Diagnosis of invasive fungal disease in coronavirus disease 2019: approaches and pitfalls

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Purpose of review
This review will comment on the current knowledge for the diagnosis of the main causes of COVID-19-associated invasive fungal disease (IFD); it will discuss the optimal strategies and limitations and wherever available, will describe international recommendations.

Recent findings
A range of secondary IFDs complicating COVID-19 infection have been described and while COVID-19-associated pulmonary aspergillosis was predicted, the presentation of significant numbers of COVID-19-associated candidosis and COVID-19-associated mucormycosis was somewhat unexpected. Given the range of IFDs and prolonged duration of risk, diagnostic strategies need to involve multiple tests for detecting and differentiating various causes of IFD. Although performance data for a range of tests to diagnose COVID-19-associated pulmonary aspergillosis is emerging, the performance of tests to diagnose other IFD is unknown or based on pre-COVID performance data.

Summary
Because of the vast numbers of COVID-19 infections, IFD in COVID-19 critical-care patients represents a significant burden of disease, even if incidences are less than 5%. Optimal diagnosis of COVID-19-associated IFD requires a strategic approach. The pandemic has highlighted the potential impact of IFD outside of the typical high-risk clinical cohorts, given the ever-increasing population at risk of IFD and enhanced surveillance of fungal infections is required.

Keywords
coronavirus disease 2019-associated candidosis, coronavirus disease 2019-associated mucormycosis, coronavirus disease 2019-associated pulmonary aspergillosis, invasive fungal disease, invasive fungal disease diagnosis

INTRODUCTION

The onset of the coronavirus disease 2019 (COVID-19) pandemic raised considerable concern regarding secondary invasive fungal disease (IFD) in the critical-care patient [1]. Given the clinical interventions utilized in the critical-care setting, the risk of invasive candidal disease is significant in patients receiving antibacterials, haemodialysis or parenteral nutrition or with central venous catheters, mechanical ventilation, renal insufficiency or diabetes mellitus, all of which are common in the COVID-19 critical-care patient [2]. The considerable, unavoidable pressures on critical-care during peaks of the pandemic can limit the ability to implement sufficient infection control measures and outbreaks of *Candida auris* have been documented [3]. Although there is evidence confirming the increased incidence of invasive candidosis during the COVID-19 pandemic, it is not clear whether this is directly associated with COVID-19 disease pathogenicity or the difficulty in maintaining infection control processes [4,5].

With the recent enhanced awareness of influenza-associated pulmonary aspergillosis, there was significant anxiety that a similar manifestation would arise in the critical-care COVID-19 patient [1,6,7]. Despite differences in the pathogenicity of COVID-19 and influenza, COVID-19-associated pulmonary aspergillosis (CAPA) has been diagnosed in significant numbers, although incidences vary considerably, dependent on various factors but it remains a significant secondary complication in the critical-care COVID-19 patient

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KEY POINTS

- Due to the vast numbers of COVID-19 infections, IFD in COVID-19 critical-care patients represents a significant burden of disease, even if incidences are less than 5%.
- Optimal diagnosis of COVID-19-associated IFD requires a strategic approach involving multiple mycological tests, both conventional and novel.
- The pandemic has highlighted the potential impact of IFD outside of the typical high-risk clinical cohorts, given the ever-increasing population at risk of IFD and the wider use of immunomodulatory therapies enhanced surveillance of fungal infections is required.

associated with increased mortality [8–10]. Lymphopenia is a common manifestation of COVID-19 infection, potentially associated with poor prognosis, which itself is a documented risk factor for IFD [11,12]. Although infections, such as CAPA have been regularly diagnosed, other IFD associated with lymphopenia, such as *Pneumocystis jirovecii* pneumonia (PcP), have only been diagnosed in low numbers and generally in patients with other underlying conditions that increase the risk of PcP (e.g. HIV) [13]. The use of trimethoprim/sulfamethoxazole to empirically treat ventilator-associated pneumonia may be inadvertently lowering the incidence of PcP.

A less expected, but equally concerning complication of COVID-19 infection are the significant rates of COVID-19-associated mucormycosis (CAM), particularly in patients with poorly or uncontrolled diabetes mellitus, in geographical regions with higher background incidences of mucormycosis (e.g. India) [14▪]. With severe COVID-19 infection, frequently associated with obese patients with type 2 diabetes mellitus (T2DM), the considerable rates of T2DM in certain countries, the capacity of COVID-19 to cause, or worsen hyperglycaemia and given the use of corticosteroids to manage COVID-19, there is a considerable population at risk of CAM, which can also occur post-recovery from COVID-19 infection [14▪]. Other forms of IFD have been documented (e.g. *Rhodotorula* fungaemia, *Fusarium* and *Trichosporon* infections) but remain rare, likely a result of the low pre-COVID-19 incidence combined with limited diagnostics [10,15,16].

This review will comment on the current knowledge for the diagnosis of the main causes of COVID-19-associated IFD (Fig. 1), it will discuss the optimal strategies and limitations and wherever available will describe international recommendations for diagnosing/defining IFD. Currently, rates of COVID-19-associated IFD vary considerably between centres, likely influenced by the diagnostic strategy employed by individual centres [8]. Although some reports consider the rates of secondary IFD to be low, it is important to remember the number of patients infected by COVID-19 requiring critical-care management, subsequently at enhanced risk of IFD and when these are compared with estimates for IFD outside the setting of the pandemic it highlights the significant impact of COVID-19 (Table 1) [19]. Given the established treatments for the management of COVID-19 (e.g. Dexamethasone and/or Tocilizumab) result in suppression of the patient's innate

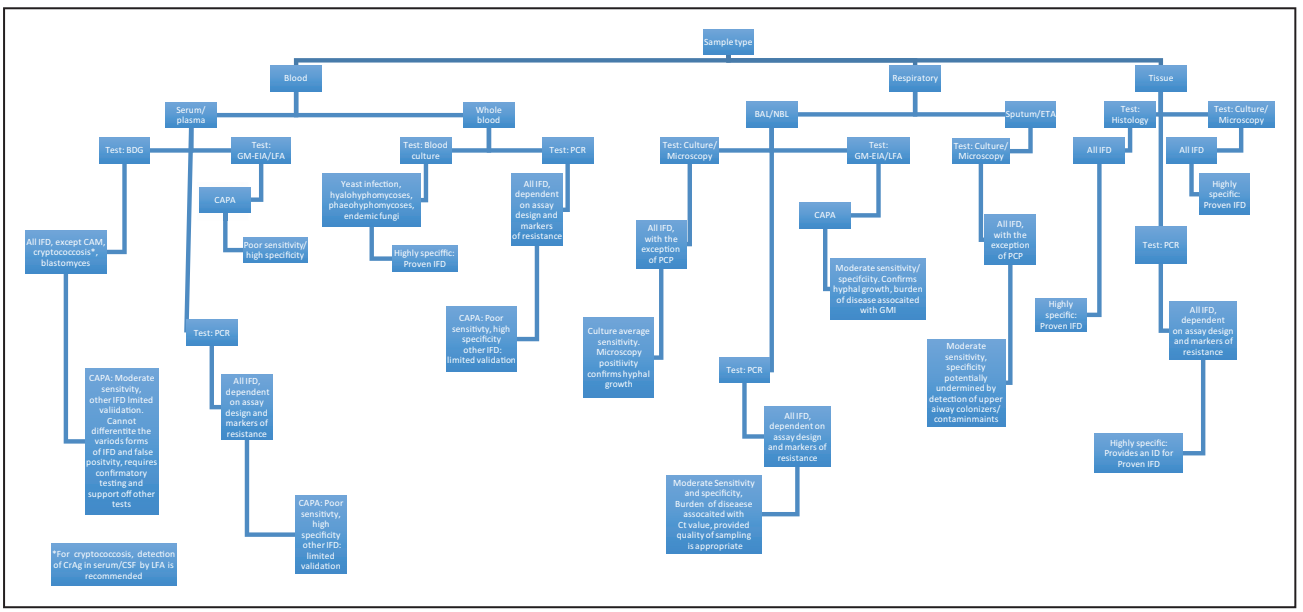

FIGURE 1. The range of mycology options for diagnosing various invasive fungal disease (with the exception of endemic fungi) in the coronavirus disease 2019 patient according to sample type.

Table 1. Global estimates for various invasive fungal disease in coronavirus disease 2019 patients according to ICU admission rates

Manifestation	Annual global estimate [a]	Annual global ICU estimate [a]	COVID-19 ICU estimates according to ICU admission rate [b]				
			1.0%	2.5%	5.0%	10.0%	20.0%
IA [c]	>450 000	>10 000	>145 000	>363 000	>726 000	>1 452 000	>2 904 000
Mucormycosis [c]	>160 000	>5000	>34 900	>87 000	>174 000	>348 000	>696 000
IC [c]	>1 000 000	>360 000	>67 900	>169 000	>338 000	>676 000	>1 352 000
PCP [c]	>500 000	NA	>1900	>4800	>9600	>19 200	>38 400
Cryptococcosis [c]	>250 000	NA	>1900	>4800	>9600	>19 200	>38 400
Total	>2 360 000	>465 000	>251 600	>628 000	>1 257 000	>2 514 000	>5 028 000

The numbers reflect estimates for each IFD, both pre-COVID-19 pandemic and during the pandemic dependent on a variable admission rate (1.0–20.0%) to the critical care unit, using the total number of COVID-19 cases documented in 27 July 2021 as an initial figure. When calculating the estimates for COVID-19-associated IFD the incidence of each, individual IFD was taken from comprehensive reviews of each manifestation, whenever available. As an example, at an ICU admission rate of 2.5%, 4 852 000 of 194 080 019 COVID-19 cases required critical care management, if the incidence of CAPA is 7.5% then a total of 363,900 cases of CAPA would be estimated. CP, *Pneumocystis* pneumonia; IA, invasive aspergillosis; IC, invasive candidosis.
[a]Estimates taken from Leading International Fungal Education (http://www.life-worldwide.org/awareness-advocacy).
[b]Based on 194 080 019 cases of COVID-19 (WHO COVID-19 dashboard 27/07/2021).
[c]The following incidences were applied: IA: 7.5% [8]; mucormycosis: 1.8% [63]; IC: 3.5% [38▪]; PCP and cryptococcosis: less than 1.0% (0.1% applied).

immune response, the risk of secondary opportunistic infection, including IFD will likely be increased and subsequent accurate diagnosis is critical to patient management.

DIAGNOSIS OF CORONAVIRUS DISEASE 2019-ASSOCIATED PULMONARY ASPERGILLOSIS

A range of testing options are available for the diagnosis of invasive aspergillosis but determining accurate test performance for the diagnosis of CAPA is difficult when results are used to classify the entity. When comparing test positivity for a range of assays across both blood and respiratory samples, it is clear that no single test detects all cases. Collating the data from 68 cases of CAPA described in six studies published early in to the course of the pandemic but with CAPA redefined using a single classification is shown in Table 2 [10,20–24]. It confirms no single test generates sensitivity close to 100%, highlighting the potential need for combined testing. Positivity rates are greater when testing respiratory samples, with galactomannan enzyme immunoassay (GM-EIA) and *Aspergillus* PCR providing the greatest sensitivity. Interestingly, rates of *Aspergillus* cultured from the respiratory tract were also moderate but could reflect the recovery of *Aspergillus* from the upper respiratory tract, which while confirming the presence of *Aspergillus* within the patient is not necessarily specific to disease and should be supported with mycological positivity in samples from deeper within the respiratory tract or in blood. Positivity in blood samples is generally lower, reflecting limited invasion by *Aspergillus* in COVID-19 patients, who commonly lack the host factors considered to impart risk for

Table 2. Performance of various mycological tests for the diagnosis of COVID-19-associated pulmonary aspergillosis in 68 cases combined from six studies[a] with cases reclassified according to a single case definition[b]

Assay type	Sample type	No of centres performing specific test ($n=6$)	Test positivity rate (%, $n=68$)
Respiratory culture	BAL/NBL/TA	6	65%
Respiratory GM-EIA	BAL/NBL	6	79%
Respiratory *Aspergillus* PCR	BAL/NBL/TA	4	73%
Blood GM-EIA	Serum	6	9%
Blood *Aspergillus* PCR	Serum/plasma	2	21%
Blood BDG	Serum	2	64%

BAL, bronchoalveolar lavage fluid; BDG, (1–3)-β-D-glucan; GM-EIA, galactomannan enzyme-immuno-assay; NBL, nondirected bronchial lavage fluid; TA, tracheal aspirate.
[a]Six studies: [10,18–22].
[b]Single case definition: [10].

invasive aspergillosis. However, serum (1–3)-β-D-Glucan (BDG) positivity appears to provide greater sensitivity over other blood biomarkers. Given the broad fungal detection range of BDG but lack of capacity to differentiate different IFD and numerous sources of BDG false positivity, combining BDG testing with other mycological tests is paramount [10,25▪,26]. A recent prospective, multicentre evaluation by the European Confederation of Medical Mycology (ECMM) confirmed the findings in Table 1 when testing up to 109 patients with CAPA defined using recent international consensus definitions [27▪▪,28▪]. Seventy-seven percent of CAPA cases were positive (index ≥1.0) by GM-EIA on broncholalveolar lavage (BAL) fluid, compared with 73% by *Aspergillus* PCR, with *Aspergillus* being cultured from the respiratory tract in up to 62% of case-based samples. The culture of *Aspergillus* is also pivotal to performing azole susceptibility testing, with cases of azole-resistant CAPA documented [29]. Serum galactomannan was only positive (index ≥0.5) in 19% of cases, but specificity of GM-EIA was excellent in both serum (99.5%) and BAL fluid (97.6%) [27▪▪]. The use of *Aspergillus* lateral flow assays may enhance access to antigen testing of both serum and BAL fluid, whereas performance outside the COVID-19 cohort is similar to GM-EIA testing validation for CAPA is currently limited [28▪].

The median time to CAPA presentation is 10 days (range 0–51 days) post-ICU admission, highlighting the need for prolonged and frequent mycological testing to ensure an earlier diagnosis [9]. Bartoletti *et al.* [24] demonstrated that although 47% of CAPA patients had GM-EIA positivity in BAL fluid within the first 2 days of admission, the majority demonstrated positivity over a longer period (>5 days). The subsequent prolonged testing period questions the suitability of BAL sampling that is invasive to the patient and raises infection control concerns in the COVID-19 patient. Testing nondirected bronchial lavage (NBL) fluid is a possible alternative respiratory sample to BAL fluid, requiring less invasive sampling using a closed suction catheter that minimizes infection control risks. The sensitivity/specificity of GM-EIA testing of NBL was 86 and 95%, respectively, with higher index greater than 4.5 values increasing specificity further (99%), performance, which is comparable with GM-EIA testing of BAL fluid in the non-COVID-19 critical-care patient [10,30,31]. Recommended thresholds for determining GM-EIA positivity in NBL are currently higher than those for BAL fluid, highlighting the uncertainty in specificity associated with NBL testing [28▪].

The incidence of CAPA is obviously dependent on the diagnostic strategy applied, and significant variation in incidence has been reported [8]. Classification based on single positive mycology results will likely generate higher incidences but the confidence in classification will vary considerably dependent on the source of positivity and its subsequent signal strength. Recovery of *Aspergillus* spp. from the upper respiratory tract may indicate airway contamination/colonization but should be used as a trigger for a diagnostic work-up [32]. Although obtaining consecutive positive upper respiratory tract cultures does increase confidence in a diagnosis of CAPA, it is far from conclusive [32]. Positivity in lower respiratory samples increases the likelihood of CAPA but false-positive GM-EIA results can occur in BAL fluid. Ideally GM-EIA BAL fluid positivity should be supported with additional mycological evidence, although GM-EIA specificity is proportional to galactomannan index value [31,33]. High index values on initial GM-EIA testing have also been associated with a poor patient prognosis [24]. Although GM-EIA and *Aspergillus* PCR positivity in blood of the COVID-19 patient is generally limited, it is likely more specific for CAPA [32]. Serum BDG positivity requires aetiological specific support to overcome the issues discussed above.

In an attempt to standardize the classification of CAPA, various diagnostic strategies have been proposed [7,10,26,28▪,32,34]. In a recent evaluation of CAPA evidence, a diagnostic work-up, based primarily on bronchoscopy and BAL fluid testing is recommended for all mechanically ventilated COVID-19 patients with unexplained respiratory deterioration or a positive *Aspergillus* culture from the respiratory tract [32]. Performing bronchoscopy also permits visualization of plaques/eschars associated with *Aspergillus* tracheobronchitis that may occur in the COVID-19 patient [28▪,32]. Undoubtedly, BAL fluid is the primary sample for the diagnosis of CAPA and the authors provide a solid basis for performing bronchoscopy, but pragmatically obtaining these samples during the peaks of the COVID-19 pandemic will be difficult, particularly in resource-limited settings. False-positive BAL fluid results will also occur and performing multiple tests (Microscopy/Culture/GM-EIA/*Aspergillus* PCR) is recommended, with multiple positive tests enhancing confidence in the CAPA diagnosis, something supported by an earlier expert opinion paper [34]. Although screening of serum with GM-EIA and BDG is not recommended because of the potential for low sensitivity, it is very difficult to facilitate screening over the required, prolonged period on the basis of BAL fluid testing. Evidence above and derived/amended from the studies included in the taskforce report demonstrates that serum-BDG sensitivity at 47% is similar to respiratory culture at 45% (currently proposed as trigger point for work-up) and may warrant BDG inclusion as a trigger alongside the testing of more

easily obtainable respiratory samples [32]. Persistent serum BDG positivity and/or mycological evidence in the non-BAL respiratory samples would trigger a diagnostic work-up, including bronchoscopy and further blood biomarkers to confirm a diagnosis.

The ECMM/ISHAM consensus CAPA definitions confirm the preference towards testing BAL fluid but incorporate the testing of more easily obtainable respiratory samples (e.g. NBL) and adjust classification accordingly [28▪]. As with all classifications proven disease is based on positive histology/microscopy/culture from a tissue biopsy, rarely obtained ante-mortem. Autopsy evidence of CAPA has provided low rates of confirmation, with a recent review confirming IFD in only 2% of deceased COVID-19 patients [35]. However, this could be indicative of limited tissue and angio-invasion in the CAPA patient, although a recent autopsy study did provide high rates (20%) of proven CAPA [36]. It is also important to remember that histological evidence is highly specific for confirming disease but sensitivity is insufficient to exclude it. In two studies, radiology typical of invasive aspergillosis was visualized in approximately 50% of CAPA patients and evidence of cavitation or well defined nodular lesions on CT should heighten the suspicion of CAPA, leading to diagnostic work-up but CT alone is not sufficient to confirm or refute CAPA [10,26,28▪,32]. Whenever present, radiology typical of invasive aspergillosis may provide clinical evidence sufficient to weight classifications so that lesser mycological evidence is required to define CAPA, compared with patients with nonspecific chest radiology [10]. It is important to remember that the ECMM/ISHAM CAPA definitions have been developed in response to urgent clinical need and through international consensus on the current information available for the diagnosis of IA, much of which has been gained outside the COVID-19 patient [28▪]. Both the incorporation of NBL testing and the current exclusion of upper respiratory culture positivity have been questioned by different groups [37,38]. The opposing views expressed, not only highlights the diagnostic dilemma encountered by centres working under different clinical pressures, including limited resources but also indicate that the current ECMM/ISHAM CAPA definitions provide a well balanced and solid platform to base studies, while awaiting further evidence required to redefine the definitions [28▪,39].

DIAGNOSIS OF CORONAVIRUS DISEASE-2019-ASSOCIATED CANDIDOSIS

CAC has been described globally at varying incidences (<1 to 23.5%), mostly presenting as candidaemia 1–2 weeks post admission, caused primarily by *Candida albicans* and *Candida glabrata*, although outbreaks caused by multidrug-resistant *C. auris* continue to be reported [3,10,18▪,40–42]. Diagnosis is primarily through the recovery of *Candida* spp. through blood culture. Although no performance data specific for CAC is currently available, it is likely that blood culture sensitivity is comparable to that in non-COVID-19 patients, detecting approximately 50% of all forms of IC, reduced when the organism causes deep-seated infection in the absence of fungaemia [43]. With higher levels of *Candida* intravenous line infection reported, regular culture of line tips may be beneficial in the deteriorating patient, and while a risk factor for deep-seated infection, additional mycological evidence is required to confirm invasive candidosis [10]. The presence of *Candida* spp. cultured from the respiratory tract likely reflect commensal organisms rather than *Candida* pneumonia, diagnosis requires histological/microscopic evidence of pseudohyphae/hyphae invading lung tissue.

Nonculture diagnostics in the form of *Candida* PCR, BDG, *Candida* antigen and antibody EIA can aid the diagnosis of invasive candidosis but performance data specific to CAC is lacking. A pre-COVID-19 meta-analysis of *Candida* PCR testing of blood generated very high sensitivity and specificity (>90%) and the development of the T2 Candida assay allows fully automated testing, with promising performance (Se: 91%/Sp: 94%) and commercial PCR assays for the detection of *C. auris* are available [44–46]. The performance of serum BDG for detection of invasive candidosis generates sensitivity and specificity of approximately 80% but an understanding of the discussed limitations of BDG testing is critical, along with combining BDG testing with *Candida* specific assays [18▪,47]. BDG testing of respiratory samples is not recommended, even when respiratory fungal infection is suspected, as commensal *Candida* spp. and other colonizing fungi will compromise assay specificity. The individual meta-analytical performance of *Candida* antibody and *Candida* antigen testing provide moderate pooled sensitivity (approximately 60%) but good pooled specificity (approximately 83–93%). Combining these two tests enhances sensitivity (approximately 83%, when either test is positive) while maintaining specificity (approximately 86%, when both tests are positive) [48].

Although *Candida* risk/colonization scores have shown potential for identifying patients at increased risk of IC, the significant number of clinical interventions and the necessary duration of admission in the ICU mean that critical-care COVID-19 patients are likely exposed to prolonged risk of invasive candidosis [18▪,40,49,50]. It remains unclear what

combination of tests will prove optimal for the diagnosis of invasive candidosis in either the COVID-19 or non-COVID-19 population, but a likely combination of molecular, serological and conventional testing will cover the range of targets potentially available in cases of invasive candidosis and when all tests are negative invasive candidosis will be unlikely [51]. In candidaemic patients, particularly those with immunosuppression or persisting blood culture positivity, transoesophageal echocardiography and fundoscopy are recommended for the diagnosis of endocarditis or ocular candidosis [52].

DIAGNOSIS OF CORONAVIRUS DISEASE 2019-ASSOCIATED MUCORMYCOSIS

CAM has emerged as a somewhat unexpected, yet devastating complication of COVID-19, with diagnosis complicated by the prolonged period of presentation (0–90 days) post COVID-19 infection, including patients who have recovered from COVID-19, coupled with the limited testing options to diagnose this IFD [14▪]. As with other IFD, proven CAM can only be diagnosed by positive histology/microscopy demonstrating broad ribbon like hyphae with limited or no septa and 90^{o} branching angles or positive culture of tissue biopsies or from other sterile sites [8,14▪]. Positive culture from respiratory tract samples (sputum, BAL fluid and tracheal aspirates) and sinus washout combined with radiology indicative of sinusitis or chest infection (nodules, reverse halo, cavities) is indicative of rhino-orbital/rhino-orbital-cerebral and pulmonary CAM, respectively. The primary presentation of CAM appears to be rhino-orbital-cerebral disease, with pulmonary disease generally presenting in patients with existing underlying conditions (e.g. haematological malignancy) that predispose to mucormycosis. Molecular testing of respiratory samples and serum may assist in the diagnosis of CAM, and can be used to aid in the identification of fungi in positive histology specimens where culture is negative. Unfortunately, the performance of Mucorales PCR is not validated for CAM, and given the wide array of species capable of causing mucormycosis may not be able to detect all causative agents. Pan-fungal PCR, with downstream processing (e.g. DNA sequencing) to provide at least a genus level identification should be used when testing positive tissue samples, although will delay the time to result. With most cases of CAM caused by *Rhizopus* species, which are usually detected by Mucorales PCR assays, this technology could play a significant role in the diagnosis of CAM, although positive culture is required for antifungal susceptibility testing [14▪]. Given the limited diagnostic options available for the diagnosis of Mucormycosis, likely exacerbated in resource limited settings and the poor sensitivity of conventional diagnostic approaches, many cases of CAM in high-risk areas (e.g. India) have been diagnosed on clinical presentation and individual underlying risk or discovered on autopsy [14▪].

DIAGNOSIS OF CORONAVIRUS DISEASE 2019-ASSOCIATED *PNEUMOCYSTIS JIROVECII* PNEUMONIA

Despite the presence of risk for developing PcP, very few cases have been reported. Case reports describing PcP in COVID-19 are generally associated with underlying conditions (e.g. HIV infection, haematological malignancy) that already predispose the patient to PcP [13]. In such patients, differentiating PcP chest radiology from that of COVID-19 infection is difficult, with manifestations, such as ground glass opacification common to both diseases. Subsequently, to avoid a misdiagnosis, it is important to consider PcP, alongside COVID-19, as part of the initial differential diagnosis when screening high-risk populations presenting with chest infection during the pandemic [53–55].

Definitive PCP diagnosis requires microscopic visualization of *P. jirovecii* in respiratory tract specimens and while immune-fluorescent microscopy improves sensitivity, it lacks the capacity to exclude PcP and interpretation remains subjective. The difficulty in culturing *Pneumocystis* precludes it from a diagnostic role [56]. PcP PCR performed on respiratory samples is a highly sensitive test, particularly when testing deeper respiratory samples (sensitivity >90%) but can detect potential *Pneumocystis* colonization/contamination of the respiratory tract rather than PcP in the COVID-19 patient, with a significant number of PcP PCR positive patients surviving despite the absence of PcP treatment [57,58]. Combining PcP PCR on respiratory samples with serum BDG (itself a very sensitive test for the diagnosis of PcP) can provide enhanced specificity when both tests are positive, and permits the PcP PCR testing of upper respiratory tract samples [59,60]. Using serum BDG alone for the diagnosis of PcP is not recommended for the reasons discussed previously, and while sensitivity is generally sufficient to exclude PcP when BDG is negative, performance outside of the HIV infected has shown reduced sensitivity, supporting a combined PcP PCR/BDG strategy. Incorporating testing of elevated serum lactate dehydrogenase, alongside PcP PCR and BDG may be useful and in a recent retrospective study, this strategy diagnosed PcP in 4/57 COVID-19 patients [60].

OTHER FUNGI

Information about other IFD associated with COVID-19 is currently limited, likely associated with not only the lesser occurrence of these infections prepandemic, similarity in symptoms between the respiratory infections but also the difficulty in diagnosing them on a global scale, outside specialist referral centres [8]. Infections associated with fungi endemic to certain geographical areas of the world have occurred, diagnosed using a range of tests including culture, microscopy, serologic antibody tests, antigen tests, and PCR. Few cases of cryptococcosis in the COVID-19 patient are documented but should be considered part of the differential diagnosis in high-risk patients (e.g. HIV infected), where testing cerebral–spinal fluid or serum for the presence of cryptococcal antigen by lateral flow assay is simple to perform and provides excellent performance (Se/Sp >90%) in the non-COVID-19 population [61–63]. Other forms of IFD (e.g. *Rhodotorula* fungaemia, *Fusarium* and *Trichosporon* infections), continue to be diagnosed in the COVID-19 patient, with diagnosis generally reliant on classical mycology [10,15,16]. Pan-fungal PCR or PCR specific to these species may play a role in the diagnosis. The detection of serum BDG may prove useful, provided BDG is present in the fungal cell wall of the specific species, while accepting that aetiological differentiation will not be feasible, which may have therapeutic consequences because of the ranging antifungal susceptibility profiles of rare yeast infections, hyalohyphomycoses and phaeohyphomycoses. Secondary infection by fungi endemic to certain geographical areas (e.g. *Histoplasma*, *Coccidioides*, *Blastomyces*) should also be considered in COVID-19 patients inhabiting or having recently traveled from such regions.

CONCLUSION

The presence of secondary IFD primarily in the critical-care COVID-19 patient, while predicted for CAPA, was somewhat unexpected for CAC and CAM. Even at low incidences (Table 1) and with only 2.5% of COVID-19 patients requiring critical-care management the combined burden of IFD exceeds what we would expect to see in the ICU by approximately 35%. With such a broad range of IFD not usually seen in the critical-care patient, outside specific high-risk populations, it highlights the need for comprehensive IFD screening algorithms during the pandemic (https://covidandfungus.org/care-step-pathways/). With an ever-increasing population at risk of fungal disease and the concerning emergence of antifungal resistance, it is time to recognize the increasing need for enhanced mycological diagnosis within microbiology, and outside specialist referral centres.

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