes
16	HTN, obesity	Methylprednisolone	Yes	Cavitation	Asp PCR NBL Positive GM-EIA NBL: 1.6 (×2)	Voriconazole, AmBisome	No	Yes	Yes	No
17	Alzheimer, HTN	None	No	Nonspecific	GM-EIA NBL: 4.2	Voriconazole	No	Yes	No	Yes
18	HTN, solid cancer	Dexamethasone	Yes	Nonspecific	GM-EIA NBL: 1.1, (0.7)	None	No	Yes	No	No
19	DM, HTN, obesity	None	Yes	Nonspecific, sinusitis	Asp PCR NBL Positive (×2) GM-EIA NBL: 2.2 *A fumigatus* and *Aspergillus versicolor* cultured from NBL, BDG: 137 (>1 month later)	Voriconazole	No[c]	Yes	Yes	No
20	CR, solid cancer	None	No	Nodules (secondary lung metastases)	GM-EIA NBL: 1.0	None	No	Yes	No	No
21	CKD, solid cancer	Hydrocortisone, fludrocortisone	Yes	Nodules	BDG: >500, 467	Voriconazole, fluconazole	No	No	Yes	No
22	CR, obesity	Prednisolone	No	Nodule	Asp PCR NBL Positive	None	No	No	Yes	Yes
23	DM, obesity, cardiac	Methylprednisolone	Yes	Nonspecific	BDG: (70) Asp PCR NBL Positive (×2) GM-EIA NBL: (0.7)	Voriconazole	No	No	Yes	Yes
24	CR, CKD, HTN	Not systemic, fluticasone nasal spray and Symbicort inhaler	Yes	Nonspecific	BDG: 103 Asp PCR plasma: Positive	Fluconazole	No	No	Yes	Yes
25	Autoimmune, HTN	Dexamethasone, hydrocortisone, prednisolone	No	Fungal ball in sinus	BDG: >500 (×2) GM-EIA serum: (0.3)	L-Amb	No	No	Yes	Yes

Seven patients with positive *Aspergillus* mycology insufficient for classification by any of the definitions have been excluded. shaded cells reflect agreement between the definitions.

[a]Antifungal therapy was deemed appropriate if it were in line with international guidelines. For instance, if a patient was diagnosed with CAPA but had only received caspofungin, then this would be considered inappropriate because it is not recommended as frontline therapy for invasive aspergillosis.

[b]IAPA guidelines have been modified to accepted NBL GM-EIA positivity in place of testing bronchoalveolar lavage fluid.

[c]Cases did not meet AspICU definitions as the patient lacked a host factor and the *Aspergillus* culture was not performed in quantifiable manner.

Abbreviations: Asp PCR, *Aspergillus* polymerase chain reaction; BDG, (1-3)-β-D-Glucan; CKD, chronic kidney disease; CR, chronic respiratory illness; DM, diabetes mellitus; GM-EIA, Galactomannan enzyme immunoassay; HM, hematological malignancy; HTN, hypertension; L-Amb, liposomal amphotericin B; NBL, nondirected bronchial lavage.

BDG tests (7/16) compared with 23/116 patients without multiple positive results (OR, 3.15; 95% CI, 1.06–9.34; P = .05). There were no significant associations between underlying conditions/comorbidities and yeast infections (results not shown).

Procalcitonin, C-reactive protein, total leukocytes, neutrophils, and lymphocytes were similar across cohorts (Table 1).

Timing of Mycology Positivity

In the 16 patients with multiple *Aspergillus*-positive results, the median time to positivity postadmission to the ICU was 8 days, although this ranged from 0 to 35 days (90th percentile, 23.8 days). After PCR diagnosis of COVID-19 infection, the median time to positivity was 6.5 days, with a range of −20 to 22 (90th percentile, 19.9 days). In the 17 patients with yeast infection, the median time to culture positivity after ICU admission was 9 days (range, 0–38 days; 90th percentile, 26 days) and time elapsed after PCR diagnosis of COVID-19 infection was 10 days (range, 1–38 days; 90th percentile, 29 days). Positive mycology results extended the ICU admission duration (Table 2).

Radiological Evidence of IPA

In 7/16 of patients with multiple *Aspergillus*-positive results, CT scan of the thorax/CT pulmonary angiogram was nonspecific, indicative of progressing respiratory infection and indistinguishable from COVID-19 pneumonia (eg, bilateral airspace opacification). However, in 56%, chest CT scan was typical of IPA (cavities [n = 5], nodules [n = 5], and "tree in bud" [n = 1]) (Table 4). Seven patients had 1 typical chest sign and 2 patients had 2 signs. Three patients (6, 14, and 16 in Table 4) had potential bacterial respiratory infection, possibly explaining the CT evidence, although each patient had 3–5 mycological positive tests supportive of IPA. Four patients with typical chest radiology also had CT evidence of sinusitis. One additional patient with nonspecific chest radiology had evidence of sinusitis. In total, 62.5% of patients with multiple *Aspergillus*-positive tests had radiology that could be attributed to IFD.

Of the 14 patients with a single *Aspergillus*-positive test, 2 had nodules and 1 patient had evidence of sinusitis. One patient had *Aspergillus* cultured from an NBL, with a Galactomannan index (GMI) value of 0.5, another was *Aspergillus* PCR positive on NBL, and the third had a single NBL with a GMI of 1.0, but also had a potential bacterial pneumonia and likely lung metastases. None of the patients received antifungal therapy and 2 died. Two of the 4 patients that were positive by BDG alone had radiological evidence (1 potential fungal ball in the sinuses, 1 lung nodule). Two of the 84 patients who were negative for mycology had evidence of chest cavitation. Comparing the chest radiology typical of IPA from patients with multiple *Aspergillus*-positive results (n = 16) to those with yeast infection (radiology typical of IPA: 0/17) combined with patients with negative mycology (radiology typical of IPA: 2/84) generates sensitivity and specificity of 56.3% (95% CI, 33.2–76.9) and 98.0% (95% CI, 93.1–99.5), respectively. The subsequent positive likelihood ratio (28.2) was highly predictive of IPA (probability, 82.2% at a 14.1% incidence).

Defining IPA in ICU COVID-19 Patients

The incidence of IPA varies, dependent on the definitions used to classify disease (Tables 1 and 4). Using the ICU (AspICU), IAPA, and novel CAPA definitions the incidence of IPA was 5.9% (8/135), 14.8% (20/135) and 14.1% (19/135), respectively [2, 18]. Among the 3 methods, 25 patients were classified with IPA (Table 4). The 8 patients classified by the AspICU definitions were supported by the IAPA definitions, but 1 patient was not classified using the CAPA definitions. This patient had *Aspergillus fumigatus* cultured from a single NBL sample but radiology was nonspecific. Seven of the 12 additional IPA cases classified by IAPA were supported by CAPA, 5 had radiology attributable to IPA, and 2 had nonspecific radiology; all were supported with multiple *Aspergillus*-positive results (Table 4). Three patients classified by IAPA alone had nonspecific radiology with a single *Aspergillus*-positive result. Two patients had radiology that could be attributed to IFD (1 with sinusitis and 1 with nodules), both had a single supporting mycology result. Given the broad etiological diversity associated with sinusitis, including COVID-19, the lack of multiple positive *Aspergillus* markers prevented classification using the CAPA definitions (Table 1). The patient with nodules had secondary lung metastases and a bacterial pathogen, possibly explaining the radiology; subsequently multiple positive *Aspergillus* results would be required to classify CAPA. Of the 5 IPA patients classified by CAPA alone, 2 had nodules detected on chest CT scan with supporting mycological evidence and 2 had nonspecific radiology but multiple *Aspergillus*-positive results. The final patient had radiological evidence of a fungal ball in the sinuses and was supported by multiple strongly positive BDG results.

Patient Prognosis

The overall mortality rate for COVID-19 patients in the ICU was 38% (Table 1). Mortality rates in patients with negative mycology were similar irrespective of AFT (P = 1.000). The mortality rate in patients defined with CAPA was 57.9% (95% CI, 36.3–76.9), ranging from 46.7% (95% CI, 24.8–69.9) in patients receiving appropriate AFT to 100% (95% CI, 51.1–100) in patients not receiving appropriate AFT. In patients with invasive yeast infection, mortality was 47.1% (95% CI, 26.2–69.0), ranging from 27.3% (95% CI, 9.8–56.6) in patients on appropriate AFT to 83.3% (95% CI, 43.7–97.0) in those not receiving appropriate AFT (P = .0498). For combined IFD (CAPA and yeast infections), the mortality rate was 52.8% (95% CI, 37.0–68.0), being 38.5% (95% CI, 22.4–57.5) in patients receiving appropriate AFT and 90.0% (95% CI, 59.6–98.2) in those not receiving appropriate AFT (P = .008). All 4 patients with unspecified IFD died; 2 received appropriate AFT.

DISCUSSION

There is urgent need for structured IFD testing in COVID-19 patients given the likely poor prognosis in untreated patients [5, 10, 12]. BDG was incorporated as a primary test because it provides broad fungal detection in easily obtainable samples and has been associated with improved sensitivity over serum GM-EIA testing for the diagnosis of ICU-associated IPA [23]. Unfortunately, BDG testing is not universally available and although GM-EIA screening of blood is highly specific it cannot be used to exclude CAPA, leaving the testing of respiratory samples as the preferred option. Although testing BAL would be preferable, obtaining this invasive sample from a large number of COVID-19 patients represents a significant infection control risk. Obtaining NBL is less intrusive and is a routine microbiological investigation in many Welsh ICU units. Mycological testing of NBL is less validated and could be associated with the detection of upper airway fungal colonization/contamination, but a recent evaluation in COVID-19 supports NBL fungal testing, and there is an argument for a low threshold for initiating AFT, given early AFT significantly improves prognosis of IA [26, 27]. In this study, the mortality rate of IFD patients on appropriate AFT (38.5%) was comparable to patients suffering from COVID-19 alone (31.0%).

The incidence of CAPA varied according to the definitions applied (Table 1). Using the definitions proposed in this manuscript, the CAPA incidence was 14.1% of patients screened (7.4% of all COVID-19 ICU admissions), lower than 2 previous studies in France (30%) and Germany (26%). Patient numbers in this current study were 5- to 7-fold higher and its prospective, consecutive multicenter nature should provide more robust data, although geographical differences need to be considered [10, 11]. A limitation of our study is that not all ICU patients were screened, and of those that were < 50% were tested by the optimal combined respiratory/circulatory approach. Consequently, the incidence of CAPA is likely underestimated, nevertheless considerable. Although 257 COVID-19 patients were admitted to the ICU during the testing period, not all would have met the inclusion criteria (Figure 1), so calculating incidence based on all patients, or extrapolating the incidence to entire population to determine a total disease burden would not be accurate. Given 68.4% and 84.2% of CAPA-defined cases were positive by serum BDG and NBL testing, respectively, it is possible that CAPA cases were missed when combination testing was not performed, accounting for this increases the incidence to 31%, in line with other studies [10, 11, 28].

The AspICU definitions significantly underestimate the rate of CAPA (5.9%), with classification based on respiratory culture that lacks sensitivity, is slow and of limited utility in the ICU, with mortality rates similar in patients with positive *Aspergillus* respiratory culture, irrespective of AFT [21]. Applying the IAPA definitions, which incorporate GM-EIA, increases the incidence to 14.8%, similar to the proposed CAPA definitions (14.1%), but considerable discordance was evident (Table 4). Given the IAPA definitions allow nonspecific radiology with a single GM-EIA positive result, it is hoped that the CAPA definitions would provide enhanced specificity. Overall mortality rates from cases classified according to the CAPA and IAPA definitions were 58% and 45%, respectively; 46.7% of CAPA patients died despite AFT, whereas 100% not receiving AFT died. In IAPA-defined patients, 42.9% died on AFT and 50% died while not receiving AFT. As a high mortality would be expected in untreated IPA patients, it appears that the IAPA definitions are misclassifying patients. While this could be a result of testing NBL over BAL, the utility of NBL testing has been demonstrated [27]. Receiver operator characteristic analysis of NBL GM-EIA testing, with CAPA defined using the proposed definitions showed that using GM threshold of 1.2 generated a specificity of 97.4%, with values >4.5 associated with 99% specificity, implying a high likelihood of IPA (positive likelihood ratio >16) and performance comparable to BAL testing [29]. In patients with no mycological evidence of IFD, the use of AFT did not improve patient outcome, indicating that the CAPA definitions were not missing cases. The prognosis of untreated CAPA is unclear; subacute or chronic disease could occur in this heterogeneous group. The overuse of AFT is obviously of concern, but the incidence of CAPA was not excessive, and the administration of AFT on the basis of radiology typical of IPA or positive mycology represents an improvement over empirical AFT use (29% of mycology negative patients in this study received empirical AFT, without improving prognosis).

CT scan of the chest and head provided signs that could be attributed to IFD in 15 patients. However, many patients presented with nonspecific radiology, which makes diagnosing an additional respiratory infection in a patient with underlying respiratory disease challenging. In this scenario, progression of nonspecific radiology can be suggestive of IFD. Chest signs more typical of IA are highly specific (98%) and should increase concern of IA, unless they can be attributed to alternative clinical reason (eg, lung metastases) [19]. Given the variability in reporting of chest radiology, independent review of images by a radiologist with experience of discriminating IFD is recommended.

Clinical risk factors associated with CAPA included an underlying chronic respiratory condition and the use of corticosteroids. The latter has implications in the UK COVID-19 treatment randomized control trial in which 1 arm recommends the use of dexamethasone, which been associated with reduced COVID-19 mortality (RECOVERY trial, www.recoverytrial.net). Adverse outcomes in this arm could be potentially attributable to CAPA rather than COVID-19, and the benefits of this approach could be enhanced if CAPA was systematically screened for and treated. In 31 COVID-19 patients requiring ventilator support in the ICU in the Netherlands,

the incidence of putative CAPA was (10%) [30]. In this current study, 79% of CAPA patients were ventilated. As noted previously, there was a significant incidence of invasive yeast infections (13%) [31]. The reasons for this are unclear, but may be a consequence of difficult working conditions, rather than COVID-19. Given cases of IFD present 1–5 weeks after ICU admission' frequent and prolonged testing of easily obtained specimens is recommended.

To conclude, there is substantial IFD in ICU COVID-19 patients, potentially associated with poorer prognosis. The proposed systematic screening program using a combination of markers from easily obtainable samples provides a sufficiently sensitive and specific way of identifying IFD in patients with COVID-19 and has the potential to reduce mortality from this relatively frequent complication. Radiology, when typical of IA, is highly specific for CAPA, and AFT should be administered and further investigation considered. Multiple positive mycology results are also indicative of IFD. The CAPA definition provided enables clinicians to use a strategic approach for identifying and classifying IPA in critically unwell COVID-19 patients. It provides a framework for introduction of AFT in this cohort, which is likely to confer a survival benefit, if initiated early, but prospective validation is required. The use of steroids and an underlying chronic respiratory condition increase the likelihood of developing CAPA, and prophylactic AFT may benefit this group.

Note

Potential conflicts of interest. P. L. W. performed diagnostic evaluations and received meeting sponsorship from Bruker, Dynamiker, and Launch Diagnostics; speakers fees, expert advice fees, and meeting sponsorship from Gilead; and speaker and expert advice fees from F2G and speaker fees MSD and Pfizer; he is a founding member of the European *Aspergillus* PCR Initiative. M. B. reports speakers fees, expert advice fees, and meeting sponsorship from Gilead, and meeting sponsorship form Abbvie. R. D. reports educational grants from Gilead and MSD, and expert opinion fees from MSD. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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