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Practice Guidelines for the Diagnosis of COVID-19-Associated Pulmonary Aspergillosis in an Intensive Care Setting

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Abstract

Coronavirus disease-2019 (COVID-19)-associated pulmonary aspergillosis (CAPA) is a new disease characterized by secondary *Aspergillus* mold infection in patients with COVID-19. It primarily affects patients with COVID-19 in critical state with acute respiratory distress syndrome, requiring intensive care and mechanical ventilation. CAPA has a higher mortality rate than COVID-19, posing a serious threat to affected individuals. COVID-19 is a potential risk factor for CAPA and has already claimed a massive death toll worldwide since its outbreak in December 2019. Its second wave is currently progressing towards a peak, while the third wave of this devastating pandemic is expected to follow. Therefore, an early and accurate diagnosis of CAPA is of utmost importance for effective clinical management of this highly fatal disease. However, there are no uniform criteria for diagnosing CAPA in an intensive care setting. Therefore, based on a review of existing information and our own experience, we have proposed new criteria in the form of practice guidelines for diagnosing CAPA, focusing on the points relevant for intensivists and pulmonary and critical care physicians. The main highlights of these guidelines include the role of CAPA-appropriate test specimens, clinical risk factors, computed tomography of the thorax, and non-culture-based indirect and direct mycological evidence for diagnosing CAPA in the intensive care unit. These guidelines classify the diagnosis of CAPA into suspected, possible, and probable categories to facilitate clinical decision-making. We hope that these practice guidelines will adequately address the diagnostic challenges of CAPA, providing an easy-to-use and practical algorithm to clinicians for rapid diagnosis and clinical management of the disease.

Keywords

COVID-19-associated pulmonary aspergillosis, diagnosis, practice guidelines, intensive care, clinical risk factors, computed tomography, non-culture-based mycological factors

Introduction

Invasive pulmonary aspergillosis (IPA) is a life-threatening fungal infection caused by *Aspergillus* species.[1] It usually occurs in immunocompromised individuals who have neutropenia, hematologic malignancies, acquired or genetic immunodeficiencies, hematopoietic stem cell or solid organ transplantation, and corticosteroid or other immunosuppressive therapies.[2] Recently, after the outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), IPA has been frequently observed in these otherwise immunocompetent patients. This new entity of the disease has been termed COVID-19-associated pulmonary aspergillosis (CAPA).[3–5] It mainly affects patients with COVID-19 in critical state with acute respiratory distress syndrome (ARDS), requiring intensive care and mechanical ventilation. Pulmonary lesions and immune dysfunctions caused by SARS-CoV-2 render patients with COVID-19 susceptible to CAPA. The risk of CAPA in patients with COVID-19 increases more after treatment with immunomodulators (eg, tocilizumab) and corticosteroids (eg, prednisolone or dexamethasone) that impairs neutrophil and lymphocyte functions and induces an immunosuppressed state.[6–8] Critical COVID-19 patients with an immunosuppressed state acquire *Aspergillus* infection from the environment including hospital air, and develop CAPA as schematically shown in Figure 1.

CAPA was initially highlighted through case reports.[3–5] Subsequent case series[9–14] and cohort studies[6,15–19] have reported its frequency as varying from 3% to 35%. CAPA is

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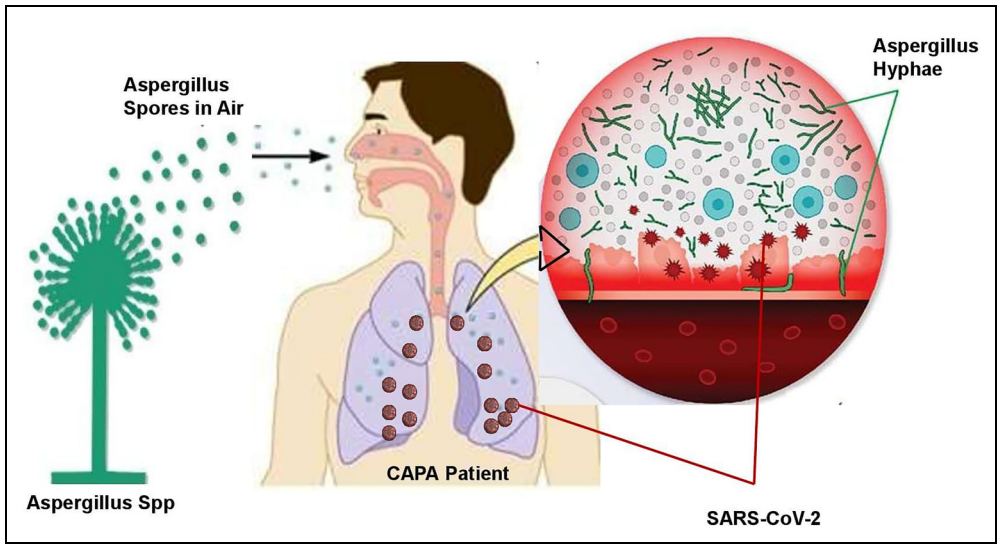

Figure 1. A schematic representation of CAPA. The immunodeficiency caused by SARS-CoV-2 infection and/or subsequent glucocorticoids/immunomodulatory treatment make the severe COVID-19 patients prone to acquire *Aspergillus* infection from the environment to develop CAPA.

Abbreviations: COVID-19, Coronavirus disease 2019; CAPA, COVID-19 associated pulmonary aspergillosis; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

responsible for >60% mortality in patients with COVID-19.[17–19] Thus, it is a frequent and fatal complication in patients with COVID-19 and poses a serious threat. COVID-19 is a potential risk factor for CAPA and has already claimed a massive death toll worldwide since its outbreak in December 2019. Its second wave is currently progressing towards a peak, while the third wave of this devastating pandemic is expected to follow. Therefore, an early and accurate diagnosis of CAPA is of utmost importance for effective clinical management of this highly fatal disease.[20–22]

The diagnosis of CAPA is challenging for intensivists, and pulmonary and critical care physicians as both IPA and COVID-19 are lower respiratory tract pulmonary infections with overlapping signs and symptoms, and no significant differences in clinical characteristics of patients with COVID-19 versus those with presumed CAPA. There are no uniform criteria for diagnosing CAPA in an intensive care setting, and hence, it is often missed or misdiagnosed in the patients.[23–26] Various studies have diagnosed CAPA either by recovering *Aspergillus* species from culture or applying diagnostic algorithms proposed for immunocompromised conditions, influenza-associated pulmonary aspergillosis (IAPA), or other patients in the intensive care unit (ICU) and have reported a widely variable incidence of the disease.[6,9–19] These non-CAPA algorithms include the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) criteria,[2] *Aspergillus* ICU (AspICU) criteria,[27] modified AspICU criteria,[28] IAPA criteria,[29] and biomarker–AspICU (BM-AspICU) criteria.[30] However, these algorithms do not take account of the unique pathology of CAPA, and CAPA-appropriate specimens for the diagnosis of the disease.

Recently, two algorithms, namely the European Confederation of Medical Mycology, the International Society for Human and Animal Mycology (ECMM/ISHAM) criteria,[31] and Asp-COVID-19 criteria[32] were proposed for the diagnosis of CAPA. However, they are quite rigid and complex for use in routine clinical practice, particularly in ICU/emergency clinics that require a rapid diagnosis. Furthermore, they rely on fungal cultures and do not include the complete profile of fungal biomarkers important for establishing a rapid diagnosis of CAPA (Table 1). In the present study, we aimed to propose clinically practicable diagnostic criteria based on CAPA-appropriate test specimens and diagnostic factors for the rapid detection of CAPA in an intensive care setting.

CAPA-Appropriate Test Specimens

An early diagnosis of CAPA is usually hampered by a lack of a consensus on the best (ie, disease-appropriate) specimens for testing in terms of sensitivity and practicability. A lung biopsy is the only specimen to demonstrate a definitive or proven *Aspergillus* infection.[2] However, being an invasive procedure, it is not feasible in the majority of CAPA patients due to significant risks associated with their critical illness and hemodynamic instability.[25]

Conventional blood or serum specimens may be helpful in diagnosing *Aspergillus* infection but they have low sensitivity when used alone because being a nonneutropenic condition, CAPA predominantly has a broncho-invasive *Aspergillus* infection with fungal growth confined mainly to lung tissues during the early phase of the disease.[9,12,24,33,34] Therefore, obtaining direct pulmonary specimens is essential, and its rationale is that the deeper the specimen, the higher the diagnostic value for CAPA. Accordingly, bronchoalveolar lavage (BAL), and non-bronchoscopic lavage (NBL) represent CAPA-appropriate specimens for early diagnosis of the disease (Table 2).

Table 1. Different Criteria for Diagnosis of CAPA.

Criteria	Specimens	Mycology Tests	CAPA Definitions	Limitation
EORTC/MSG Criteria-2020[2]	• Serum/ Plasma • BAL • Bronchial Brush • Sputum	• Microscopy and Culture • GM assay • *Aspergillus* PCR	• Proven CAPA: Histopathology or culture (+) lung biopsy. • Possible CAPA (i) Host Factors (+) (ii) Radiology (+). (iii) Mycology (−) • Probable CAPA: (i) Host Factors (+) (ii) Radiology) (+) (iii) Mycology (+)	These criteria are for immunocompromised patients and not applicable to CAPA.
AspICU Criteria[27]	• Serum • BAL • TA	Microscopy and culture	• Proven CAPA: Histopathology or culture (+) lung biopsy. • Putative CAPA: • *Aspergillus* (+) TA culture • Clinical Features (+) • Radiology (+) • *Aspergillus* (+) BAL culture	• Requires BAL and mycology cultures. • No fungal biomarkers and Aspergillus-PCR. • Originally proposed for critical ICU patients with various conditions.
Modified AspICU Criteria[28]	• Serum • BAL	• Microscopy and culture • GM assay	• Proven CAPA: Histopathology or culture (+) lung biopsy. • Probable CAPA: (i) Clinical Features (+) (ii) Radiology (+) (iii) Mycology (+)	• Requires BAL and mycology culture. • No *Aspergillus*-PCR and fungal biomarkers other than GM. • Originally proposed for IAPA
IAPA Criteria[29]	• Serum • BAL • TA • Sputum	• Microscopy and culture • GM assay	• Proven CAPA: Histopathology or culture (+) lung biopsy. • Probable CAPA: (i) Radiology (+) (ii) Mycology (+)	• Requires BAL and mycology culture. • No *Aspergillus*-PCR and fungal biomarkers other than GM. • Originally proposed for IAPA
BM-AspICU criteria[30]	• Serum • BAL	• Microscopy and culture • GM assay • *Aspergillus* PCR	• Probable CAPA: (i) Clinical Features (+) (ii) Radiology (+) (iii) ≥2 Mycology (+) • Possible CAPA: (i) No ≥1 Radiology sign (ii) No mycology other than BAL culture (+)	• Requires BAL and mycology culture. • No fungal biomarker other than GM and *Aspergillus*-PCR. • Proposed for ICU patients with various conditions
ECMM/ISHAM) criteria for CAPA[31]	• Serum • BAL • NBL	• Microscopy and culture • GM assay • GM-LFA • *Aspergillus* PCR	• Proven CAPA: Histopathology or culture (+) lung biopsy. • Probable CAPA: (i) Clinical Features (+) (ii) Radiology (+) (iii) BAL/Blood-Mycology (+) • Possible CAPA: (i) Clinical Features (+) (ii) Radiology (+) NBL (iii) Mycology (+)	• Proposed mainly for research and clinical trials • No fungal biomarker other than GM and *Aspergillus*-PCR.

(continued)

Table 1. (continued)

Criteria	Specimens	Mycology Tests	CAPA Definitions	Limitation
AspCOVID-19 criteria[32]	• Serum • NBL	• Microscopy and culture • GM assay	• Probable CAPA: (i) Clinical Features (+) (ii) Radiology (+) (iii) Mycology (+)	Requires mycology culture. No *Aspergillus*-PCR and fungal biomarkers other than GM.

Abbreviations: CAPA, COVID-19-associated pulmonary aspergillosis; GM, Galactomannan; NBL, nonbronchoscopic lavage; BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; ECMM/ISHAM, European Confederation of Medical Mycology, the International Society for Human and Animal Mycology; BM-AspICU, biomarker *Aspergillus* ICU; IAPA, influenza-associated pulmonary aspergillosis; AspICU, *Aspergillus* ICU; GM-LFA, galactomannan lateral flow assay

Due to its direct lavaging from the lungs, BAL carries negligible microbial contamination from the upper aerodigestive tract and represents the preferred test specimen for the diagnosis of CAPA.[29,34,35] The availability of BAL from patients with CAPA is often restricted because bronchoscopy, being an aerosol-generating procedure, is usually avoided for safety concerns to prevent the transmission of SARS-CoV-2 from patients to health care workers through aerosolized viral particles.[36] However, to combat this limitation, new and safe procedures of bronchoscopy using a single-use disposable bronchoscope and closed bronchosampler have been devised to obtain BAL from ventilated patients with CAPA.[37,38]

NBL, also called mini-BAL, is the next lower respiratory tract specimen of choice for CAPA diagnosis. It can be safely obtained using a closed system by deep bronchial suction without bronchoscopy and has a diagnostic value comparable to that of BAL.[39,40] The usage of NBL instead of BAL is a new approach, and its application in the diagnostic testing of CAPA is currently gaining momentum.[15,16,41,42] Recently proposed ECMM/ISHAM[31] and Asp-COVID-19[32] diagnostic guidelines for CAPA have also recommended the use of NBL. Similar to CAPA, the early diagnosis of *Aspergillus* infection is challenging in several other nonneutropenic conditions, including IAPA, chronic obstructive pulmonary disease, lung transplant recipients, and critically ill ICU patients.[27–30] Therefore, once the diagnostic utility of NBL is validated in CAPA, its use can be further extended to diagnose the *Aspergillus* infection in these conditions.

Although CAPA is predominantly a bronchoinvasive disease, it may progress to be angioinvasive during the advanced stage, leading to appearance of disease-associated fungal biomarkers in the peripheral blood as seen in nonsurviving patients with CAPA.[32] Thus, in line with recent international opinion, the mycological testing of both blood and CAPA-appropriate deep respiratory specimens (ie, BAL or NBL) is essential for optimal diagnostic workup of the disease.[43]

CAPA-Appropriate Diagnostic Factors

The pathogenesis of IPA differs between patients with neutropenia (neutropenic) and those with COVID-19 (nonneutropenic), thereby affecting the clinical presentation, radiological findings, and other diagnostic factors. Patients with CAPA

Table 2. CAPA-Appropriate Test Specimens and Diagnostic Factors.

Test Specimens	Clinical Features	Risk Factors	CT Findings	Mycological Evidence
• BAL/NBL • Heparinized Blood • Plain Blood	• ARDS • Respiratory insufficiency despite ventilatory support • Refractory Fever • Pleuritic chest pain • Dyspnea • Hemoptysis	• Severe COVID-19 requiring ICU care: and Mechanical ventilation, NIV, HFNC • Corticosteroid therapy • Tocilizumab/Other immunomodulatory therapy • Uncontrolled diabetes • CAD • CKD • COPD • Cirrhosis, hepatic insufficiency • Other (chronic alcohol abuse, chronic diseases, cardiac surgery, etc) • Severe Immunodeficiency diseases like AIDS and CGD.	• Nodules or consolidation with cavitary or air crescent changes • Consolidation in "reverse halo" or "bird nest" appearance" • Consolidation in broncho-vascular distribution • Appearance of new consolidation patch • Multiple nodular lesions • Rounded lung lesion with nonenhancing centre • Tree-in-bud pattern	• BDG assay • Galactomannan assay • Mannoprotein lateral flow assay • Direct Microscopy • Asp-PCR

Abbreviations: COVID-19, coronavirus disease-2019; CAPA: COVID-19 associated pulmonary aspergillosis; BAL, bronchoalveolar lavage; NBL, nonbronchoscopic lavage; ARDS, acute respiratory distress syndrome; ICU, intensive care Unit; NIV, noninvasive ventilation; HNFC, high-flow nasal cannula; CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; AIDS, acquired immunodeficiency disease syndrome; CG,: chronic granulomatous disease; BDG, 1 to 3-β-D-glucan; Asp-PCR, *Aspergillus* polymerase chain reaction.

have none of the host factors present in neutropenic or immunosuppressed conditions. Thus, the diagnosis of CAPA is established mainly on the presence of mycological evidence of *Aspergillus* infection, while clinical and radiological features of the disease serve as supporting diagnostic factors.[31] An *Aspergillus*-positive culture is conventionally used as mycological evidence of CAPA. Nevertheless, fungal culture is a time-consuming test with low sensitivity and specificity, and recovery of *Aspergillus* species from the culture of clinical specimens is relatively infrequent.[44,45] Hence, such diagnostic methods are not appropriate for CAPA, which is a medical emergency critically requiring a rapid diagnosis for effective treatment.[42] In recent years, several biomarkers serving as non-culture-based mycological evidence of fungal infection have been identified, and they may be appropriate for rapidly diagnosing CAPA.[46,47] Here we discuss these nonculture-based rapid diagnostics, including clinical and radiological factors, fungal biomarkers, direct microscopy, and *Aspergillus* DNA (Table 2).

Clinical Risk Factors

Clinical risk factors commonly observed in CAPA include refractory fever, pleural rub, chest pain, hemoptysis, respiratory insufficiency, and acute respiratory distress syndrome. These findings also exist in patients with COVID-19 and thus are of limited value in diagnosing CAPA.[13,23–26] In patients with severe COVID-19, ICU care and mechanical ventilation, treatment with corticosteroids or immunomodulators, and presence of comorbidities such as uncontrolled diabetes or chronic diseases of the liver, kidney, and heart may serve as potential risk factors for CAPA.[48,49] Therefore, it is essential to consider these clinical risk factors in the diagnostic workup of CAPA.

Radiological Imaging

Although bedside chest radiography can be easily performed in an ICU setting, to our experience it has low sensitivity for detecting super-added *Aspergillus* infection. Hence, a high-resolution computed tomography (CT) scan should be preferred whenever feasible.

The CT scan is an essential diagnostic tool for detecting pulmonary lesions, and most often serves as the first sign of IPA in patients with neutropenia.[50,51] However, CAPA is a nonneutropenic condition, and in addition to *Aspergillus* infection, several factors, including SARS-CoV-2 infection, host inflammation, and drug induced toxicity, may be involved in the lung damage.[4,6] Due to these background lung changes in patients with COVID-19, CT images must be carefully reviewed to detect signs of super-added *Aspergillus* infection. Different CT patterns reported in patients with CAPA include nodules with cavities and dendritic sign, reverse halo sign, nodular consolidation, crazy paving, nodular infiltrations, air-crescent sign, pleural effusion, and some indeterminate and atypical signs.[3,52] Patients with CAPA usually do not have bilateral and peripheral ground-glass opacities alone, which are typical CT findings of COVID-19.[53,54] Nevertheless, if present, it may be challenging to detect a superimposed *Aspergillus* infection in COVID-19-infected lungs due to the opacities.

Of 50 patients with suspected CAPA identified at our center, CT scan could be performed only in 15 patients. We observed that the development of nodular lesions (possibly cavitating), abscess formation, new consolidation patch, consolidation with cavitation, and cavitary lesion over the background of COVID-19 lung changes led to a suspicion of fungal infection (Figure 2). These lesions commonly appear as nodules, consolidation, or reverse halo patterns during the early stages of CAPA, and some of these may develop cavitation over the next 1 to 2 weeks (Figure 3). Thus, despite specific overlapping COVID-19-related lung changes, many CT findings raise the possibility of CAPA, prompting further investigation for diagnostic confirmation.

Certain CT findings may reflect occlusion of pulmonary arteries in IPA. Typical vessel occlusion sign (VOS) manifested on CT scan consists of pulmonary nodules surrounded by a halo of ground-glass attenuation (halo sign) or pleura-based wedge-shaped areas of consolidation. Nodules are due to coagulation necrosis, whereas the halo of ground glass is due to surrounding alveolar hemorrhage. Pleura-based consolidation areas correspond to hemorrhagic infarcts.[55,56] These CT findings should be confirmed by CT pulmonary angiography (CTPA) that provides direct evidence of vessel occlusion by detecting filling defect or interruption of vessels. However, CTPA is only useful to perform if large vessels are involved or dense nodules (>10 mm) are present on a basal CT scan.[57,58] Detection of VOS by CT/CTPA is helpful in diagnosing angioinvasive pulmonary aspergillosis that occurs almost exclusively in immunocompromised patients with neutropenia.[55–58] However, this form of IPA is rare in immunocompetent patients including CAPA and we also observed no CT findings of vessel occlusion in our CAPA patients.

Mycological Evidence

Non-culture-based mycological evidence of CAPA includes the presence of fungal cell wall antigens and *Aspergillus* hyphae or DNA in clinical specimens. *Aspergillus* cell wall antigen tests serve as indirect biomarkers, while direct microscopy and *Aspergillus* DNA testing serve as direct mycological evidence of the disease.[46] The rate of release of fungal antigens in the extracellular milieu is not uniform and varies with time of infection, type of *Aspergillus* species involved, and other conditions. Accordingly, CAPA is usually diagnosed within an average of 10 days (range: 0-51 days) after the COVID-19 diagnosis.[17] Therefore, it is recommended that mycological testing for suspected CAPA be performed on a weekly or bi-weekly basis. The mycological factors appropriate for the rapid diagnosis of CAPA are mentioned here.

β-D-Glucan

1-3-β-D-glucan (BDG) is a conserved polysaccharide abundantly found in the cell wall of most fungi, including

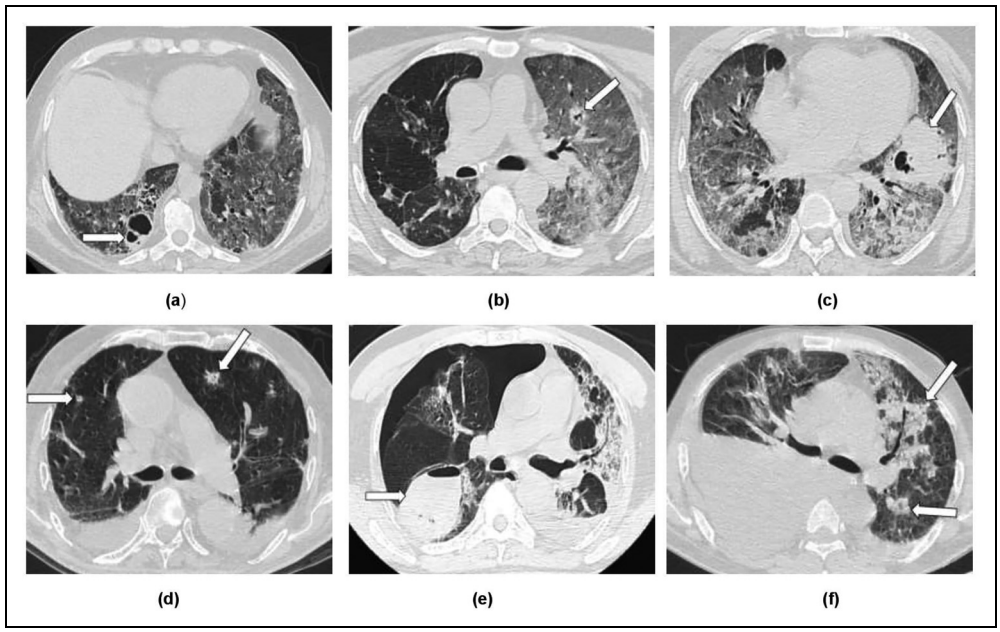

Figure 2. Representative patterns of chest CT of CAPA patients observed at our institute. (a) multiple small nodules with presence of cavitation in left lung nodule, (b) small nodular lesion left upper lobe with presence of cavitation, (c) consolidation in lingular segment with cavitary changes, (d) cavitary lesion, (e) rounded abscess with air fluid level with presence of pneumothorax, and (f) multiple poorly marginated nodular lesions coalescing to form a consolidation patch. The arrows show the case-specific lung lesions.
Abbreviations: CT, computed tomography; CAPA, COVID-19 associated pulmonary aspergillosis.

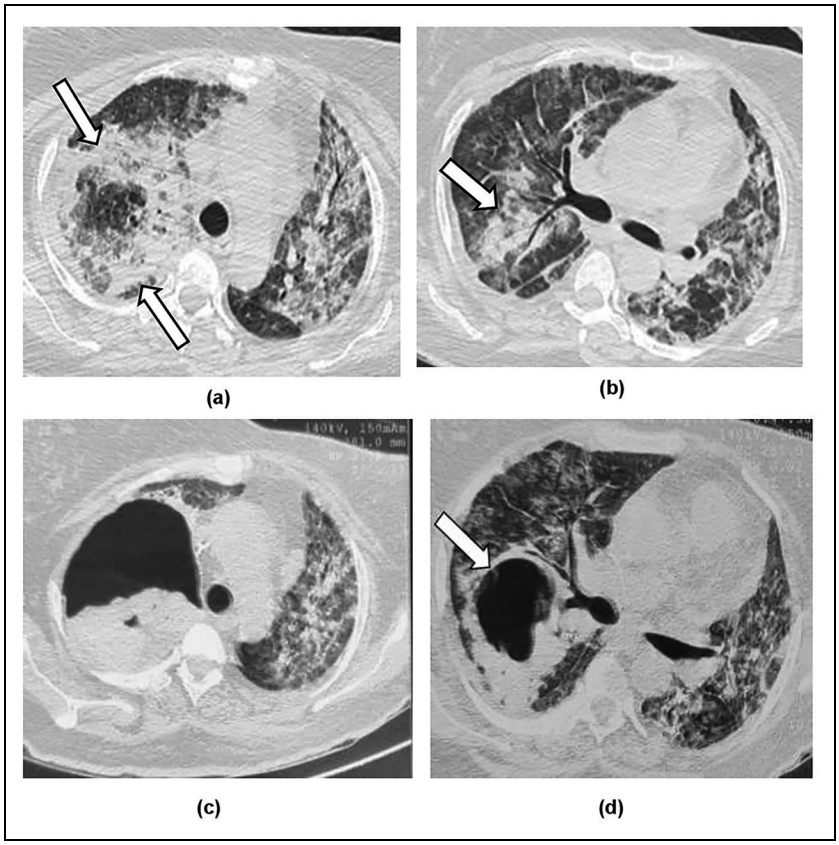

Figure 3. The CT chest of a 48-year-old diabetic female with COVID-19 pneumonia, (a) CT images of cranial section showing rounded area of consolidation in the form of reverse halo or bird's nest appearance (arrows). (b) Section just below the carina shows patchy consolidation patch (arrow). (c, d) Corresponding sections showing development of cavitation at the interval of 3 weeks. Mycological tests of the patients were suggestive of CAPA.
Abbreviations: CT, computed tomography; CAPA, COVID-19 associated pulmonary aspergillosis.

Aspergillus, *Candida*, and *Pneumocystis*.[59] The United States Food and Drug Administration (US-FDA) in 2004 has approved serum BDG testing by Fungitell Assay (Associates of Cape Cod, Inc., Falmouth, Massachusetts, USA) as a common biomarker of fungal infections. Since 2008, it has been included as a mycological criterion in EORTC/MSG definitions for the diagnosis of fungal diseases in immunocompromised patients.[2] Although BDG is not specific for *Aspergillus* infection and serves as a pan-fungal screening test, along with other *Aspergillus* biomarkers, it may be helpful in the diagnosis of aspergillosis.[60] A few recent studies have investigated BDG in NBL, reporting its promising utility in CAPA diagnosis.[15,16]

Galactomannan

Galactomannan (GM) is another major polysaccharide of the *Aspergillus* cell wall, and it is released into body fluids during active hyphal growth of the mold in host tissues.[61] A GM test on serum and BAL is more sensitive than culture and since 2002, it has been included as a mycological criterion in the EORTC/MSG definitions for the diagnosis of IPA in immunocompromised patients. The US-FDA in 2003 approved GM detection using Platelia *Aspergillus* enzyme immunoassay (EIA) kit (BioRad Laboratories) as an in vitro diagnostic test for *Aspergillus* infection.[2]

In CAPA, although BAL is the preferred specimen for GM testing, its availability is unfortunately restricted in these cases. The sensitivity of a GM test in serum is low in CAPA (~20%), and a significant fraction of patients with proven disease have negative GM in serum.[24,62] Detection of GM in NBL (cut-off $\geq$1.2) is considered to be clinically relevant mycological evidence for CAPA,[16] and recent diagnostic guidelines of CAPA, namely ECMM/ISHAM criteria and AspCOVID-19 criteria, have recommended mycological investigations including GM and other biomarkers in NBL.[31,32] Our laboratory has also observed a comparable GM index in NBL and BAL samples of patients with IPA, supporting the utility of NBL in the diagnosis of CAPA.

Mannoprotein

Mannoprotein (MP) is a glycoprotein of the outermost layer of the *Aspergillus* cell wall, and it is secreted into the external milieu during active hyphal growth of the mold.[63] It can be detected in serum and BAL by immunochromatographic lateral flow assay using a Conformitè Europëenne (CE)-marked AspLFD (lateral flow device) kit (OLM Diagnostics). This serves as a point-of-care test offering results within <30 min and requires no specific laboratory setup. The ECMM/ISHAM criteria have recommended MP testing in NBL for the definition of CAPA.[31] The GM antigen test sometimes results in false positivity due to its cross-reactivity with non-*Aspergillus* fungi or with contaminating GM in β-lactam antibiotics and certain food products. In such cases, this non-GM *Aspergillus* biomarker may be especially helpful to enhance the diagnostic accuracy of CAPA.[64,65]

Direct Microscopy

Direct microscopy of CAPA-appropriate clinical specimens is the most straightforward and rapid method for detecting *Aspergillus* and other fungal infections based on their distinctive morphological characteristics. It allows the detection of fungal elements before the growth of fungal cells is apparent in culture. In addition, unlike cultures, direct microscopy excludes the possibility of environmental contamination by airborne fungi. If the specimen has a sufficient number of fungal elements, microscopy may offer a presumptive or even definitive diagnosis within 2 to 3 h of the specimen collection and can thus provide enough information to intensive care physicians for prompt initiation of anti-fungal therapy. The traditional potassium hydroxide (KOH) wet mount method of microscopy has low sensitivity (50% maximum).[66] The combination of KOH with Calcofluor or Blankophor (optical brighteners) and subsequent examination by fluorescence microscope enhances the sensitivity of direct microscopy.[67,68] Therefore, direct fluorescent microscopy of BAL/NBL/blood using optical brighteners may be useful for the optimal diagnostic yield of *Aspergillus* infection in CAPA (Figure 4).

Aspergillus DNA

Detection of the *Aspergillus* DNA by polymerase chain reaction (PCR) (Asp-PCR) is a rapid and sensitive (>90%) mycological test, offering direct evidence of the presence of *Aspergillus* species in clinical specimens. After its inclusion as mycological evidence in recent EORTC/MSG criteria,[2] cultures with low sensitivity and long turnaround time are being consistently replaced by the Asp-PCR.[44] The identification of *Aspergillus* DNA in culture-negative clinical samples further establishes the diagnostic superiority of Asp-PCR over mycological culture and even microscopy.[69–72] The advent of multiplex Asp-PCR has further improved the diagnostic value of *Aspergillus* DNA testing. It performs a quantitative analysis of the DNA of involved *Aspergillus* species (A. flavus, A. terreus, and A. niger) to offer an etiological diagnosis of the disease.[73,74] However, as with other biomarkers, the detection of *Aspergillus* DNA in the bloodstream of nonneutropenic patients is low; hence, PCR in respiratory specimens is preferred for enhanced sensitivity.[75,76] Although evidence is scarce, available studies exhibit the superiority of Asp-PCR in NBL over blood in CAPA diagnosis.[15,16] The ECMM/ISHAM criteria for the definition of CAPA have also recommended NBL for Asp-PCR.[31]

Practice Guidelines for the Diagnosis of CAPA

A few months before the outbreak of the COVID-19 pandemic, we established the Microbial Hematology Laboratory at our institute for diagnosing invasive fungal diseases in patients

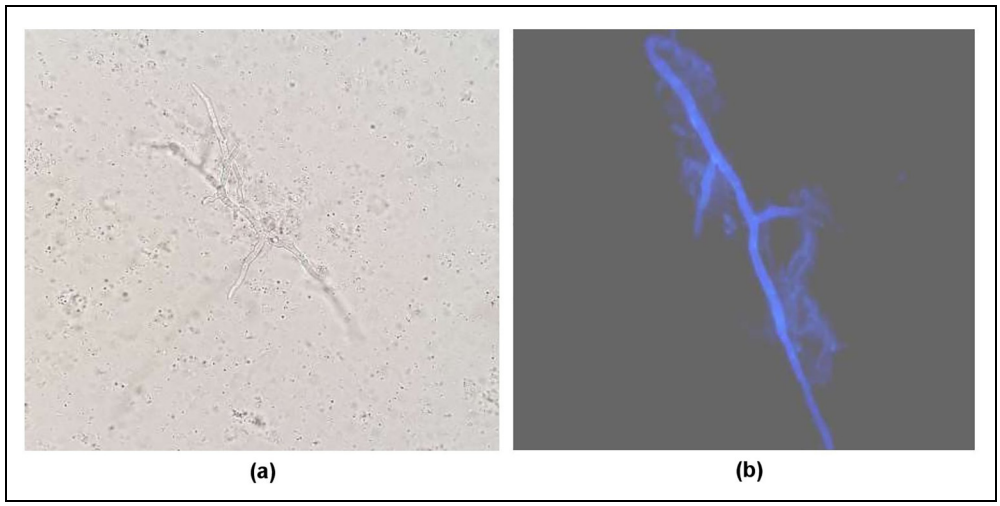

Figure 4. Direct microscopy showing thin hyaline septate acute angle branching fungal hyphae of *Aspergillus* spp. (a) 10% KOH wet mount, (b) Calcofluor white staining. Magnification: 40×.

with hematological malignancies. The laboratory has GM and MP testing in serum, BAL, and NBL in place, while BDG assay and Asp-PCR are currently being established. Direct microscopy and fungal cultures of blood and respiratory specimens are routine microbiological investigations at our institute. We presumed that diagnosis of alarmingly increasing CAPA cases could be improved and expedited for clinical care using these non-culture-based mycological assays and CAPA-appropriate specimens (ie, BAL, NBL). Therefore, based on the survey of existing diagnostic guidelines and our own experience, we have proposed new criteria for practically easy and rapid diagnosis of CAPA in an intensive care setting. We have named these criteria practice guidelines.

Although the presence of clinical and radiological factors is indicative of an invasive disease, it is difficult to distinguish CAPA from COVID-19 without mycological evidence. In the intensive care setting and routine clinical practice, a definitive or proven diagnosis of CAPA is almost impossible to make due to the lack of lung biopsy. Therefore, to facilitate easy clinical decision-making, our practice guidelines have classified the diagnosis of CAPA into suspected, possible, and probable categories based on clinical and radiological factors, and non-culture-based mycological biomarkers of the disease (Figure 5).

Suspected CAPA

We have defined a suspected CAPA as (i) presence of clinical risk factors, and (ii) abnormal CT findings. The clinical risk factors and typical CT patterns indicative of CAPA are the same as described above in the section on diagnostic factors of the disease.

Since this category lacks mycological evidence, the suspected CAPA needs to be upgraded to a possible or probable level by additional investigations for considering antifungal treatment.

Possible CAPA

A possible CAPA is defined as suspected CAPA (ie, presence of clinical risk factors and abnormal CT findings) with a positive GM or MP in BAL, NBL, or serum as mycological evidence of the *Aspergillus* infection. In addition, if CT scanning of a patient is deemed unfeasible, the positivity of GM or MP along with the presence of clinical risk factors may also be considered to indicate possible CAPA.

If the mycological biomarker does not test positive in a patient suspected to have CAPA, it would be essential to repeat the test in a second sample drawn after an interval of 24 to 48 h to confirm the result. In settings where BDG, GM, and MP tests are possible, it would be optimal to test any two of these biomarkers at regular intervals for maximizing the timely diagnosis of possible CAPA.

Since possible CAPA represents an intermediate certainty of the disease and includes mycological evidence of *Aspergillus* infection, we can consider it for empirical antifungal treatment. Concurrently, direct mycological evidence, ie, direct microscopy or Asp-PCR, is attempted to upgrade the diagnosis of possible CAPA to a probable level.

Probable CAPA

Probable CAPA represents a high certainty of CAPA diagnosis. Its definition includes (i) presence of clinical risk factors, (ii) abnormal CT findings, (iii) positivity of GM or MP biomarker in BAL, NBL, or serum, and (iv) direct microscopy or Asp-PCR (preferably multiplex) detecting *Aspergillus* species in BAL, NBL, or blood samples. The diagnosis of probable CAPA is sufficient to initiate targeted antifungal treatment.

We recommend that testing of all the three proposed fungal biomarkers (ie, BDG, GM, and MP), direct microscopy, and Asp-PCR be performed to improve the diagnostic yield and exclude fungal infections other than *Aspergillus*. When

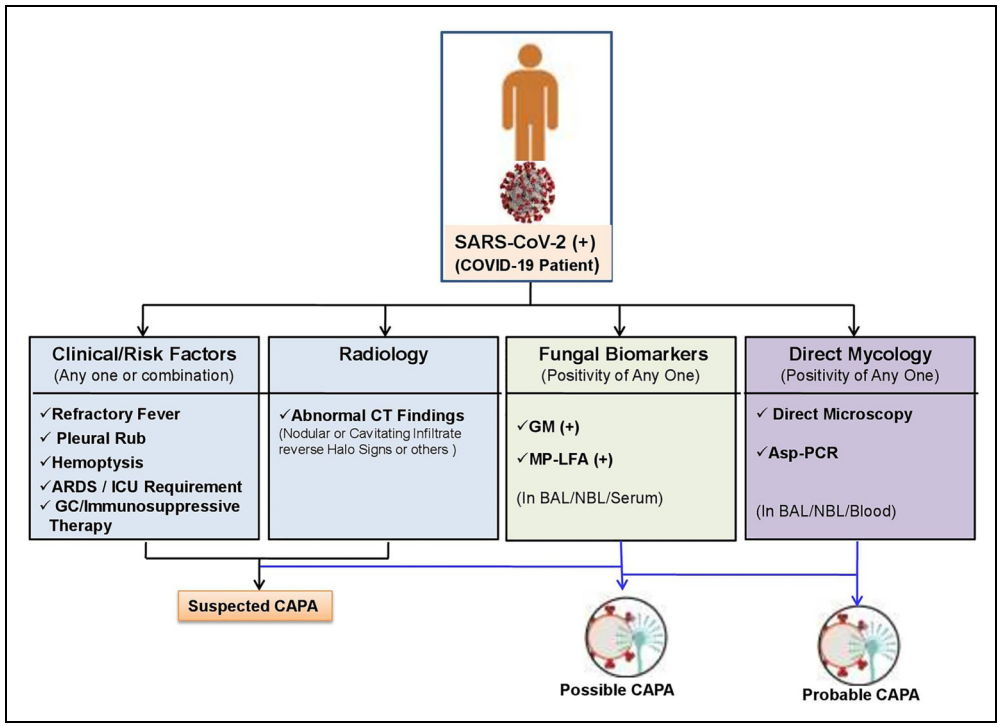

Figure 5. Definition of suspected, possible and probable diagnosis of CAPA. Presence of clinical factors, abnormal CT findings together defines suspected CAPA. Clinical factors, abnormal CT findings and mycological biomarkers together define possible CAPA. The possible CAPA factors, plus direct mycology evidenced by direct microscopy or Asp-PCR together define probable CAPA.

Abbreviations: SARSCoV-2, severe acute respiratory syndrome coronavirus-2; COVID-19, coronavirus disease-2019; CAPA, COVID-19 associated pulmonary aspergillosis; ARDS, acute respiratory distress syndrome; CT, computed tomography; BAL, bronchoalveolar lavage; NBL, nonbronchoscopic lavage; BDG, 1,3-β-D-glucan; GM, galactomannan; MP-LFA, mannoprotein lateral flow assay; Asp, *Aspergillus*; PCR, polymerase chain reaction; MP, Mannoprotein; ICU, intensive care unit; GC, glucocorticoids.

resources are limited and the tests have to be applied selectively, the combination of Asp-PCR and GM assay is preferred to obtain the greatest level of diagnostic confidence.[77,78]

Distinction Between CAPA and *Aspergillus* Colonization

Aspergillus can cause several forms of pulmonary diseases, ranging from colonization to invasive aspergillosis depending upon the pulmonary and immune functions of the host. Colonization is defined as the isolation of *Aspergillus* or its DNA from a respiratory sample without radiological and clinical findings of an invasive infection.[1,72] Distinguishing benign airway colonization from invasive *Aspergillus* infection has been challenging because *Aspergillus* conidia are common inhabitants of our airways and do not always cause an invasive disease. Clinical and radiological findings of CAPA not only support the diagnosis but also indicative of an invasive fungal disease.[49,54] Similarly certain fungal biomarkers such as GM and MP are signals of active hyphal growth of the *Aspergillus* in host tissues and show the presence of fungal infection.[61,64] Detection of *Aspergillus* in a respiratory test specimen by direct microscopy or Asp-PCR alone (ie, in the absence of clinical and radiological features or GM and MP biomarkers of the disease) may reflect colonization rather than infection and has limited diagnostic value.[79,80] Therefore, it is always important to define the diagnosis of CAPA taking clinical and radiological features, and fungal biomarkers of the disease into consideration to exclude the possibility of *Aspergillus* colonization and avoid unnecessary antifungal treatment and unwanted toxicity to patients.

Conclusion & Perspectives

Patients with severe COVID-19 are at high risk of CAPA due to immunosuppression caused by SARS-CoV-2 infection and certain medications. Definitions of CAPA available in the literature are complex and not suitable for intensive care settings, where early therapy is essential to save patients.[24,31] Our practice guidelines, which include comprehensive diagnostic interventions comprising fungal biomarkers (BDG, GM, and MP), direct microscopy, and Asp-PCR, are meant explicitly for intensive care application. To the best of our knowledge, these guidelines represent the first purely nonculture-based criteria providing an easy-to-use and practicable algorithm for rapid diagnosis of CAPA to provide urgent clinical management of the disease. The outbreak of COVID-19 has led to the establishment of PCR in almost all major hospitals, and microscopy is a routine microbiology investigation. Other diagnostics such as BDG, GM, and MP included in our guidelines are commercial

kit-based easy-to-perform tests producing rapid results and need no specific laboratory infrastructure. Even if the Asp-PCR is not yet established in some limited-resource laboratories, possible CAPA can be identified or ruled out using simple mycological tests like BDG, GM, and MP in BAL, NBL, or serum. Thus, our practice guidelines could serve as widely applicable criteria for a precise and rapid diagnosis of CAPA. Although we have not included mycological culture in our diagnostic criteria owing to their low sensitivity and long turnaround time, it is a significant pillar of fungal diagnostics and therapeutics. Therefore, once a CAPA diagnosis is achieved, the culture of the *Aspergillus*-positive specimen would be essential for identification of the involved *Aspergillus* species and antifungal susceptibility testing for individualized therapy.[81,82] Although several studies have reported the utility of NBL and other respiratory specimens in the diagnosis of CAPA,[15,21,43,45] validation of *Aspergillus* infection by histopathology of lung autopsy of patients with CAPA will be helpful for authentication and routine application of these guidelines. Thus, we believe that establishing a consensus definition of CAPA requires more efforts, particularly those directed towards the performance of existing biomarkers and identification of more sensitive new biomarkers for the disease.

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Declaration of Conflicting Interests

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Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

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