COVID-19-Associated Invasive Aspergillosis: Data from the UK National Mycology Reference Laboratory

Andrew M. Borman,[a,b] Michael D. Palmer,[a] Mark Fraser,[a] Zoe Patterson,[a] Ciara Mann,[a] Debra Oliver,[a] Christopher J. Linton,[a] Martin Gough,[a] Phillipa Brown,[a] Agnieszka Dzietczyk,[a] Michelle Hedley,[a] Sue McLachlan,[a] Julie King,[a] Elizabeth M. Johnson[a,b]

[a]UK National Mycology Reference Laboratory, Public Health England South-West, Bristol, United Kingdom
[b]Medical Research Council Centre for Medical Mycology (MRC CMM), University of Exeter, Exeter, United Kingdom

ABSTRACT COVID-19-associated pulmonary aspergillosis (CAPA) was recently reported as a potential infective complication affecting critically ill patients with acute respiratory distress syndrome following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, with incidence rates varying from 8 to 33% depending on the study. However, definitive diagnosis of CAPA is challenging. Standardized diagnostic algorithms and definitions are lacking, clinicians are reticent to perform aerosol-generating bronchoalveolar lavages for galactomannan testing and microscopic and cultural examination, and questions surround the diagnostic sensitivity of different serum biomarkers. Between 11 March and 14 July 2020, the UK National Mycology Reference Laboratory received 1,267 serum and respiratory samples from 719 critically ill UK patients with COVID-19 and suspected pulmonary aspergillosis. The laboratory also received 46 isolates of *Aspergillus fumigatus* from COVID-19 patients (including three that exhibited environmental triazole resistance). Diagnostic tests performed included 1,000 (1-3)-β-D-glucan and 516 galactomannan tests on serum samples. The results of this extensive testing are presented here. For a subset of 61 patients, respiratory specimens (bronchoalveolar lavage specimens, tracheal aspirates, and sputum samples) in addition to serum samples were submitted and subjected to galactomannan testing, *Aspergillus*-specific PCR, and microscopy and culture. The incidence of probable/proven and possible CAPA in this subset of patients was approximately 5% and 15%, respectively. Overall, our results highlight the challenges in biomarker-driven diagnosis of CAPA, especially when only limited clinical samples are available for testing, and the importance of a multimodal diagnostic approach involving regular and repeat testing of both serum and respiratory samples.

Invasive aspergillosis (IA) complicating severe influenza pneumonia is well described from anecdotal case reports, and more recently from cross-center and large cohort studies (1–5). Of particular note, mortality was significantly higher in patients with influenza/aspergillosis than in those with influenza alone (2, 4). There exists significant variation in the reported incidence of IA in critically ill influenza patients, with incidence rates between 7.2 and 28.1% reported from different studies (1–5). These variations in calculated incidence are likely to reflect a combination of real regional differences in *Aspergillus* predominance/exposure, variable access to different diagnostic testing regimens, different clinical approaches to managing influenza patients in intensive care units (ICUs) and diagnosing IA, and variations in awareness of influenza-associated pulmonary aspergillosis (IAPA) (5). In addition, direct comparisons of the incidence have

been hindered by the employment of nonstandardized reference definitions and diagnostic algorithms for IAPA, since the European Organization for Research and Treatment of Cancer Mycoses (EORTC/MSG) criteria for the diagnosis of IA in immuno-compromised patients are not directly applicable to nonneutropenic patients in ICUs (5–7). In an attempt to circumvent these limitations in existing reference definitions, modified diagnostic algorithms have been proposed for IA in nonimmunocompro-mised ICU patients (2, 5, 6, 8, 9), including the AspICU definition (6), which showed promising sensitivity and specificity for critically ill ICU patients in whom *Aspergillus* spp. had been recovered from respiratory specimens (7). Based on current evidence, galac-tomannan (GM) testing on serum samples lacks sensitivity compared to testing with fluid recovered from bronchoalveolar lavage (BAL), serum (1-3)-β-D-glucan (BDG) test-ing lacks specificity, and there is currently insufficient evidence to support the role of *Aspergillus* lateral flow devices (LFD) and PCR on serum samples in the diagnosis of IAPA (6, 7). Thus, IAPA classification frequently relies at least in part upon a positive BAL GM test (7).

Since late 2019, a novel *Betacoronavirus* (severe acute respiratory syndrome coro-navirus 2 [SARS-CoV-2]) has rapidly spread around the world, causing a global pan-demic of coronavirus-19 disease (COVID-19) with over 13 million cases and in excess of 500,000 deaths to date (14 July 2020). The disease spectrum in COVID-19 ranges from asymptomatic infection to severe illness with acute respiratory distress syndrome (ARDS) requiring critical care, with 20 to 40% of hospitalized symptomatic patients developing ARDS (10–12). An increasing number of anecdotal case reports and case series have identified COVID-19-associated pulmonary aspergillosis (CAPA) as an addi-tional infective complication of COVID-19 (12–22). Depending on the study and the region of origin, reported incidence rates for CAPA ranged from approximately 5% (14) to 20 to 35% (17–21). Similar to IAPA, many questions remain concerning the diagnostic criteria used to define CAPA (12), and diagnosis remains challenging. Radiological findings on COVID-19 and IA in nonneutropenic patients are nonspecific and show considerable overlap. Moreover, GM testing of BAL fluids is often unavailable due to the restricted role recommended for bronchoscopy in COVID-19 and local reluctance to perform bronchoalveolar lavages due to the aerosol-generating nature of this inter-vention (12, 23, 24). Currently, the diagnostic value of serum GM and BDG testing or *Aspergillus* LFDs remains largely unknown in CAPA, although initial studies suggest that GM/BDG might lack sensitivity in this context (12, 15, 19, 23). While upper respiratory tract specimens (tracheal aspirates [TA], nonbronchoscopic lavage [NBL] specimens) are more readily available than BAL fluids from intubated COVID-19 patients, GM testing is not validated on such specimens, and isolation of an *Aspergillus* sp. in the absence of other positive biomarkers should be interpreted with a degree of caution (23). Finally, due to fears surrounding potential aerosol generation during autopsy, postmortem proof of fungal infection to substantiate putative cases of CAPA is absent in all but very rare reports (22). Due to the above limitations, the diagnosis of a substantial proportion of the putative cases of CAPA reported to date (10/35) has been based solely upon recovery of *Aspergillus* spp. from respiratory secretions, with no substantiating evidence from additional positive biomarkers (reviewed in reference 12). Similar to IAPA, varia-tions in diagnostic algorithms employed in different centers are likely to contribute to the widely variable incidence rates reported for CAPA to date.

As described abroad (18), due to ease of access to samples, many centers in the United Kingdom have adopted a weekly or twice weekly screening approach using serum BDG and GM testing, either alone or in conjunction with culture of upper respiratory tract specimens and *Aspergillus*-specific PCR, to diagnose CAPA in intubat-ed/ventilated COVID-19 ICU patients. Between 11 March and 14 July 2020, during the first "peak" of SARS-CoV-2 infections in the United Kingdom, the Public Health England UK National Mycology Reference Laboratory (MRL) received a substantial number of specimens from critically ill COVID-19 patients across the entire United Kingdom, including over 1,000 serum samples and about 90 BAL, NBL, and TA samples from over

700 patients. This report details the results of biomarker and mycological analyses of those samples.

MATERIALS AND METHODS

Inclusion/exclusion criteria for patients/samples and test selection. Samples were included in the current study only if the corresponding patient had been admitted to an ICU and had tested positive by RT-PCR for SARS-CoV-2 RNA. Samples from patients who had a clinical diagnosis of COVID-19 but repeatedly tested negative for SARS-CoV-2 were excluded. Between 11 March and 14 July 2020, the MRL received a total of 1,267 diagnostic specimens (1,178 serum samples and 89 respiratory specimens) from 719 critically ill UK patients diagnosed with COVID-19. Serum samples were subjected to BDG or GM testing as indicated by the requesting physician. However, if only one of the two tests was requested and the sample tested positive, it was subsequently retested for the presence of the other fungal biomarker. A minor proportion of serum samples were also subjected to *Aspergillus*-specific PCR if requested. Respiratory secretions (BAL fluids, NBL fluids, TA, and secretions) were subjected to GM testing, *Aspergillus*-specific PCR and microscopic examination and mycological culture (provided sufficient sample was received for all tests). A limited selection of serum or BAL fluid samples were also subjected to *Aspergillus*-specific LFD testing as described below.

GM and BDG assays. GM antigen detection in serum and BAL fluid and BDG antigen detection in serum were performed exactly as described previously (25), using the Platelia *Aspergillus* Ag kit (Bio-Rad Laboratories Ltd., Watford, UK) and the Fungitell assay (Associates of Cape Cod, Inc., East Falmouth, MA, USA), respectively. Cutoff values for positivity were >80 pg/ml (BDG), index value >0.5 (GM on serum) and index value >1.0 (GM on BAL fluid) as suggested previously (25, 26).

Aspergillus-specific PCR. Molecular testing was conducted as described previously, using an *Aspergillus*-specific real-time PCR method that is European *Aspergillus* PCR Initiative (EAPCRI)-compliant (27, 28). Briefly, total DNA was extracted from 1-ml volumes of serum or respiratory samples (BAL, NBL fluids, tracheal and secretions) as described previously (28) except that post-bead-beating, DNA was column-purified using the QIAmp DNA minikit (Qiagen Ltd., Manchester, UK). Eluted DNA was subjected to real-time *Aspergillus*-specific PCR targeting the 28S rRNA gene as described previously (27, 28).

Aspergillus-specific LFD. For a subset of samples from patients with probable CAPA (recovery of *Aspergillus* spp. from respiratory secretions and/or several simultaneously positive fungal biomarkers), BAL fluid or serum samples where available were also subjected to testing using the *Aspergillus*-specific LFD (Asp-LFD, OLM Diagnostics, Newcastle Upon Tyne, UK) exactly as directed by the manufacturer. Results were interpreted semiquantitatively by 3 independent investigators on the basis of test band intensity and scored as strong positive (+++), positive (++), weak positive (+), or negative (−).

Microscopic examination and culture of BAL fluids and tracheal secretions. Respiratory secretions from COVID-19 patients were processed for microscopic examination and culture essentially as described previously (29) with the following minor modifications. Volumes of 10 ml of respiratory fluids were centrifuged at 3,000 rpm (1,500 × *g*) for 10 min, and supernatants were discarded, leaving approximately 0.5 ml of fluid and pelleted sediment. Sedimented material was resuspended in the approximately 0.5 ml of remaining fluid, and aliquots of 0.2 ml each were inoculated onto duplicate Sabouraud's glucose peptone agar flasks containing chloramphenicol (Oxoid, Basingstoke, UK), which were incubated at 30°C and 37°C for 2 weeks. Cultures were examined and reported after 48 h and then reincubated for a further 12 days and reexamined weekly. Molds were identified by examination of macroscopic and microscopic features according to standard descriptions. The remaining 0.1 ml of resuspended sediment/fluid was mixed with an equal volume of 20% KOH and a single drop of calcofluor (Bactidrop, Remel) and examined by fluorescence microscopy with a V-2A filter (excitation, 380– to 420 nm; dichromatic mirror, 430 nm; barrier filter, 450 nm). All procedures were performed in a class 1 safety cabinet in an HG3/containment level 3 laboratory. Moreover, due to the high risk posed by the presence of SARS-CoV-2 in respiratory fluids from COVID-19 patients, the following additional precautions were taken: all operators were double-gloved while processing specimens in the cabinet, with removal of the outer pair of gloves within the safety cabinet at the end of processing; all culture flasks and other material removed from the safety cabinet were thoroughly wiped with 10% Virusolve+ (Amity International Ltd., South Yorkshire, UK); prior to microscopic examination of slides, coverslips were sealed in place with clear nail varnish, which was allowed to dry before slides were disinfected with Virusolve+ prior to removal from the safety cabinet and microscopic examination.

Antifungal susceptibility testing of A. fumigatus isolates. All 46 isolates of *A. fumigatus* from respiratory secretions of COVID-19 patients were subjected to susceptibility testing using CLSI method M38-A2 (30). Azole-resistant isolates were subjected to the AsperGenius PCR (PathoNostics B.V., Maastricht, The Netherlands) for the detection of environmental azole resistance mutations, exactly as described by the manufacturers.

Definition of possible (putative), probable, and proven cases of CAPA used in this study. As discussed above, several modified diagnostic algorithms have been proposed for IA in nonimmunocompromised ICU patients (2, 5, 6, 8, 9), as the EORTC/MSG criteria for the diagnosis of IA in immunocompromised patients cannot be applied to nonneutropenic patients in ICU. Essentially, here, we have employed the modified AspICU algorithm incorporating PCR, serology, and angioinvasion biomarkers as proposed by Gangneux and colleagues (31), with some additional subtleties as described below. Due to the nonspecific and overlapping radiological appearances of patients with COVID and CAPA, the clinical criterion of abnormal thoracic medical imaging was extended to include worsening clinical symptoms despite optimal management (increasing inflammatory markers, new chest changes, persistent fever, deteriorating pulmonary function, new episodes of unexplained sepsis, etc.). Cases were defined as

possible (putative) and proven CAPA if, in addition, the patients had one or more than one, respectively, of the following: positive *Aspergillus* PCR test on respiratory secretions or blood, recovery of *Aspergillus* spp. after culture of respiratory samples, positive GM test on BAL fluid or serum, positive BDG test on serum, or positive LFD test on serum or BAL fluid. However, since multiple and repeated tests were performed on many of the patients described in the current study, a degree of common sense was also applied to rule out likely erroneous results; for example, a single positive BDG test that was flanked by multiple negative tests in the same patient in the absence of any other mycological or biomarker evidence of infection despite multiple tests would not be considered sufficient to change the case definition (see, for example, patients 8, 49, and 52 [Table 1]). Since all current definitions of proven IAPA/CAPA require histopathological evidence of infection, which is difficult to obtain in critically ill COVID patients, none of the cases described in this study could be categorized as proven CAPA.

RESULTS

Between 11 March and 14 July 2020, the MRL received 1,267 samples from 719 critically ill UK patients with COVID-19, including 1,178 serum samples, and 89 respiratory samples. From a total of 1,000 BDG antigen tests performed, 818 (81.8%) were negative (BDG concentration, <80 pg/ml), with 655 (65.5%) BDG concentrations below the lower limit of detection of the assay (<30 pg/ml). BDG concentrations in positive serum samples were 80 to 249 pg/ml in 130 samples (13.0%), 250 to 500 pg/ml in 30 samples (3.0%), and >500 pg/ml in 22 serum samples (2.2%). Similarly, the vast majority (508/516; 98.4%) of serum samples that were subjected to GM antigen testing were negative (index value, <0.5). Multiple samples and specimen types were available for a subset of the 719 patients for whom samples were submitted, allowing a rudimentary analysis of which biomarkers might have better power in diagnosing CAPA. Table 2 summarizes the results of BDG and GM testing on serum samples and GM testing on respiratory secretions for patients in whom at least 2 different tests (BDG, serum GM, BAL GM, *Aspergillus* PCR, microscopy, and culture of respiratory secretions) were performed. The majority of patients (230/340 [67.6%] for BDG; 209/295 [70.8%] for serum GM; 40/54 [74.1%] for BAL GM) had no evidence of invasive fungal infection (no positive biomarkers). In patients with a single fungal positive biomarker, that biomarker was most likely to be BDG antigen (83/97; 85.6%), with 30% (3/10) of patients having a positive BAL GM antigen test as the sole positive biomarker and no patients having a positive serum GM antigen test as the single positive biomarker. Unsurprisingly, the relative proportions of serum BDG and serum and BAL GM tests that were positive were substantially increased in patients with multiple positive fungal biomarkers (Table 2).

Table 3 summarizes the results of biomarker tests and mycological examinations for 15 selected patients with possible (putative), probable, or proven CAPA, stratified according to recent guidelines for IAPA and aspergillosis in nonimmunocompromised ICU patients (6, 7). Patients were selected for inclusion on the basis of multiple positive biomarker(s) in serum and/or respiratory specimens. Serum BDG tests were positive at least once in 12/15 patients with suspected CAPA, whereas serum GM testing appeared less sensitive (positivity in 5/15 patients). BAL fluids were submitted to the MRL for 5/15 patients with suspected CAPA listed in Table 3. Filamentous fungal elements were seen in direct microscopy and *A. fumigatus* recovered in culture in two successive BAL specimens from a single patient (patient 1), who was positive by GM, *Aspergillus*-specific PCR, and *Aspergillus* LFD with BAL fluid, but serum BDG and serum GM negative. BAL GM tests were positive in 4/5 patients for whom BAL fluid was available for testing. Finally, *Aspergillus*-specific PCR was positive on serum or BAL fluid in 3/15 patients, and the *Aspergillus*-specific LFD test was strongly positive or positive in 3/15 patient samples and weakly positive in a further 6 (total positivity, 9/15 cases). Overall, the results of fungal biomarker testing and mycological examination of samples submitted from these 15 patients with likely CAPA underscore the importance of employing a multi-faceted approach, with no single biomarker or even a 2-biomarker combination being capable of detecting all potential cases. Since histopathological proof of infection was not available, no cases fulfilled current AspICU guidelines for proven CAPA, despite some patients having multiple positive biomarkers plus recovery of *Aspergillus* spp. from respiratory secretions.

The MRL also received 46 isolates of *A. fumigatus*, 3 of which were resistant to the

TABLE 1 Summary of biomarker testing and mycological examination of respiratory secretions for 61 patients for whom both respiratory secretions and serum samples were available for testing[a]

Case	Age (yrs)/sex	Sample	BDG	Serum GM	BAL GM	AspPCR	MICR/CULT	Clinical details	CAPA
1	46/F	TA ×2	NA	NA	INAP	Neg ×2	Neg/neg	ICU, pneumonitis, intubated	
2	52/M	BAL	<30	0.10	0.18	IMT	Neg/neg	ICU, post-ECMO, intubated, febrile, ongoing CT changes	
3	42/M	BAL	<30	0.04	0.16	Neg	Neg/neg	ICU, respiratory failure, LUL cavitation	
4[b]	64/F	BAL ×2	44	0.06	**>6.0, 4.62**	**Pos ×2**	**FF × 2/A. fumigatus × 2**	ICU, pneumonia, febrile, worsening sepsis	**Probable**
5	43/M	TA	<30	0.05	**1.43**	Neg	Neg/neg	ICU, cavitating lung lesions on CXR	Possible
6	58/M	BAL ×2	<30 ×4	0.11, 0.15	**2.55**, 0.59	Neg ×2	Neg/neg ×2	ICU, pneumonia, intubated and ventilated	Possible
7	32/M	BAL	NA	NA	0.07	Neg	Neg/neg	ICU, respiratory failure	
8	54/M	TA ×3, BAL	<30 ×2, **190**	0.04, 0.05, 0.06	0.05 ×2, 0.06, 0.10	Neg ×4	Neg/neg ×4	ICU, ventilated, slow to wean	
9	43/F	BAL	NA	NA	0.08	Neg	Neg/neg	ICU, cystic fibrosis	
10	66/F	BAL	NA	NA	0.06	Neg	Yeast/yeast	ICU	
11	64/M	TA	**197**, <30	0.04	**5.13**	Neg	Yeast/yeast	ICU, CXR changes, raised IM, continually spiking	**Probable**
12	67/M	TA	<30	0.05	INAP	Neg	Neg/neg	ICU	
13	21/F	BAL	ND	0.22	0.18	IMT	Neg/neg	ICU, pneumonia, T1RF	
14	60/M	BAL	ND	0.09	0.14	Neg	Yeast/yeast	ICU	
15	64/M	BAL	<30 ×2	ND	0.11, 0.25	Neg	Yeast/yeast	ICU, pneumonia, bilateral infiltrates on CXR	
16	62/M	BAL ×4	<30 ×5, 69	0.08, 0.10, 0.05	0.35, 0.17, 0.06, 0.08	Neg ×4	Neg/neg ×4	ICU, atypical pneumonia, T1RF, spiking, raised inflammatory markers	
17	61/M	SPU	<30 ×5	0.13 ×2, 0.04, 0.08	INAP	INAP	Neg/neg	ICU, intubated, raised inflammatory markers	
18	72/F	BAL	**95**	0.06 ×2	0.19	IMT	Neg/neg	ICU, septic	Possible
19	45/M	SPU	NA	NA	0.05	Neg	Neg/neg	ICU	
20	56/F	BAL	<30 ×6	0.04, 0.06, 0.14	0.05	IMT	Neg/neg	ICU, pneumonia, T1RF, intubated, febrile, raised IM	
21	67/M	TA	<30	0.24	0.19	Neg	Neg/neg	ICU	
22	55/M	TA	<30	0.04	0.10	Neg	Neg/neg	ICU, pneumonia, T1RF, ventilated, CXR changes, raised IM	
23	39/M	BAL	<30	0.06	0.07	Neg	Neg/neg	ICU, T1RF	
24	64/M	BAL	NA	NA	0.19	Neg	Neg/neg	ICU, pneumonia, ventilated	
25	59/M	BAL	NA	NA	0.98	IMT	Neg/yeast	ICU	
26	50/M	BAL	<30	ND	0.08	Neg	Neg/neg	ICU, atypical pneumonia, intubated, CXR changes	

(Continued on next page)

TABLE 1 (Continued)

Case	Age (yrs)/sex	Sample	BDG	Serum GM	BAL GM	AspPCR	MICR/CULT	Clinical details	CAPA
27	68/M	TA	<30	ND	0.02	Neg	Neg/neg	ICU, pneumonia, T1RF, intubated and ventilated, persistent fevers	
28	63/M	BAL	NA	NA	0.23	IMT	Neg/neg	ICU	
29	58/M	BAL	NA	NA	0.16	Neg	Neg/neg	ICU	
30	64/M	BAL ×3, TA	<30	0.26	0.22, 0.11, 0.11, 28	Neg ×4	Neg/yeast ×4	ICU, pneumonia	
31	47/M	TA ×2	<30	ND	0.05, 0.05	Neg ×2	Neg/neg ×2	ICU, intubated and ventilated, ex-ECMO, persistently febrile	Possible
32	49/M	BAL ×2	**80, 100**	0.07, 0.08	0.14, 0.07	Neg ×2	Neg/neg ×2	ICU, ARDS, T1RF, intubated and ventilated, ECMO, pyrexial	Possible
33	42/M	BAL	NA	NA	IMT	**Pos**	Neg/neg	ICU, ECMO	
34	31/M	BAL	NA	NA	0.06	Neg	Neg/neg	ICU, intubated and ventilated	
35	53/M	TA, BAL	<30	0.11	0.21, 0.16	Neg ×2	Neg/neg ×2	ICU	
36	43/M	BAL	<30	0.16	0.11	Neg	Yeast/yeast	ICU, pneumonitis	
37	29/M	BAL	NA	NA	0.07	Neg	Neg/neg	ICU	
38	57/M	BAL	**137**	0.04	0.08	**Pos**	Neg/yeast	ICU, not improving	**Probable**
39	66/M	SPU	<30 ×2	0.06	INAP	INAP	Neg/neg	ICU	
40	49/M	BAL	<30, 74	0.03, 0.09	0.08	Neg	Neg/neg	ICU, ARDS	
41	53/M	TA ×2	<30 ×2	ND	0.32, 0.06	**Pos**	Yeast/yeast	ICU, atypical pneumonia, raised IM, CXR changes, spiking	Possible
42	54/M	BAL	<30	NA	0.06	Neg	Neg/neg	ICU	
43	49/M	SPU	NA	NA	INAP	INAP	Neg/neg	ICU	
44	62/F	BAL	51	0.04	0.11	Neg	Neg/neg	ICU, pneumonia, intubated and ventilated, raised IM, febrile	
45	55/M	BAL	<30 ×2	0.05, 0.05	0.14	Neg	Neg/neg	ICU	
46	53/M	TA ×2,	<30	0.09	IMT, IMT	IMT, Neg	Neg/neg, Neg/yeast	ICU, ARDS, intubated and ventilated, long wean, bilateral infiltrates	
47	47/M	BAL	52	0.13	0.05	Neg	Neg/neg	ICU, ventilated, poor wean	
48	57/F	BAL	NA	NA	0.14	**Pos**	Neg/neg	ICU	Possible
49	76/M	TA, BAL ×2	<30, **>500,** <30 ×7	0.04 ×2	0.07, 0.06, 0.06	Neg ×3	Yeast/yeast ×3	ICU, pneumonia, intubated and ventilated, slow wean, RLL shadowing	
50	45/M	BAL	ND	0.06	0.07	Neg	Neg/neg	ICU, intubated, CXR changes: consolidation and cavitation	

TABLE 1 (Continued)

Case	Age (yrs)/sex	Sample	BDG	Serum GM	BAL GM	AspPCR	MICR/CULT	Clinical details	CAPA
51	71/U	BAL	**445**	0.12	0.04	IMT	Neg/neg	ICU	Possible
52	62/M	TA, BAL	<30, **156**, <30, <30	0.09, 0.08, 0.14, 0.11	0.37, 0.06	**Pos,** neg	Neg/neg ×2	ICU, pneumonia, T1RF, intubated, raised IM	Possible
53	35/M	BAL	<30	0.21	0.15	Neg	Neg/neg	ICU	Possible
54	52/M	BAL ×2	<30	0.04	**2.15/4.16**	IMT/neg	Yeast/yeast ×2	ICU	
55	48/M	BAL ×3	<30	0.23	0.11, 0.18, 0.04	Neg ×3	Neg/neg ×3	ICU, pneumonia, ventilated, ECMO	
56	57/F	BAL	<30	0.05, 0.04, 0.06	0.13	IMT	Neg/neg	ICU, pneumonitis, SRF, intubated and ventilated, raised IM	
57	56/F	BAL	<30	ND	0.09	Neg	Neg/neg	ICU, ventilated	
58	46/M	BAL ×4	ND	0.12, 0.08, 0.04	0.13, 0.98, 0.48, 0.25	Neg ×3	Neg/neg ×4	ICU, pneumonitis, SRF, ECMO, bilateral PEs, necrotic RLL	
59	51/F	BAL	72	0.14	0.15	Neg	Neg/neg	ICU, T1RF, bilateral consolidation	
60	37/M	BAL	45	ND	0.19	IMT	Neg/neg	ICU, pulmonary nodules	
61	54/F	BAL	<30	0.04, 0.03, 0.04	0.18	Neg	Neg/neg	ICU, SRF, ECMO	

[a]Serum (1-3)-β-D-glucan antigen concentrations (BDG; pg/ml) and galactomannan (GM) index values in serum or BAL fluid are shown, together with the results of mycological examination of respiratory secretions (MICR/CULT) and the results of Aspergillus-specific PCR (AspPCR). Positive test results are indicated in boldface text. The 87 respiratory secretions examined comprised 62 BAL fluid samples, 21 tracheal aspirates, and 4 sputum samples. Abbreviations and presentation conventions are as in Table 3 in addition to the following: IMT, insufficient material for testing; INAP, inappropriate sample for the test; ICU, intensive care unit; ECMO, extracorporeal membrane oxygenation; LUL, left upper lobe; CXR, chest X-ray; IM, inflammatory markers; T1RF, type 1 respiratory failure; ARDS, acute respiratory distress syndrome; SRF, severe respiratory failure; RLL, right lower lobe; PE, pulmonary embolism. When successive samples were submitted for a single patient, the test results are presented in chronological order.

[b]This patient is case 1 in Table 3, who was also AspLFD strongly positive.

TABLE 2 BDG and GM antigen testing of serum samples and GM testing of BAL fluids for patients for which samples were submitted for ≥2 different diagnostic tests[a]

No evidence of IFI (no positive biomarkers)			1 positive biomarker	2 positive biomarkers	>3 positive biomarkers
Serum BDG					
Patients (n = 340)		230	97	9	4
BDG neg		230	14	1	1
BDG pos			83[b]	8	3
Serum GM					
Patients (n = 295)		209	71	11	4
GM neg		209	71[b]	7	1
GM pos			0	4	3
BAL GM					
Patients (n = 54)		40	10	3	1
GM neg		40	7	2	0
GM pos			3	1	1

[a]IFI, invasive fungal infection; BDG, serum (1-3)-β-D-glucan antigen testing; GM, *Aspergillus* antigen (galactomannan) testing; neg, negative; pos, positive.
[b]Includes 3 cases of blood-culture-proven candidemia.

triazole antifungals and had mutations consistent with resistance development due to environmental exposure (i.e., the presence of the typical tandem repeat and single nucleotide polymorphisms in the *cyp51A* gene were detected using the AsperGenius PCR assay), 2 isolates of *Aspergillus niger*, and 1 isolate of *Rhizopus arrhizus*, all of which had been cultured from respiratory secretions from COVID-19 patients across the United Kingdom (Table 4). According to the clinical information submitted with isolates in conjunction with additional biomarker testing that was requested for some of the patients from which organisms were isolated, at least 6/46 isolates of *A. fumigatus* had been recovered from patients who fulfilled the diagnostic criteria for probable/proven CAPA (Table 4). Insufficient clinical information was available for all other isolates to be able to make a judgment on clinical significance.

While the data presented in the above sections provide a preliminary insight into the diagnostic utility of different fungal biomarkers in patients suspected of having CAPA, they do not contribute to our understanding of the potential prevalence of

TABLE 3 Summary of biomarker testing and mycological examination of respiratory secretions for 15 patients with possible or probable CAPA[a]

Case	Age (yrs), sex	BDG concn (pg/ml)	Serum GM index	BAL GM index	MICR/CULT/Site	AspLFD[d]	AspPCR[d]	CAPA
1	64, F	44	**0.06**	**>6.0, 4.62**	**FF seen/A. fumigatus/BALx2**[b]	**Pos +++ (B)**	**Pos (B)**	Probable
2	55, M	**>500**	**4.34**	NA	***A. fumigatus*/BAL**[c]	**Pos ++ (S)**	**Pos (S)**	Probable
3	54, M	**278, >500**	0.37	NA	***A. fumigatus*/ETT**[c]	**Pos + (S)**	Neg (S)	Probable
4	**80, M**	**>500, >500**	**1.64/1.70**	NA	***A. fumigatus*/TA ×2**[c]	**Pos ++ (S)**	Neg (S)	Probable
5	64, M	<30, **197**, <30	0.04	**5.13**	Yeast/ETT	ND	Neg (S)	Probable
6	66, M	**261**	**4.6**	NA	NA	ND	NA	Probable
7	57, M	42, **84**	**0.57**	**1.57**	ND	Neg (B)	ND	Probable
8	31, F	**>500, 389, 314**	0.17, 0.21	NA	***A. fumigatus*/SPU**[c]	Neg (S)	Neg (S)	Probable
9	38, F	<30	**0.65**	NA	NA	**Pos + (S)**	Neg (S)	Probable
10	76, M	**>500, >500**	0.05, 0.43	NA	NA	**Pos + (S)**	Neg (S)	Probable
11	47, F	<30, **>500, >500**	0.09, 0.21, 0.34, 0.26	NA	NA	**Pos + (S)**	Neg (S)	Probable
12	67, F	**222**	0.44, 0.40	NA	NA	**Pos + (S)**	ND	Probable
13	48, F	**283**	0.46	NA	NA	**Pos + (S)**	ND	Probable
14	57, M	**137**	0.04, 0.06	0.08	Yeast/BAL	ND	**Pos (B)**	Probable
15	52, M	<30	0.06, 0.04	**1.69/5.21**	Yeast ×2/BAL	Neg (B)	Neg (B)	Possible

[a]Serum (1-3)-β-D-glucan antigen concentration (BDG; pg/ml) and galactomannan (GM) index values in serum or BAL fluid are shown together with the results of mycological examination of respiratory secretions (MICR/CULT/Site) and the results of *Aspergillus*-specific LFD and PCR (AspLFD and AspPCR, respectively).
[b]Filamentous fungus (FF) was observed and *A. fumigatus* recovered in two consecutive BAL fluids processed at the MRL.
[c]Results of cultures performed by the referring laboratory and included in the clinical details that accompanied samples submitted to the MRL.
[d]Positive (Pos) or negative (Neg) results obtained with the *Aspergillus*-specific LFD and PCR (AspLFD and AspPCR, respectively) using either BAL fluids (B) or serum (S) samples. M, male; F, female; ETT, endotracheal tube; TA, tracheal aspirate; SPU, sputum sample; NA, sample not available; ND, test not done. Positive results for each patient/test are highlighted in bold text.

TABLE 4 Isolates of filamentous fungi recovered from COVID-19 patient respiratory secretions and submitted to the MRL by referring laboratories[a]

| Organism (n) and finding (n) | Sample type | | | | | |
	Cough swab	Sputum	ETA/ETT	NBL	BAL	Other
Aspergillus fumigatus (46)	1	21	7	2	14	1 (unspecified)
Probable/proven CAPA (6)	0	1	3	0	2	0
Aspergillus fumigatus azole[R] (3)	0	2	0	0	0	1 (unspecified)
Probable/proven CAPA (0)	0	0	0	0	0	0
Aspergillus niger (2)	0	1	0	0	0	1 (vasc. line tip)
Probable/proven CAPA (0)	0	0	0	0	0	0
Rhizopus arrhizus (1)	0	0	0	0	1	0
Proven fungal infection (0)	0	0	0	0	0	0

[a]Proven CAPA was scored on the basis of additional information supplied by the referring laboratory in conjunction with the results of additional testing of samples submitted to the MRL for analyses. Abbreviations are as in Table 2 in addition to the following: NBL, nonbronchoscopic lavage; azole[R], pan-triazole resistant isolates; vasc. line tip, vascular line tip.

CAPA, since the full panel of samples with diagnostic utility (repeat serum samples plus BAL fluid/other respiratory secretions) was not submitted to the MRL for the vast majority of patients. In an attempt to begin to address this issue, we examined a subset of 61 patients who at least partly fulfilled the criteria of serum plus respiratory samples. Table 1 summarizes the results of mycological examination and biomarker testing for these 61 patients. *Aspergillus fumigatus* was recovered in culture from only 2 BAL specimens out of the 83 respiratory samples analyzed (case 4 in Table 1 = patient 1 in Table 3). Of the 61 patients, 13 (21.3%) had at least 1 positive fungal biomarker (possible CAPA), with only 2/61 patients (3.3%) having 2 or more independent positive biomarkers (probable) and 1/61 patients (1.6%) with multiple positive biomarkers and recovery of an *Aspergillus* sp. from BAL fluid. Taken together, these data suggest that approximately 5% of patients had probable/proven CAPA, with a further ~15% having possible CAPA.

DISCUSSION

Here, we have presented the results of fungal biomarker testing on serum and respiratory samples and mycological examination of respiratory secretions performed on samples from over 700 critically ill COVID-19 patients across the United Kingdom, including a subset of 61 patients for which multiple sample types were available. In the majority of patients, there was no mycological evidence of CAPA (all fungal biomarkers were negative and respiratory samples failed to yield growth of *Aspergillus* spp.). The data obtained with samples from patients with possible/probable and proven CAPA (Tables 1 and 3) allow a preliminary and somewhat crude analysis of the diagnostic value of individual biomarkers and testing combinations. Measurement of serum BDG antigen levels appears potentially promising in detecting CAPA in that this biomarker was positive in 13/15 patients with possible/probable/proven CAPA (Table 3) and appears to be more sensitive than serum GM testing. In addition to CAPA, candidemia is another potential complication of severe COVID, and the MRL received 25 isolates of yeast from confirmed cases of candidemia (recovery of the organism from blood cultures) in ICU patients diagnosed with COVID-19. Serum BDG was elevated in all four of the patients from whom we received serum samples (data not shown). However, none of the patients listed in Tables 1 and 3 had any evidence of invasive or disseminated *Candida* infections, ruling out the possibility that elevated BDG was due to candidemia in those patients. However, in general, BDG positivity is not specific for CAPA in critically ill patients with COVID. Similar to BDG antigen testing on serum, GM antigen tests on BAL fluids (where samples were available) were positive in 4/5 of the patients with probable/proven CAPA who had multiple positive biomarkers (Table 3) but were positive in only 5 of 12 patients with possible/probable/proven CAPA when the 61 patients with both serum and respiratory samples available were analyzed (Table 1).

It is possible that discrepant serum biomarkers (negative serum BDG/GM) in the

single patient who was BAL GM positive and microscopy and culture positive is an indicator of the general lack of sensitivity of serum biomarkers. However, we cannot rule out that these results are an indication of heavy colonization of damaged pulmonary epithelium with *Aspergillus* sp. hyphae prior to the development of active invasive fungal disease. Indeed, distinguishing between *Aspergillus* colonization as opposed to invasive infection is likely to be difficult in this patient population and is likely to be further confounded in those COVID-19 patients with preexisting chronic pulmonary disease (32). Moreover, as has been suggested previously (12, 23, 32), recovery of *Aspergillus* spp. in culture from respiratory secretions in COVID-19 patients should be interpreted with caution given the ubiquitous nature of *A. fumigatus* spores. The MRL always performs fluorescence microscopy for the mycological examination of all respiratory samples to aid in the interpretation of the results of conventional culture. Here, we have described a safe method for the microscopic examination of respiratory secretions from COVID-19 positive patients to aid regional laboratories in diagnostic decision making. Of concern is that 3 of the 46 isolates (6.5%) of *A. fumigatus* from respiratory secretions of COVID-19 patients were resistant to azole antifungal agents. The presence of the mutations associated with emergent resistance due to environmental exposure to agricultural azoles was confirmed by the AsperGenius assay. This is a concerning finding, as it rules out the use of voriconazole as first-line therapy in these patients and highlights the necessity of susceptibility testing of all isolates from suspected infections.

The results from the current study suggest that the incidence of probable/proven and possible CAPA is approximately 5% and 15%, respectively. However, there are a number of limitations to this study. It is not a case series that describes successive patients admitted to a single institution, which would be better suited to addressing incidence rates for CAPA. In addition, since samples were submitted to the MRL for analysis, accurately establishing a denominator to aid incidence calculations is impossible due to two confounding scenarios. On the one hand, it is likely that samples were preferentially submitted on certain patients that local clinicians strongly suspected of having CAPA. Conversely, for a large number of patients (especially those where only weekly BDG testing was requested), it is likely that sampling formed part of a routine surveillance program in long-stay ICU patients. Further limitations include (i) the possibility of missing or incomplete data since the MRL relied upon information that accompanied the samples that was supplied by the referring laboratory, (ii) the fact that MRL had no control over sample types/frequency of sampling in hospitals and microbiology laboratories nationwide, with the result that there were insufficient or inappropriate samples for comprehensive diagnostic testing for many patients, and (iii) the fact that with very few exceptions, information on antifungal therapy and clinical outcome was missing. However, despite these limitations, the current study highlights the risk of invasive pulmonary aspergillosis in critically ill COVID-19 patients and adds to the growing literature concerning CAPA.

Finally, a recent study failed to find histological evidence of CAPA in postmortem biopsy samples from six patients diagnosed with probable CAPA on the basis of positive GM tests on BAL fluids with or without recovery of *Aspergillus* spp. in culture (33). All 6 patients had received combination antifungal therapy with voriconazole plus anidulafungin following the diagnosis of probable CAPA. However, given the short duration of their disease and treatment, it would seem unlikely that negative histological results reflected clearance of genuine fungal infections. Rather, such reports lend support to suggestions that a more stringent classification may be required for CAPA cases, compared to existing ones which were developed for IAPA (32, 34). At the very least, the present study underscores the challenges surrounding the diagnosis of CAPA and the importance of the serial evaluation of multiple fungal biomarkers in both serum and respiratory samples in addition to conventional mycological examination of respiratory samples as part of a comprehensive multimodal approach (26, 32–34). The precise determination of the best testing algorithm to accurately diagnose CAPA will require further studies. However, from the results presented here, we would agree with

the recent suggestion (35) that in addition to radiological imaging, serial screening for CAPA in ICU patients with deteriorating respiratory function should include (i) regular (at least weekly) *Aspergillus* antigen testing of serum samples, (ii) regular (at least weekly) BDG testing of serum samples, (iii) *Aspergillus* antigen testing of BAL fluids (where available) or nondirected lavages/tracheal aspirates, and (iv) *Aspergillus* PCR in conjunction with conventional mycological examination (microscopy and culture) of respiratory secretions if available.

ACKNOWLEDGMENTS

REFERENCES

1. Schwartz IS, Friedman DZP, Zapernick L, Dingle TC, Lee N, Sligl W, Zelyas N, Smith SW. 2020. High rates of influenza-associated invasive pulmonary aspergillosis may not be universal: a retrospective cohort study from Alberta. Can Clin Infect Dis 6:ciaa007. https://doi.org/10.1093/cid/ciaa007.
2. Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, Lagrou K, Verwij PE, Van de Veerdonk FL, Gommers D, Spronk P, Bergmans DCJJ, Hoedemaekers A, Andrinopoulou ER, van den Berg CHSB, Juffermans NP, Hodiamont CJ, Vonk AG, Depuydt P, Boelens J, Wauters J, Dutch-Belgian Mycosis Study Group. 2018. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. Lancet Respir Med 6:782–792. https://doi.org/10.1016/S2213-2600(18)30274-1.
3. Wauters J, Baar I, Meersseman P, Meersseman W, Dams K, De Paep R, Lagrou K, Wilmer A, Jorens P, Hermans G. 2012. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study. Intensive Care Med 38:1761–1768. https://doi.org/10.1007/s00134-012-2673-2.
4. Ku YH, Chan KS, Yang CC, Tan CK, Chuang YC, Yu WL. 2017. Higher mortality of severe influenza patients with probable aspergillosis than those with and without other coinfections. J Formos Med Assoc 116:660–670. https://doi.org/10.1016/j.jfma.2017.06.002.
5. Verweij PE, Rijnders BJA, Brüggemann RJM, Azoulay E, Bassetti M, Blot S, Calandra T, Clancy CJ, Cornely OA, Chiller T, Depuydt P, Giacobbe DR, Janssen NAF, Kullberg BJ, Lagrou K, Lass-Flörl C, Lewis RE, Liu PW, Lortholary O, Maertens J, Martin-Loeches I, Nguyen MH, Patterson TF, Rogers TR, Schouten JA, Spriet I, Vanderbeke L, Wauters J, van de Veerdonk FL. 2020. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. Intensive Care Med 46:1524–1512. https://doi.org/10.1007/s00134-020-06091-6.
6. Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselaers N, Dimopoulos G, Paiva JA, Misset B, Rello J, Vandewoude K, Vogelaers D, AspICU Study Investigators. 2012. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med 186:56–64. https://doi.org/10.1164/rccm.201111-1978OC.
7. Bassetti M, Giacobbe DR, Grecchi C, Rebuffi C, Zuccaro V, Scudeller L, FUNDICU investigators. 2020. Performance of existing definitions and tests for the diagnosis of invasive aspergillosis in critically ill, adult patients: a systematic review with qualitative evidence synthesis. J Infect 81:131–146. https://doi.org/10.1016/j.jinf.2020.03.065.
8. Bulpa P, Dive A, Sibille Y. 2007. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Eur Respir J 30:782–800. https://doi.org/10.1183/09031936.00062206.
9. Vandewoude KH, Blot SI, Depuydt P, Benoit D, Temmerman W, Colardyn F, Vogelaers D. 2006. Clinical relevance of Aspergillus isolation from respiratory tract samples in critically ill patients. Crit Care 10:R31. https://doi.org/10.1186/cc4823.
10. Xu Z, Li S, Tian S, Li H, Kong LQ. 2020. Full spectrum of COVID-19 severity still being depicted. Lancet 395:947–948. https://doi.org/10.1016/S0140-6736(20)30308-1.
11. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J, Song Y. 2020. Risk factors associated with acute respiratory distress syndrome and death in pa-

tients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 180:934–911. https://doi.org/10.1001/jamainternmed.2020.0994.
12. Arastehfar A, Carvalho A, van de Veerdonk FL, Jenks JD, Koehler P, Krause R, Cornely OA, S Perlin D, Lass-Flörl C, Hoenigl M. 2020. COVID-19 associated pulmonary aspergillosis (CAPA): from immunology to treatment. J Fungi 6:91. https://doi.org/10.3390/jof6020091.
13. Blaize M, Mayaux J, Nabet C, Lampros A, Marcelin AG, Thellier M, Piarroux R, Demoule A, Fekkar A. 2020. Fatal invasive aspergillosis and coronavirus disease in an immunocompetent patient. Emerg Infect Dis 26:1636–1637. https://doi.org/10.3201/eid2607.201603.
14. Wang J, Yang Q, Zhang P, Sheng J, Zhou J, Qu T. 2020. Clinical characteristics of invasive pulmonary aspergillosis in patients with COVID-19 in Zhejiang, China: a retrospective case series. Crit Care 24:299. https://doi.org/10.1186/s13054-020-03046-7.
15. Antinori S, Rech R, Galimberti L, Castelli A, Angeli E, Fossali T, Bernasconi D, Covizzi A, Bonazzetti C, Torre A, Carsana L, Tonello C, Zerbi P, Nebuloni M. 2020. Invasive pulmonary aspergillosis complicating SARS-CoV-2 pneumonia: a diagnostic challenge. Travel Med Infect Dis 26 May:101752. https://doi.org/10.1016/j.tmaid.2020.101752.
16. Prattes J, Valentin T, Hoenigl M, Talakic E, Reisinger AC, Eller P. 2020. Invasive pulmonary aspergillosis complicating COVID-19 in the ICU: a case report. Med Mycol Case Rep https://doi.org/10.1016/j.mmcr.2020.05.001.
17. Alanio A, Dellière S, Fodil S, Bretagne S, Mégarbane B. 2020. Prevalence of putative invasive pulmonary aspergillosis in critically ill patients with COVID-19. Lancet Respir Med 8:e48–e49. https://doi.org/10.1016/S2213-2600(20)30237-X.
18. Rutsaert L, Steinfort N, Van Hunsel T, Bomans P, Naesens R, Mertes H, Dits H, Van Regenmortel N. 2020. COVID-19-associated invasive pulmonary aspergillosis. Ann Intensive Care 10:71. https://doi.org/10.1186/s13613-020-00686-4.
19. Koehler P, Cornely OA, Böttiger BW, Dusse F, Eichenauer DA, Fuchs F, Hallek M, Jung N, Klein F, Persigehl T, Rybniker J, Kochanek M, Böll B, Shimabukuro-Vornhagen A. 2020. COVID-19 associated pulmonary aspergillosis. Mycoses 63:528–534. https://doi.org/10.1111/myc.13096.
20. van Arkel ALE, Rijpstra TA, Belderbos HNA, van Wijngaarden P, Verweij PE, Bentvelsen RG. 2020. COVID-19-associated pulmonary aspergillosis. Am J Respir Crit Care Med 202:132–135. https://doi.org/10.1164/rccm.202004-1038LE.
21. Lamoth F, Glampedakis E, Boillat-Blanco N, Oddo M, Pagani JL. 2020. Incidence of invasive pulmonary aspergillosis among critically ill COVID-19 patients. Clin Microbiol Infect. https://doi.org/10.1016/j.cmi.2020.07.010.
22. Santana MF, Pivoto G, Alexandre MAA, Baía-da-Silva DC, Borba MGDS, Val FA, Brito-Sousa JD, Melo GC, Monteiro WM, Souza JVB, Pinheiro SB, Ferreira LCL, Naveca FG, Nascimento VA, Corado ALG, Hajjar LA, Silva Neto JR, Siva GAV, Pasqualotto AC, Lacerda MVG. 2020. Confirmed invasive pulmonary aspergillosis and COVID-19: the value of postmortem findings to support antemortem management. Rev Soc Bras Med Trop 53:e20200401. https://doi.org/10.1590/0037-8682-0401-2020.
23. Verweij PE, Gangneux J-P, Bassetti M, Brüggemann RJM, Cornely OA, Koehler P, Lass-Flörl C, van de Veerdonk FL, Chakrabarti A, Hoenigl M. 2020. Diagnosing COVID-19-associated pulmonary aspergillosis. Lancet Microbe 1:e53–e55. https://doi.org/10.1016/S2666-5247(20)30027-6.
24. Wahidi MM, Lamb C, Murgu S, Musani A, Shojaee S, Sachdeva A,

Maldonado F, Mahmood K, Kinsey M, Sethi S, Mahajan A, Majid A, Keyes C, Alraiyes AH, Sung A, Hsia D, Eapen G. 2020. American Association for Bronchology and Interventional Pulmonology (AABIP) statement on the use of bronchoscopy and respiratory specimen collection in patients with suspected or confirmed COVID-19 infection. J Bronchology Interv Pulmonol 10:1097. https://doi.org/10.1097/LBR.0000000000000681.

25. Talento AF, Dunne K, Joyce EA, Palmer M, Johnson E, White PL, Springer J, Loeffler J, Ryan T, Collins D, Rogers TR. 2017. A prospective study of fungal biomarkers to improve management of invasive fungal diseases in a mixed specialty critical care unit. J Crit Care 40:119–127. https://doi.org/10.1016/j.jcrc.2017.03.025.

26. Eigl S, Hoenigl M, Spiess B, Heldt S, Prattes J, Neumeister P, Wolfler A, Rabensteiner J, Prueller F, Krause R, Reinwald M, Flick H, Buchheidt D, Boch T. 2107. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. Med Mycol 55:528–534. https://doi.org/10.1093/mmy/myw102.

27. White PL, Linton CJ, Perry MD, Johnson EM, Barnes RA. 2006. The evolution and evaluation of a whole blood polymerase chain reaction assay for the detection of invasive aspergillosis in hematology patients in a routine clinical setting. Clin Infect Dis 42:479–486. https://doi.org/10.1086/499949.

28. White PL, Mengoli C, Bretagne S, Cuenca-Estrella M, Finnstrom N, Klingspor L, Melchers WJ, McCulloch E, Barnes RA, Donnelly JP, Loeffler J, European Aspergillus PCR Initiative (EAPCRI). 2011. Evaluation of Aspergillus PCR protocols for testing serum specimens. J Clin Microbiol 49:3842–3848. https://doi.org/10.1128/JCM.05316-11.

29. Borman AM, Palmer MD, Delhaes L, Carrère J, Favennec L, Ranque S, Gangneux JP, Horré R, Bouchara JP. 2010. Lack of standardization in the procedures for mycological examination of sputum samples from CF patients: a possible cause for variations in the prevalence of filamentous fungi. Med Mycol 48 Suppl 1:S88–S97. https://doi.org/10.3109/13693786.2010.511287.

30. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, 2nd ed; approved standard. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

31. Gangneux JP, Reizine F, Guegan H, Pinceaux K, Le Balch P, Prat E, Pelletier R, Belaz S, Le Souhaitier M, Le Tulzo Y, Seguin P, Lederlin M, Tadié JM, Robert-Gangneux F. 2020. Is the COVID-19 pandemic a good time to include Aspergillus molecular detection to categorize aspergillosis in ICU patients? A monocentric experience. J Fungi 6:E105. https://doi.org/10.3390/jof6030105.

32. Boch T, Reinwald M, Spiess B, Liebregts T, Schellongowski P, Meybohm P, Rath PM, Steinmann J, Trinkmann F, Britsch S, Michels JD, Jabbour C, Hofmann WK, Buchheidt D. 2018. Detection of invasive pulmonary aspergillosis in critically ill patients by combined use of conventional culture, galactomannan, 1-3-beta-D-glucan and Aspergillus specific nested polymerase chain reaction in a prospective pilot study. J Crit Care 47:198–203. https://doi.org/10.1016/j.jcrc.2018.07.001.

33. Flikweert AW, Grootenboers MJJH, Yick DCY, Du Mée AWF, van der Meer NJM, Rettig TCD, Kant MKM. 2020. Late histopathologic characteristics of critically ill COVID-19 patients: different phenotypes without evidence of invasive aspergillosis, a case series. J Crit Care 59:149–155. https://doi.org/10.1016/j.jcrc.2020.07.002.

34. Fekkar A, Poignon C, Blaize M, Lampros A. 2020. Fungal infection during COVID-19: does Aspergillus mean secondary invasive aspergillosis? Am J Respir Crit Care Med 202:902–903. https://doi.org/10.1164/rccm.202005-1945LE.

35. Armstrong-James D, Youngs J, Bicanic T, Abdolrasouli A, Denning DW, Johnson E, Mehra V, Pagliuca T, Patel B, Rhodes J, Schelenz S, Shah A, van de Veerdonk FL, Verweij PE, White PL, Fisher MC. 2020. Confronting and mitigating the risk of COVID-19 associated pulmonary aspergillosis. Eur Respir J 56:2002554. https://doi.org/10.1183/13993003.02554-2020.