BRIEF REPORT

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Mini-Bronchoalveolar Lavage for Diagnosing Coronavirus Disease 2019–Associated Invasive Pulmonary Aspergillosis

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OBJECTIVES: To evaluate the yield of mini-bronchoalveolar lavage compared with that of directed bronchoalveolar lavage in critically ill patients with suspected coronavirus disease 2019–associated pulmonary aspergillosis.

DESIGN: A retrospective cohort study.

SETTING: The ICU of the Amsterdam University Medical Centers.

PATIENTS: Patients with confirmed coronavirus disease 2019 screened for coronavirus disease 2019–associated pulmonary aspergillosis.

INTERVENTIONS: Mini-bronchoalveolar lavage and/or directed bronchoalveolar lavage.

MEASUREMENTS AND MAIN RESULTS: In total, 76 patients were included, 20 of whom underwent bronchoalveolar lavage, 40 mini-bronchoalveolar lavage, and 16 both mini-bronchoalveolar lavage and bronchoalveolar lavage. The percentage of samples with one or more positive *Aspergillus* detecting test (galactomannan, culture, polymerase chain reaction) did not differ significantly between bronchoalveolar lavage and mini-bronchoalveolar lavage (16.7% vs 21.4%). However, in mini-bronchoalveolar lavage samples, this was more frequently driven by a positive polymerase chain reaction than in bronchoalveolar lavage samples (17.9% vs 2.8%; $p = 0.030$). In 81% of patients (13/16) with both mini-bronchoalveolar lavage and bronchoalveolar lavage, the test results were in agreement. In 11 of 12 patients (92%) with first a negative mini-bronchoalveolar lavage, the subsequent bronchoalveolar lavage sample was also negative.

CONCLUSIONS: We found a similar percentage of positive test results in mini-bronchoalveolar lavage and bronchoalveolar lavage samples in patients with suspected coronavirus disease 2019–associated pulmonary aspergillosis. Our findings indicate that mini-bronchoalveolar lavage could be a useful tool for coronavirus disease 2019–associated pulmonary aspergillosis screening in ICU patients.

KEY WORDS: aspergillosis; bronchoalveolar lavage; bronchoscopy; critical care; diagnosis; severe acute respiratory syndrome coronavirus 2

Approximately 5% of patients with coronavirus disease 2019 (COVID-19) require admission to the ICU (1). In this patient group, an increased risk of invasive pulmonary aspergillosis (IPA) has been observed, with occurrence rates ranging from 20% to 35% (2, 3).

Microbiological evidence from respiratory samples is crucial for diagnosing COVID-19–associated pulmonary aspergillosis (CAPA) and the preferred sampling modality is bronchoalveolar lavage (BAL) (4). However, it has been advised that bronchoscopy should be used sparingly in patients with COVID-19 since airborne transmission might occur.

Mini-BAL is a nonbronchoscopic bedside method for performing a small-volume bronchial lavage. Even though the procedure is blind, research has shown

DOI: 10.1097/CCE.0000000000000601

that the microbiological results show good concordance with those obtained via bronchoscopic BAL and can be used to successfully guide treatment (5). We hypothesized that mini-BAL could be a useful alternative to BAL for CAPA diagnostics in critically ill patients. To evaluate this hypothesis, we compared the microbiological results of mini-BAL and BAL with respect to detection of *Aspergillus* species in critically ill patients with severe acute respiratory syndrome coronavirus 2 infection.

MATERIALS AND METHODS

All patients who were admitted to the ICU of the Amsterdam University Medical Center (AUMC) between March 1, 2020, and May 31, 2020, because of polymerase chain reaction (PCR)-confirmed COVID-19 and underwent either BAL and/or mini-BAL for CAPA diagnostics within 7 days after COVID-19 diagnosis were included in this retrospective cohort study.

Ethical approval was waived by the VU University Medical Center Medical Ethics Review Committee, approval number 2020-6339. No patients or legal representatives objected to the use of the patients' data, which were extracted from the patients' electronic medical files.

All patients were on invasive ventilatory support when the BAL or mini-BAL was performed. For mini-BAL, a sterile suction catheter with a suction trap was used. The catheter was blindly advanced through the endotracheal tube into a distal airway to a random endpoint, 1 × 20 mL of sterile saline was instilled and retrieved. Flexible bronchoscopy using a single-use bronchoscope (Ambu aScope; Ambu A/S, Ballerup, Denmark) was performed according to the guidelines of the Dutch Society of Pulmonologists. Samples were sent to the microbiology laboratory for culture, galactomannan detection (Platelia; Bio-Rad, Marnes La Coquette, France), and *Aspergillus* PCR. A galactomannan optical density index greater than or equal to 0.8 was considered positive.

Patients who received antifungal treatment for 6 weeks or longer and/or with a diagnosis of CAPA at discharge as mentioned in their letter of discharge from the ICU were counted as CAPA cases.

Data are shown as median with interquartile range or mean with SD. Continuous variables were compared using the Mann-Whitney *U* test, Kruskal-Wallis test or one-way analysis of variance. Categorical variables were compared using Pearson chi-square test, if more than 20% of cells had an expected frequency of less than five, the Fisher exact test was used.

RESULTS

One-hundred forty-six patients with PCR confirmed COVID-19 were admitted to the ICUs of the AUMC between March 1, 2020, and May 31, 2020. After exclusion of those that did not receive mini-BAL or BAL, 76 patients remained. A patient flowchart is available as Supplemental Digital Content (**S1**, http://links.lww.com/CCX/A881). Baseline characteristics, subdivided by diagnostic group, are summarized in **Table 1**. Overall mortality was 38.2%. A total of six patients (7.9%) had host factors for IPA (6).

Test Results in Total Population

Table 2 shows the number of BAL and mini-BAL samples that were positive for one or more *Aspergillus* detecting test and gives the combination of positive test results in these samples. This is further illustrated in **Figure S1**, ***a*** and ***b*** (http://links.lww.com/CCX/A880; legend, http://links.lww.com/CCX/A894). The percentage of positive samples did not differ significantly between the two diagnostic methods (BAL: 16.7% vs mini-BAL: 21.4%; $p = 0.574$). All eight positive cultures showed growth of *Aspergillus fumigatus*.

Test Results in Patients With Both Mini-BAL and BAL

In 16 patients, mini-BAL as well as BAL were performed, with a mean interval of 4.3 days (SD = 2.1 d). None of the patients received antifungal treatment between mini-BAL and BAL. In 14 cases, mini-BAL was followed by BAL, in one patient both were obtained on the same day and in one patient BAL was performed first. The results of the mini-BAL and BAL agreed in 13 of 16 cases (81%), with both mini-BAL and BAL being negative in 12 cases. Of those, three cases in which the results did not coincide, two had a positive *Aspergillus* PCR in the mini-BAL followed by a completely negative BAL and one had negative mini-BAL followed by a positive galactomannan in the BAL. In 11 of 12 patients (92%) with a negative mini-BAL, the subsequent BAL sample was also negative.

CAPA Cases

Eleven of the 146 patients (7.5%) with PCR confirmed COVID-19 were classified as CAPA cases. Three cases (27%) had one or more risk factors for invasive

TABLE 1.
Baseline Characteristics per Diagnostic Group

Characteristic	Mini-BAL, *n* = 40	BAL, *n* = 20	Mini-BAL + BAL, *n* = 16
Sex, *n* (%)			
Male/female	25/15 (62/38)	15/5 (75/25)	16/0 (100/0)
Age, yr	65 (59–73)	66 (58–70)	63 (52–72)
Body mass index, kg/m^2	28 (24–31)	28 (25–31)	26 (23–30)
Medical history, *n* (%)			
Hypertension	20 (50)	6 (30)	6 (38)
Cardiovascular disease	13 (33)	1 (5)	2 (13)
Cerebrovascular disease	6 (15)	0 (0)	0 (0)
Diabetes	13 (33)	5 (25)	6 (38)
Asthma	5 (13)	4 (20)	0 (0)
Chronic obstructive pulmonary disease	3 (8)	2 (10)	1 (6)
Invasive pulmonary aspergillosis host factor, *n* (%)	2 (5.0)	1 (5.0)	3 (18.8)
Total length of ICU stay, d	14 (10–22)	21 (15–43)	19 (14–35)
Mechanical ventilation, d	15 (9–21)	18 (12–32)	17 (12–32)
Systemic corticosteroids, *n* (%)	9 (23)	6 (30)	8 (50)
CT score, mean (SD)	17 (5)	17 (4)	14 (3)
Deaths, *n* (%)	20 (50)	5 (25)	4 (25)
Coronavirus disease 2019–associated pulmonary aspergillosis cases, *n* (%)	7 (17.5)	2 (10)	2 (12.5)

BAL = bronchoalveolar lavage, *n* = number of patients.
Data are median (interquartile range) unless specified otherwise.

Aspergillus disease. The median time between ICU admission and CAPA diagnosis was 9 days (8–14 d). All 11 CAPA cases received antifungals. Mortality in CAPA cases (63.6%) was significantly higher than in non-CAPA cases (28.9%; $p = 0.037$). The median duration between CAPA diagnosis and death was 3 days (1–6 d). In three cases, an autopsy was performed, with confirmation of pulmonary *Aspergillus* superinfection in one patient. This patient had a positive mini-BAL culture for *Aspergillus* and a galactomannan of 7.9. The microbiological test results in the CAPA cases are shown in Online Supplemental Digital Content (**Fig. S1**, ***c*** and ***d***, http://links.lww.com/CCX/A880).

DISCUSSION

This is one of the first studies comparing the microbiological results of mini-BAL and BAL for *Aspergillus* detection in critically ill COVID-19 patients. We found that, in 76 ICU patients with suspected CAPA, both methods yielded a similar percentage of positive test results. However, in mini-BAL, the positive samples were more frequently driven by a positive PCR compared with BAL, which more frequently showed positive galactomannan or culture results. It is known that the sensitivity of *Aspergillus* PCR on BAL is higher than that of culture but with a lower specificity for invasive aspergillosis (4).

Even though recent data indicates BAL can be safely performed in patients with COVID-19 (7), mini-BAL has other advantages over regular BAL. First, a mini-BAL is technically simple and requires no extra personnel. Additionally, the catheters used for mini-BAL are smaller in diameter than a bronchoscope, minimizing the risk of complications. Last, mini-BAL can easily be obtained in intubated patients, which could make it a useful screening modality, considering that in this study, a negative mini-BAL for *Aspergillus* was followed by a negative BAL in more than 90% of cases.

The CAPA occurrence rate and high mortality are in line with those reported in a larger retrospective

TABLE 2.
Positive Test Results per Diagnostic Method

Test Results	BAL, *n* = 36	Mini-BAL, *n* = 56
≥ 1 positive result, *n* (%)	6 (16.7)	12 (21.4)
One positive result		
PCR	0	4
Galactomannan	3	1
Culture	0	0
Two positive results		
PCR + galactomannan	1	1
Culture + galactomannan	2	1
PCR + culture	0	0
Three positive results		
PCR+ galactomannan+ culture	0	5
Galactomannan value, median (interquartile range)	0.15 (0.10–0.48)	0.10 (0.10–0.30)

BAL = bronchoalveolar lavage, *n* = number of samples, PCR = polymerase chain reaction.

analysis of CAPA cases (8). Additionally, the current study demonstrates that most ICU patients with CAPA do not have classic host factors for IPA. This is in accordance with the high proportion of host factor negative CAPA patients reported in other studies (9).

A limitation of our study is the small percentage of patients who underwent both BAL and mini-BAL and the selection of patients who were identified as "CAPA cases." Since definitions for proven IPA require histopathological evidence, only one of our patients can be categorized as proven CAPA. In the remaining 10 cases, the diagnosis was based on the clinical picture and was importantly driven by microbiological results. Validated criteria for the diagnosis of CAPA were lacking at the time of our data collection.

CONCLUSIONS

Our data indicate that mini-BAL could be a useful tool for CAPA screening in ICU patients. This is in line with the statement in the European Confederation of Medical Mycology and the International Society for Human and Animal Mycology working document that mini-BAL testing is most likely sufficient to initiate antifungal therapy, even though it is not considered equal to BAL for diagnosing CAPA (10). Considering the limited specificity of PCR positive mini-BAL for diagnosis of IPA, clinicians should proceed with caution when diagnosing CAPA and obtain a follow-up mini-BAL or directed BAL if there are any doubts on the clinical relevance of a positive *Aspergillus* PCR in a mini-BAL sample.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccejournal).

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The authors have disclosed that they do not have any potential conflicts of interest.

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