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MYCOLOGY

Frequency of Positive *Aspergillus* Tests in COVID-19 Patients in Comparison to Other Patients with Pulmonary Infections Admitted to the Intensive Care Unit

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ABSTRACT The aim of this study was to describe the frequency of positive *Aspergillus* tests in COVID-19 patients and investigate the association between COVID-19 and a positive *Aspergillus* test result. We compared the proportion of positive *Aspergillus* tests in COVID-19 patients admitted to the intensive care unit (ICU) for >24 h with two control groups: patients with community-acquired pneumonia with (i) a PCR-confirmed influenza infection (considered a positive control since the link between influenza and invasive aspergillosis has been established) and (ii) *Streptococcus pneumoniae* pneumonia (in whom positive *Aspergillus* tests are mostly considered as colonization). During the study period, 92 COVID-19 patients (mean [standard deviation] age, 62 [14] years; 76.1% males), 48 influenza patients (55 [14]; 56.2% males), and 65 pneumococcal pneumonia patients (58 [15], 63,1% males) were identified. Any positive *Aspergillus* test from any respiratory sample was found in 10.9% of the COVID-19 patients, 6.2% of the patients with pneumococcal pneumonia, and 22.9% of those infected with influenza. A positive culture or PCR or galactomannan test on bronchoalveolar lavage (BAL) fluid only was found in 5.4% of COVID-19 patients, which was lower than in patients with influenza (18.8%) and comparable to that in the pneumococcal pneumonia group (4.6%). Using logistic regression analysis, the odds ratio (OR) (95% confidence interval) for a positive *Aspergillus* test on BAL fluid for COVID-19 patients was 1.2 (0.3 to 5.1; $P = 0.8$) compared to the pneumococcal pneumonia group, while it was 0.2 (0.1 to 0.8; $P = 0.02$) compared to the influenza group. This difference remained significant when corrected for age and sex. In conclusion, in COVID-19 patients, the prevalence of a positive *Aspergillus* test was comparable to that in patients admitted for pneumococcal pneumonia but substantially lower than what we observed in patients with influenza.

KEYWORDS COVID-19, *Aspergillus*, invasive aspergillosis, galactomannan

Invasive pulmonary aspergillosis (IPA) is a life-threatening disease that typically occurs in severely immunocompromised patients (1). The mortality of IPA in patients with hematological disease is estimated to be 30% (2) but is substantially higher in critically ill patients (3). More recently, intensive care unit (ICU) admission for severe influenza has been shown to be a risk factor for IPA, with an incidence varying between 7 and 18% and overall mortality around 50% (4–6).

Like influenza, COVID-19 is predominantly a pulmonary disease, and COVID-19 has been linked with *Aspergillus* detection in respiratory samples by several authors, who have coined the term COVID-19-associated pulmonary aspergillosis (7, 8). Assessing clinical significance of a positive *Aspergillus* test is difficult in general (9) and is even

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more difficult in ICU patients. Clinically, it is impossible to differentiate pulmonary aspergillosis from COVID-19 based on clinical signs and symptoms, especially in patients admitted to the ICU. All of them have pulmonary infiltrates. Moreover, chest computed tomography (CT) scans of COVID-19 patients that show bilateral widespread ill-defined and ground-glass opacification (10) may also obscure any eventual findings of IPA.

Studies showing prevalent positive *Aspergillus* tests in COVID-19 so far are mostly case reports or observational studies without control groups (8, 11–18). While small case series are prone to publication bias, the lack of any control group precludes a valid conclusion (19). The link between *Aspergillus* and COVID-19 might, for example, also be explained by the high frequency of bronchoalveolar lavage (BAL) performed in COVID-19 patients admitted to the ICUs to exclude bacterial superinfection. The more diagnostic procedures are performed, the higher the chances of detecting microorganisms that may be colonizers rather than true pathogens, which also applies to the ubiquitous mold *Aspergillus*. Detection of *Aspergillus* in respiratory samples in nonimmunocompromised patients is often considered to indicate colonization and does not require antifungal therapy (20). By considering any positive *Aspergillus* test in COVID-19 patients clinically significant, antifungal treatment will be initiated in these patients, leading to higher costs, possible adverse events, and toxicity (21).

Based on these observations, we hypothesized that the number of positive *Aspergillus* tests in COVID-19 patients would not differ from that in a control group but would be lower than in a group of ICU patients that is now broadly considered to be at increased risk for IPA.

Therefore, the aim of this study was to describe the frequency of positive *Aspergillus* tests in COVID-19 patients and to investigate the association between COVID-19 status and positive *Aspergillus* tests in a case-control study using two different control groups, one with an established link with IPA (influenza) and another without (pneumococcal pneumonia).

MATERIALS AND METHODS

Study design. This was a case-control study performed in Erasmus University Medical Centre (Erasmus MC), Rotterdam, The Netherlands. Erasmus MC is a large tertiary hospital. It hosted the national coordination center for COVID-19 patient distribution during the first wave of the COVID-19 outbreak in The Netherlands (between March and June 2020). During the first wave, it had 102 ICU beds allocated for COVID-19 (7% of all Dutch ICU beds for COVID-19).

Included were adult patients (>18 years old) admitted to the ICU for >24 h. COVID-19 cases were those with confirmed COVID-19 based on a positive PCR from respiratory samples between 1 March and 21 April 2020. We used two control groups selected in the period between January 2010 and April 2020 admitted to the same ICU: (i) patients with community-acquired pneumonia due to influenza confirmed by a positive PCR on respiratory samples and (ii) patients with community-acquired pneumococcal pneumonia based on positive respiratory culture with *Streptococcus pneumoniae* or positive urine antigen test and a negative influenza test. All included patients had infiltrates on the radiograph or CT scan of the chest.

Demographic (age and gender) and microbiology data were obtained from hospital and laboratory information system. This study was a noninterventional observational study using only limited demographic data and was performed under institutional review board approval (METC-2015-306).

Test algorithm. In COVID-19 cases and controls, BAL or other respiratory samples were collected within the first week of ICU admission. BAL fluid was collected when patients showed clinical deterioration leading to differential diagnosis of secondary infection based on clinicians' judgment. At Erasmus MC, it is the standard procedure to do a fungal culture on all BAL samples from ICU patients. *Aspergillus* antigen test (galactomannan) and *Aspergillus* DNA detection by PCR are occasionally performed as well. Due to the invasive nature of BAL, and because BAL fluid is used to make a diagnosis and not for follow-up of the pulmonary infections, BAL was rarely done more than once.

Microbiological tests. Galactomannan tests were performed on the BAL fluid and other types of respiratory samples (when BAL was not performed) or serum of the patients using an immunoenzymatic sandwich microplate assay (Platelia Aspergillus Ag; Bio-Rad Laboratories B.V., Veenendaal, The Netherlands). A galactomannan index of >0.6 was considered positive for serum as well as for BAL fluid. The performance of this test depends on the study population, and studies in nonneutropenic patients reported sensitivities of 22% in serum (22) and 76% in BAL fluid to diagnose invasive pulmonary aspergillosis (IPA) at a cutoff galactomannan index of ≥0.5 (23). PCR for *Aspergillus* was performed using the commercial kit AsperGenius (PathoNostics, Maastricht, The Netherlands), which detects and identifies *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus* spp., and azole resistance markers TR34 and TR46, or

in-house real-time PCR assay as described before (24). The sensitivity of the in-house real-time PCR was 80% in nonneutropenic patients in diagnosing IPA (24). *Aspergillus* cultures were performed on BBL Sabouraud dextrose agar with chloramphenicol (BD Diagnostics, Erembodegem, Belgium) and incubated at 26°C and 35°C for 21 days. The sensitivity of culture in detecting invasive pulmonary aspergillosis is between 20% and 50% (25, 26). These tests were performed in an accredited ISO 15189 microbiology lab. PCR for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targeting E and RNA-dependent RNA polymerase (RdRp) was performed in the Department of Viroscience of Erasmus MC, which is one of the WHO referral laboratories.

Statistical analysis. Data were analyzed using SPSS Statistics 26 (SPSS Inc., Chicago, IL). Continuous variables are presented as means with standard deviations (SD). Means among group were compared using analysis of variance (ANOVA). Number and proportion of gender and positive tests were calculated and chi-squared test was used to compared proportions between groups.

We determined several possibilities as proxies in defining clinical significance of pulmonary aspergillosis based on microbiology results (culture, PCR, and *Aspergillus* antigen in respiratory samples), from low probability to higher probability: (i) any positive *Aspergillus* tests performed in any type of respiratory or serum samples, (ii) any positive *Aspergillus* tests from BAL samples only, (iii) positive PCR and positive *Aspergillus* antigen in BAL samples, (iv) positive culture and positive *Aspergillus* antigen in BAL samples, (v) positive culture and positive PCR from BAL samples, and (vi) positive PCR, positive *Aspergillus* antigen, and positive culture.

Further, we used logistic regression analysis to calculate the odds ratio (OR) with 95% confidence interval (CI) to determine whether COVID-19 pneumonia was independently associated with positive *Aspergillus* tests. We performed two analyses: first, by considering positive *Aspergillus* tests in all type of respiratory samples (BAL fluid, sputum aspirates, and lung tissue), and second, by considering positive *Aspergillus* tests in BAL fluid only. We adjusted both associations for age and sex.

RESULTS

Demographic characteristics and microbiological tests. During the study period, 92 patients were admitted to the ICU with COVID-19, 48 with influenza, and 65 with pneumococcal pneumonia (Table 1). COVID-19 patients tended to be older (mean age [SD], 62 [14] years) than patients with influenza or pneumococcal pneumonia ($P = 0.1$) and were more often male ($P = 0.04$).

Respiratory samples of approximately one-third of the COVID-19 patients were obtained. BAL sampling was more often performed in COVID-19 patients (29.3%) than in patients with pneumococcal pneumonia (18.5%) but less frequently than in patients with influenza (45.8%). The numbers of performed *Aspergillus* PCRs and *Aspergillus* antigen tests were significantly different between the COVID-19 and the control groups, but no statistical difference was found between the groups for numbers of performed fungal cultures.

Positive *Aspergillus* tests. Any positive *Aspergillus* test from any respiratory samples was found in 10.9% of the COVID-19 patients, and this proportion was higher than in patients with pneumococcal pneumonia (6.2%) but lower than in the influenza patients (22.9%; $P = 0.02$) (Table 2). When only the *Aspergillus* tests performed on BAL fluid were considered, the proportion of positive tests in COVID-19 patients was reduced to 5.4% and comparable to that of the pneumococcal pneumonia group (4.6%) but much lower than what was observed in patients with influenza (18.8%; $P = 0.01$). Stricter criteria for positivity (e.g., positive PCR and positive *Aspergillus* antigen in BAL samples or positive culture and positive *Aspergillus* antigen in BAL samples) delivered a limited number of cases to allow statistical comparisons.

Association between detection of *Aspergillus* and COVID-19. The OR (95% CI) of having any positive *Aspergillus* test in any respiratory sample for COVID-19 patients compared with pneumococcal pneumonia patients was 1.9 (0.6 to 6.2; $P = 0.3$). Taking age and gender into account did not change the estimate. Compared this group with the influenza group, the OR (95% CI) was 0.4 (0.2 to 1.1; $P = 0.06$) without and 0.4 (0.1 to 0.9, $P = 0.04$) when age and sex were taken into account.

When only a positive *Aspergillus* test from BAL fluid was taken into account, the OR was 1.2 (0.3 to 5.1, $P = 0.8$) for the comparison of COVID-19 patients with pneumococcal pneumonia, while it was 0.2 (0.1 to 0.8; $P = 0.02$) compared with the influenza group despite the fact that a fungal culture had been performed less often on BAL samples from patients with influenza than from COVID-19 patients. This difference remained significant when corrected for age and sex (OR of 0.2 (0.01 to 0.7; $P = 0.01$).

TABLE 1 Demographic characteristics and microbiological tests performed in COVID-19 patients and controls

Characteristic or test	Total ($n = 205$)	COVID-19 ($n = 92$)	Influenza ($n = 48$)	Pneumococcal pneumonia ($n = 65$)	*P* value for differences between groups
Mean age, yrs (SD)	62 (14)	60 (14)	55 (14)	58 (15)	0.1
Male, *n* (%)	138 (67.3)	70 (76.1)	27 (56.2)	41 (63.1)	0.04
No. of patients with (deep) respiratory samples available (%)	69/205 (33.7)	32 (34.8)	24 (50.0)	13 (20.0)	0.6
Bronchoalveolar lavage fluid	61 (29.8)	27 (29.3)	22 (45.8)	12 (18.5)	
Sputum aspirates	8 (3.9)	5 (5.4)	2 (6.3)	1 (1.5)	
No. of fungal cultures performed among available respiratory samples (%)	61/69 (88.4)	28/32 (87.5)	22/24 (91.7)	11/13 (84.6)	0.08
Bronchoalveolar lavage fluid	55/61 (90.2)	24/27 (88.9)	20/22 (90.9)	11/12 (91.7)	
Sputum aspirates	6/8 (75.0)	4/5 (80.0)	2/2 (100)	0/1 (0)	
No. of positive cultures (%)	8/61 (13.1)	3/28 (10.7)	4/22 (4.3)	1/11 (9.0)	0.1
Bronchoalveolar lavage fluid	5/55 (9.1)	2/24 (8.3)	2/20 (10.0)	1/11 (9.1)	
Sputum aspirates	3/6 (50.0)	1/4 (25.0)	2/2 (100)	0 (0)	
No. of *Aspergillus* PCRs performed among available respiratory samples (%)	38/69 (55.1)	15/32 (46.9)	18/24 (75.0)	5/13 (38.5)	0.02
Bronchoalveolar lavage fluid	30/61 (52.5)	10/27 (37.0)	16/22 (72.7)	4/12 (33.3)	
Sputum aspirates	8/8 (100)	5/5 (100)	2/2 (100)	1/1 (100.0)	
No. of positive *Aspergillus* PCRs (%)	23/38 (60.5)	10/15 (66.7)	9/18 (50.0)	4/5 (80.0)	0.3
Bronchoalveolar lavage fluid	16/30 (53.3)	5/10 (50.0)	8/16 (50.0)	3/4 (75.0)	
Sputum aspirates	7/8 (87.5)	5/5 (100.0)	1/2 (50.0)	1/1 (100)	
No. of *Aspergillus* antigen tests performed among available respiratory samples	42/69 (62.7)	22/32 (68.8)	14/24 (56.0)	6/13 (46.2)	0.05
Bronchoalveolar lavage fluid	38/61 (62.3)	20/27 (74.1)	13/22 (59.1)	5/12 (41.7)	
Sputum aspirates	4/8 (50.0)	2/5 (40.0)	1/2 (50.0)	1/1 (100)	
No. of positive tests for *Aspergillus* antigen in respiratory samples	14/42 (33.3)	4/22 (18.2)	6/14 (42.9)	4/6 (66.7)	0.07
Bronchoalveolar lavage fluid	12/38 (31.2)	3/20 (15.0)	6/13 (46.2)	3/5 (60.0)	
Sputum aspirates	2/4 (50.0)	1/2 (50.0)	0/1 (0)	1/1 (100)	
No. of positive *Aspergillus* antigen tests/no. performed on serum samples	5/44 (11.4)	0/14 (0)	3/23 (13.0)	2/7 (28.6)	

DISCUSSION

In this study, which included 92 patients admitted to the ICU with COVID-19, the proportion of patients in which *Aspergillus* was detected was comparable to that of patients admitted with pneumococcal pneumonia while significantly lower than in patients with influenza, a patient population in which the link with IPA is well established (4).

In critically ill patients admitted for respiratory failure, such as COVID-19 patients, the suspicion of a hospital-acquired superinfection typically arises when a clinical deterioration is observed. In COVID-19 patients, clinicians are often reluctant to perform BAL (27) and rely on upper respiratory tract specimens to diagnose ventilator-associated pneumonia. Yet the detection of *Aspergillus* in the upper respiratory tract more often represents colonization than infection (27). The majority of publications on *Aspergillus* in COVID-19 are case series (11–16, 18) and often included the detection of *Aspergillus* in the upper airway (6) or included the detection of β-D-glucan as a mycological criterion, a test that is not specific for *Aspergillus* spp. and it has never been

TABLE 2 Number and proportion of positive *Aspergillus* tests in COVID-19 patients and control groups

Type of positive tests (from 1 to 6) representing an increasing probability of true invasive *Aspergillosis*	Total ($n = 205$)	COVID-19 ($n = 92$)	Influenza ($n = 48$)	Pneumococcal pneumonia ($n = 65$)
1. Any positive test results in any type of respiratory samples or serum sample, *n* (%)	25 (12.2)	10 (10.9)	11 (22.9)	4 (6.2)
2. Any positive test results in BAL samples only, *n* (%)	17 (8.3)	5 (5.4)	9 (18.8)	3 (4.6)
3. Positive PCR and positive *Aspergillus* antigen test in BAL samples, *n* (%)	11 (5.4)	3 (3.3)	5 (10.4)	3 (4.6)
4. Positive culture and positive *Aspergillus* antigen in BAL samples, *n* (%)	4 (2.0)	2 (2.2)	1 (2.1)	1 (1.5)
5. Positive culture and positive PCR from BAL samples, *n* (%)	4 (2.0)	2 (2.2)	1 (2.1)	1 (1.5)
6. Positive PCR, positive *Aspergillus* antigen, and positive culture, *n* (%)	4 (2.0)	2 (2.2)	1 (2.1)	1 (1.5)

shown to be useful to diagnose IPA in ICU patients (9). Bartoletti and coworkers did perform a study with systematic sampling of BAL fluid in COVID-19 patients admitted to the ICU. In their study, 27.7% of the patients had a serum galactomannan index of >0.5, BAL galactomannan index of >1.0, growth of *Aspergillus* spp. in BAL fluid, or a cavitating infiltrate on CT scan of the chest (17). We agree that the 27.7% incidence is surprisingly high, and it is higher than the number in our study. It is very likely that the high incidence is at least partially explained by the very invasive BAL sampling protocol in this study. Indeed, a BAL was performed on admission, on day 7, and at the time of clinical deterioration.

Our observations illustrate that a positive test for *Aspergillus* in COVID-19 should not automatically lead to the initiation of antifungal therapy, a treatment that does not come without risk (21). Exactly in the ICU patient population, diagnosing IPA is most challenging. Indeed, the typical radiological findings are often absent, and in ICU patients, the specificity of any mycological test is lower than it is in well-defined patient population like those with longstanding neutropenia. In this setting, nonspecific tests such as panfungal β-D-glucan should not be used.

Due to nonspecific clinical symptoms and radiological findings, we considered a comparison of objective microbiological test results in COVID-19 patients with 2 other ICU populations with different respiratory infections a reasonable first comparator in order to estimate the clinical significance of the different *Aspergillus* tests available. We chose pneumococcal pneumonia as a control group since a comparable group in which invasive respiratory samples were obtained was needed. Patients admitted to the ICU with influenza were chosen as a patient group because ICU admission for respiratory insufficiency due to influenza was repeatedly shown to increase the risk for IPA (4). Healthy patients as a control group would be ideal, but this was not feasible.

The most reliable data will come from studies in which pre- or postmortem lung biopsies or full autopsies are performed and correlated with diagnostic test results that preceded the findings on biopsy or autopsy. Few published studies of this kind are available. In one such study, postmortem lung biopsies could not conform the premortem IPA diagnosis (28), and autopsies studies did not report the presence of possible *Aspergillus* (29–31).

In this study, we also observed several clinical practices in diagnosing and treating COVID-19 patients, such as the increased tendency to perform BAL in COVID-19 patients in comparison to pneumococcal pneumonia patients, despite initial fear of SARS-CoV-2 infection of medical personnel during the act of bronchoscopy (27). For the majority of the BAL fluid samples, an *Aspergillus* test was requested and performed. Furthermore, we noticed that a positive *Aspergillus* /\\test in our setting was common when samples were obtained from the upper respiratory tract, which suggests isolation of *Aspergillus* that may represent colonization.

The strength of the present study is the use of a control group and the fact that all patients originated from the same hospital, which should minimize selection bias. It

can be assumed, for example, that the reasons to perform BAL are comparable among the groups. Yet we cannot control for all possible confounders and there is still some bias, since cases and controls needed to be selected from different calendar years. Another limitation is that only one-third of the COVID-19 patients' respiratory samples were tested for the presence of *Aspergillus*.

In conclusion, the frequency of positive *Aspergillus* tests from BAL fluid of COVID-19 patients was comparable to that of pneumococcal pneumonia patients. Since in this control group positive *Aspergillus* tests are often considered to indicate colonization, a positive test for *Aspergillus* in COVID-19 should be interpreted with caution and not automatically lead to the start of an antifungal, especially considering its possible side effects. To get a more reliable estimate of the incidence of IPA in patients with COVID-19, studies are needed in which systematic (minimally invasive) autopsies or directed postmortem lung biopsies are performed.

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