Open Forum Infectious Diseases

MAJOR ARTICLE

An Observational Cohort Study of Bronchoalveolar Lavage Fluid Galactomannan and *Aspergillus* Culture Positivity in Patients Requiring Mechanical Ventilation

Catherine A. Gao,[1] Nikolay S. Markov,[1] Chiagozie Pickens,[1] Anna Pawlowski,[2] Mengjia Kang,[1] James M. Walter,[1] Benjamin D. Singer,[1] and Richard G. Wunderink[1]; for the NU SCRIPT Study Investigators

[1]Division of Pulmonary and Critical Care, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA, and [2]Northwestern Medicine Enterprise Data Warehouse, Chicago, Illinois, USA

Background. Critically ill patients who develop invasive pulmonary aspergillosis (IPA) have high mortality rates despite antifungal therapy. Diagnosis is difficult in these patients and incidences vary in the literature. Bronchoalveolar lavage (BAL) fluid galactomannan (GM) is a helpful marker, although the optimal cutoff is unclear.

Methods. This was a single-center cohort study of patients requiring mechanical ventilation in the medical intensive care unit (ICU) from June 2018 to March 2023. Demographics, BAL, and outcome data were extracted from the electronic health record and compared between groups of patients who grew *Aspergillus* from BAL, those who had elevated BAL GM levels (>0.5, >0.8, or >1.0) but did not grow *Aspergillus*, and those with neither.

Results. Of >1700 BALs from 688 patients, only 18 BALs from 15 patients grew *Aspergillus*. Patients who grew *Aspergillus* had more intubated days (29 vs 11, $P = .002$) and more ICU days (34 vs 15, $P = .002$). BAL GM level was higher from samples that grew *Aspergillus* than those that did not (median optical density index: 7.08 vs 0.11, $P < .001$).

Conclusions. In this large cohort of critically ill patients, we found a low rate of *Aspergillus* growth and variable BAL GM elevation. These data suggest that the pretest probability of IPA should be considered low in a general ICU population undergoing BAL evaluation to define the etiology of pneumonia. Elevated BAL GM may not reliably indicate invasive disease, but lack of culture positivity may also miss true infection. Improved scoring systems are needed to enhance pretest probability for diagnostic test stewardship purposes, and tests must be interpreted in their own clinical contexts.

Keywords. *Aspergillus*; bronchoalveolar lavage fluid; fungal pneumonia; galactomannan; severe pneumonia.

Invasive pulmonary aspergillosis (IPA) is a significant cause of morbidity and mortality, particularly in immunocompromised hosts, such as those with neutropenia and a history of organ transplantation [1]. Critically ill patients who present with IPA or develop IPA during the course of another lung infection have high mortality rates despite antifungal therapy [2].

The classification of true IPA is difficult in patients in the intensive care unit (ICU), as the gold standard of biopsy-proven infection often cannot be safely performed in these critically ill patients. Radiography lacks sufficient sensitivity and specificity, and even clinical judgment by expert clinicians can have disagreement or unclassifiable results. Until recent years, definitions focused primarily on immunocompromised hosts outside of the ICU setting. The European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) proposed invasive fungal disease definitions that were divided into (1) proven: detected by blood culture or histology and culture from a normally sterile clinical site; (2) probable: having a host factor that puts the patient at risk, clinical features of disease, and positive diagnostic tests (including indirect ones such as antigen testing); and (3) possible: having a risk factor and clinical feature but lacking mycological evidence [3]. However, chest X-rays (CXRs) obtained in the ICU are unreliable in detecting subtle nodules, halo signs, and cavities classically associated with IPA in non-ICU settings [4, 5]. Computed tomography (CT) scans, especially high-resolution CT, offer greater sensitivity than CXRs in detecting signs of IPA and are the recommended imaging modality of choice [6]. However, these are sometimes unable to be obtained in critically ill patients due to patient instability. Thus, clearly defining and categorizing critically ill patients into traditional IPA categories is even more difficult.

Received 26 July 2024; editorial decision 07 February 2025; accepted 11 February 2025; published online 15 February 2025

Correspondence: Catherine A. Gao, MD, MS, Division of Pulmonary and Critical Care, Northwestern University Feinberg School of Medicine, 625 N Michigan Ave, 15th Floor, Chicago, IL 60611 (catherine.gao@northwestern.edu).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofaf090

Groups have proposed different algorithms, such as AspICU, allowing for broader radiologic criteria in ICU patients, to include any imaging abnormality rather than the traditional imaging findings of cavity, dense lesions with or without a halo sign, and air-crescent signs [7]. They compared this broader categorization, which also included clinical symptoms and endotracheal aspirates with positive *Aspergillus* growth, to pathology obtained from lung biopsy or autopsy as a gold standard. This approach confirmed IPA with a moderate area under the receiver operating curve of 0.76, compared with only 0.57 using EORTC/MSG criteria. Newer scores, such as BM-Asp-ICU [8], classify cases using slightly modified criteria designed to reduce the number of unclassifiable cases.

Critically ill patients with severe viral pneumonia, such as influenza or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), who develop acute IPA often lack traditional host risk factors [9]. The reported prevalence of invasive fungal infections (IFIs) ranged widely across the literature, with a range of 2.5%–47% in patients with coronavirus disease 2019 (COVID-19) reported in 1 systematic review [10]. A French study of 145 critically ill patients with COVID-19 (with 475 respiratory samples) found that only 20 (14%) had traditional risk factors for IFI [11].

Given the difficulty in achieving gold standard criteria for "proven" IPA, biomarkers such as measurement of galactomannan (GM) have been studied. In addition to fungal culture, measurement of GM, a component of *Aspergillus* cell wall detected during active infection, in bronchoalveolar lavage (BAL) fluid or serum is helpful. Different BAL fluid GM optical density index (ODI) cutoffs (0.5 [12], 0.8 [13], or 1.0 [14]) have been proposed, and the best cutoff in critically ill patients is unknown.

At our center, fungal culture and GM measured in BAL fluid are part of our typical diagnostic panel for suspected pneumonia in mechanically ventilated patients. In this study, we examined a large cohort of mechanically ventilated patients undergoing BAL to evaluate the etiology of suspected pneumonia to determine the prevalence of BAL fluid GM positivity and *Aspergillus* growth. We hypothesized that patients who grew *Aspergillus* would have more risk factors, such as immunocompromised status or severe viral pneumonia, compared with patients who did not, and would experience worse outcomes. Given the uncertainty in clearly classifying cases of "true" *Aspergillus* infection due to the challenges in critically ill patients as outlined above, we present results at a variety of BAL GM cutoffs. Our primary objective was to characterize BAL GM and culture results in ICU patients requiring mechanical ventilation; secondary objectives include assessing these tests at varying cutoffs and their associations with outcomes.

METHODS

Study Setting and Participants

Patients were enrolled in the Successful Clinical Response in Pneumonia Therapy (SCRIPT) Systems Biology Center, a single-site, cohort study of mechanically ventilated patients hospitalized in the Northwestern Memorial Hospital medical intensive care unit (MICU) who underwent BAL for suspected pneumonia [15, 16]. This study was approved by the Northwestern University Institutional Review Board with study ID STU00204868. Current analysis includes patients hospitalized from June 2018 to March 2023. Patients were followed until the end of their hospitalization. Study participants were given a new identifier and enrolled at the level of hospitalization and are hereafter referred to as "patient." Our ICU has a ventilator care bundle as part of its routine clinical practice.

All patients in our MICU who underwent a BAL for suspected pneumonia (based on abnormal chest imaging, abnormal vitals/laboratory tests, and clinician concern) were eligible. Patients have a wide range of underlying diagnoses, but all have respiratory failure requiring mechanical ventilation. The exclusion criteria for the SCRIPT study are patients with prior lung transplant, prisoners, pregnant patients, and patients in whom bronchoscopy is deemed unsafe by the attending physician. Patients' families and legal authorized representatives were approached by our research team and consented to participate in the study, which collects residual BAL fluid obtained for routine clinical care and clinical data. The research team interviews patients or families upon study enrollment and at end of the study at hospital discharge.

Our patients are routinely sampled with BAL, either bronchoscopically or non-bronchoscopically (a respiratory therapist–driven protocol, described in previous works [17, 18] including safety profile), when clinicians suspect pneumonia. Patients with COVID-19 were only sampled bronchoscopically, with an aerosol-minimizing protocol described previously [19]. The recommended instillation volume during the procedure is 90–120 mL. These samples are typically sent for cell count and differential, amylase, bacterial and fungal cultures (incubated for 4 weeks at 30°C on inhibitory mold agar plates with identification performed by microscopic morphology and ability to grow at 45°C), BAL fluid GM (Bio-Rad Platelia *Aspergillus* Ag), and multiplex polymerase chain reaction (PCR; both viral and bacterial), as well as other studies when indicated. We do not have *Aspergillus* PCR testing at our institution. Our physicians use these data to guide antimicrobial therapy [20]. Decisions to perform testing or treat with antifungal therapy were up to the treating physician team, sometimes in discussion with infectious diseases consultation; there was no set protocol beyond currently available society/clinical guidelines.

Data Compilation

Demographics (including age, sex, race, ethnicity) and outcomes (including ICU length of stay, duration of mechanical ventilation, discharge disposition) were extracted from the electronic health record (EHR) via the Northwestern Medicine Enterprise Data Warehouse [21]. To protect patient privacy, patients from racial groups with <5 participants were masked as "unknown." Manual chart review was performed for quality control of EHR data pulls; errors in data pulls found on manual chart review were recoded before analysis. Immunocompromised state (such as patients with human immunodeficiency virus, organ transplant, or on chronic immunosuppressing medication; see the Supplementary Material for list of criteria) were categorized by the research intake team reviewing the chart and interviewing family members. Day-by-day data are aggregated for ICU days at our hospital; for patients who were transferred from an outside hospital, we do not have full day-by-day ICU details. Missing or unavailable data are reported as such. Since we analyzed BAL fluid results from routine clinical procedures, the sampling frequency was directed by physician decisions; hence, the missingness of follow-up samples (for example, improving patients are sampled less frequently) are inherently informative. Patients who required lung transplantation during hospitalizations were coded as having died, as is the practice for SCRIPT studies. An unfavorable outcome was defined as patients who died during admission (including those requiring lung transplant during hospitalization) or who were discharged to hospice. Only a small number of patients required lung transplant during hospitalization, and the exact patients are anonymized to protect patient privacy.

A panel of pulmonary and critical care physician adjudicators reviewed all patient charts and categorized patients into categories such as viral versus bacterial pneumonia based on their enrollment BAL studies and clinical syndrome (fever, leukocytosis, ventilator parameters, other clinical data such as imaging and culture results). This multistep/multiblinded reviewer adjudication process is described in detail separately [22].

We examined results both at the level of each BAL and at the level of each patient admission. We examined patients and BAL samples that had BAL fluid GM ODI <0.5, BAL fluid GM ODI >0.5, BAL fluid GM ODI >0.8, BAL fluid GM ODI >1.0, and those who did or did not grow *Aspergillus* on culture. We classified voriconazole, posaconazole, isavuconazonium sulfate, and liposomal amphotericin as anti-*Aspergillus* antifungal agents and coded based on administration of 1 of these agents; this method does not always differentiate between prophylactic and treatment intentions purposes. Serum galactomannan and Fungitell assays for (1,3)-β-D-glucan (BDG) were also available in many patients with a high clinical suspicion of fungal infection. We examined antibacterial antibiotic exposure both in terms of days of antibiotic administration and in terms of spectrum breadth as summarized by the Narrow Antibiotic Treatment (NAT) score, described in more detail in our previous work and in the Supplementary Materials [20, 23]. A NAT score of −2 indicates no antibiotics, a NAT score of 0 indicates antibiotics equivalent to guideline-recommended community-acquired pneumonia therapy such as ceftriaxone plus azithromycin, and higher NAT scores indicate broader antibiotic spectrum. We examined steroid administration summarized by day; to compare between different steroid agents, we converted all steroids into hydrocortisone anti-inflammatory equivalents [24]. All medication administration details are aggregated for ICU days only.

Statistical Analysis

Numerical values are reported as median (quartile 1 [Q1], quartile 3 [Q3]). Nonparametric continuous data were compared with Mann-Whitney *U* tests when performing pairwise comparisons, and with Kruskal-Wallis tests when comparing across 3 categories. Categorical data were compared with χ^2 tests. A $P < .05$ was the cutoff for statistical significance. Analysis and visualizations were done in Python version 3.9 with *seaborn* version 0.11.2 [25], *matplotlib* version 3.5.1 [26], *sklearn* version 1.0.2 [27], *scipy* version 1.7.3 [28], and *tableone* version 0.7.10 [29]. We employed time-dependent Cox proportional hazards models to evaluate the hazard of experiencing a composite unfavorable outcome. We performed analysis using time to first *Aspergillus* growth on BAL as the first time-dependent variable, and time to first elevated BAL GM as the second time-dependent variable. We used Kaplan-Meier methods to visualize and compare survival distributions across the 3 groups when summarized over the course of their admission. Multivariate log-rank tests were conducted to assess statistical differences in survival probabilities between the 3 categories, with the event of interest defined as the occurrence of the composite unfavorable outcome. The Python package *lifelines* version 0.30.0 [30] was used for analysis. Full code used for analysis are shared at our code repository: https://github.com/NUSCRIPT/gao_Aspergillus_2024. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting observational studies (Supplementary Materials) [31].

RESULTS

Completed hospitalization and adjudication information were available for 688 patients enrolled in SCRIPT and all were included in the analysis. Two hundred nine (30%) had SARS-CoV-2 on their enrollment BAL sample, and 208 (30%) were immunocompromised, with subgroups broken out in Table 1. The majority of patients started their hospital stay in the ICU; the median (Q1, Q3) length of hospital stay

Table 1. Cohort Demographics

Characteristic	No. (%)
No. of patients	688
Age, y, median (Q1, Q3)	62.0 (51.0, 71.0)
Sex	
Female	279 (40.6)
Male	409 (59.4)
Race	
Asian	21 (3.1)
Black or African American	134 (19.5)
Unknown or not reported	126 (18.3)
White	407 (59.2)
Ethnicity	
Hispanic or Latino	141 (20.5)
Not Hispanic or Latino	515 (74.9)
Unknown or not reported	32 (4.7)
Admission SOFA score, median (Q1, Q3)	11.0 (8.0, 14.0)
Admission APS, median (Q1, Q3)	91.0 (64.0, 109.0)
Admitted with COVID-19	209 (30.4)
Admitted with influenza	15 (2.2)
Immunocompromised[a]	208 (30.2)
Solid organ transplant	39 (5.7)
Stem cell transplant	36 (5.2)
Leukemia	30 (4.4)
Recent chemotherapy	43 (6.2)
Neutropenic during admission	62 (9.0)
Required vasopressor support with norepinephrine during admission	596 (87)
Required CRRT during admission	200 (29)
Required ECMO during admission	63 (9.2)

Data are presented as No. (%) unless otherwise indicated. There were no missing data for these variables.

Abbreviations: APS, Acute Physiology Score; COVID-19, coronavirus disease 2019; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; Q1, quartile 1; Q3, quartile 3; SOFA, Sequential Organ Failure Assessment.

[a]The "immunocompromised" category is inclusive of the subcategories solid organ transplant, stem cell transplant, leukemia, and chemotherapy; these subcategories were categorized by the research staff upon patient enrollment in the study, whereas neutropenia during admission was summarized separately.

before index MICU admission was 0 (0, 1) days. Our cohort was very ill with median (Q1, Q3) Sequential Organ Failure Assessment scores of 11.0 (8.0, 14.0) and median (Q1, Q3) Acute Physiology Score of 91.0 (64.0, 109.0) and required significant support, with 87% of the cohort requiring vasopressor support with norepinephrine, 29% requiring continuous renal replacement therapy, and 9% requiring extracorporeal membrane oxygenation.

A total of 1736 BALs were performed, 1146 (66%) of which had GM results, and 968 (55%) with fungal cultures. The breakdown of patient and BAL results are available in Figure 1*A* and BAL GM ODI cutoffs are available in Figure 1*B*. Of 1736 BALs, there were 1118 (64%) bronchoscopic BALs, 587 (34%) non-bronchoscopic BALs, 9 (0.5%) BALs with conflicting method documentation, and 22 (1.2%) without method clearly documented (result breakdown by BAL method in Supplementary Table 1). All samples that grew *Aspergillus* were obtained via bronchoscopic BAL, which is the preferred method when targeting a specific area of abnormality, and is the only technique allowed in patients with COVID-19 at our institution. At least 1 GM test was sent for 528 (76.7%) patients. Patients had a median (Q1, Q3) of 2 (1, 3) BALs sent during their ICU hospitalization, with 1 (0, 2) GM tests sent (Supplementary Figure 1). Of the 1146 BALs sent for GM testing, 403 (35%) were sent after antifungal therapy was administered, and 229 (20%) were sent after antifungal therapy with activity against *Aspergillus* was already administered in the ICU.

Eighteen BAL samples from 15 (2.2%) patients grew *Aspergillus* species on culture. Eleven (61%) of these were reported as *Aspergillus fumigatus*, 6 as *Aspergillus* species, and 1 as *Aspergillus* species, non-*fumigatus*. All 15 patients had at least 1 risk factor including underlying immunocompromise, neutropenia during admission, or severe viral pneumonia. All patients had abnormal chest imaging with ground glass opacities and consolidations, with all patients having numerous CXRs and 14 of 15 (93%) patients having a CT chest or CT angiography to evaluate for pulmonary embolism. Three of the 15 (20%) patients had possible cavitary or cystic lesions and another 2 of 15 (13%) had extensive cavitary disease. One of these patients had concurrent growth of *Klebsiella pneumoniae*, and another patient concurrently grew a *Corynebacterium* species and *Citrobacter koseri*. One of the patients who grew *Aspergillus* received a lung biopsy, which showed intra-alveolar exudate, reactive alveolar pneumocytes, aggregate of epithelial histocytes, and chronic inflammatory cells that were favored to be consistent with an infectious etiology, though acid-fast bacilli and Grocott methenamine silver stains were negative. None of the patients who grew *Aspergillus* had an autopsy.

Serum GM was sent in 171 instances in our cohort during their ICU stay; only 6 patients had elevated serum GM >0.5 ODI. The serum GM was not significantly different among patients who grew *Aspergillus* compared to those who did not, with a median (Q1, Q3) of 0.08 (0.05, 0.20) ODI compared with 0.07 (0.05, 0.14) ODI. There was a slight correlation between serum GM and BAL GM when they were checked within 5 days of each other (Spearman correlation = 0.27, $P < .01$). A serum BDG assay was sent in 129 cases in our cohort; it had a median (Q1, Q3) of 323 (286, 375) pg/mL in patients who grew *Aspergillus*, compared with 31 (our laboratory's lowest value) (31, 292) pg/mL in patients who did not grow *Aspergillus*. As BDG can be elevated in a range of fungal infections besides with *Aspergillus*, we examined our cohort for other fungal infections. All organisms recovered from BAL fungal cultures are reported in Supplementary Table 2. Three patients grew *Blastomyces* on 6 BALs; only 1 of these patients had BDG sent, and it was elevated at >500 pg/mL. Three patients had positive *Pneumocystis jirovecii* direct fluorescent antibody on BAL; 1 did not have BDG sent, 1 did not have an elevated value,

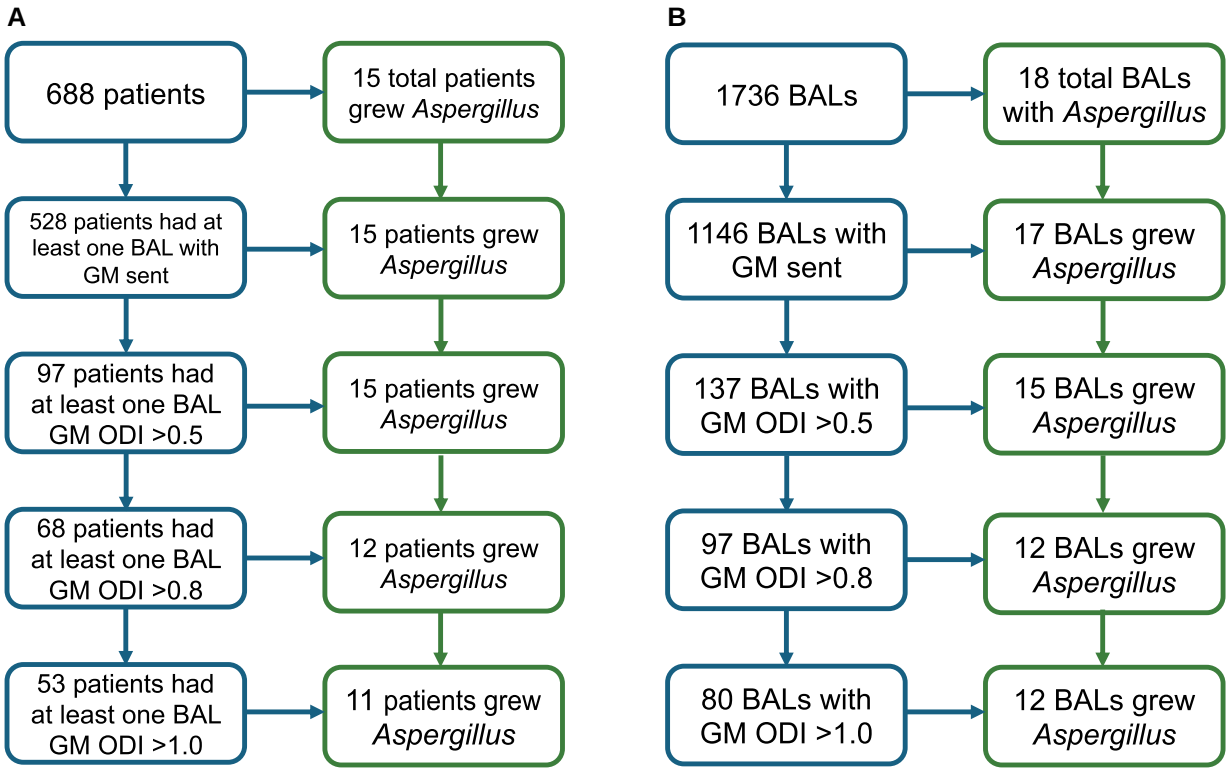

Figure 1. Bronchoalveolar lavage (BAL) fluid galactomannan and *Aspergillus* growth. *A*, Summary on a patient level. *B*, Summary of BAL samples. Abbreviations: BAL, bronchoalveolar lavage; GM, galactomannan; ODI, optical density index.

and 1 had an elevated value of >500 pg/mL. Fourteen patients had candidemia and 2 had BDG sent; 1 patient's result was mildly elevated at 97 pg/mL, and the other patient had highly elevated values of >500 pg/mL.

The median (Q1, Q3) ODI of BAL fluid GM was higher in BAL samples that grew *Aspergillus* compared with those that did not (7.08 [0.79, 7.76] vs 0.11 [0.07, 0.21]; $P < .001$). Only 1 patient who grew *Aspergillus* had BAL GM ODI <0.5. The 15 patients who grew *Aspergillus* had 54 BALs with GM assays sent, with median (Q1, Q3) BAL fluid GM ODI of 0.78 (0.24, 4.9), compared with 0.10 (0.07, 0.20) ($P < .001$) in the 1092 BAL samples from patients who never grew *Aspergillus* (Figure 2). Patients with BALs that grew *Aspergillus* often had higher GM even on different BAL samples that did not grow *Aspergillus* (example timeline graphs are shown in Figure 3).

Eleven of the 15 (73%) patients with BAL samples that grew *Aspergillus* initially presented with COVID-19, and 2 of these were immunocompromised (solid organ transplant, receiving rituximab for a lymphoproliferative disorder). Of the 4 patients who grew *Aspergillus* who did not present with COVID-19, 2 were immunocompromised (solid organ transplant, chronic corticosteroid use higher than prednisone 20 mg/day for the last month for vasculitis). Positive cultures occurred in 5.3% of patients with COVID-19 and 1.9% of immunocompromised patients in the cohort. Thirteen of the 15 patients who had BAL samples that grew *Aspergillus* were treated with antifungal therapy. One patient with a culture-positive sample who was not treated with antifungal therapy had consistently low BAL fluid GM ODI levels (0.16 and 0.05), whereas the other only had a single measurement of 0.79. Both patients who were not treated with antifungal therapy died.

Patients with at least 1 BAL sample that grew *Aspergillus* (n = 15) were older (71 vs 62 years, $P = .023$), had more days intubated (29 vs 11 days, $P = .002$), and had more ICU days (34 vs 15 days, $P = .002$) than patients who did not (n = 673) (Table 2). They received more steroids during their ICU admission (median cumulative hydrocortisone equivalents of 1660 vs 780 mg, $P = .010$). Significantly more patients with BAL that grew *Aspergillus* during their ICU stay received antifungal therapy with anti-*Aspergillus* activity compared to those who did not (87% vs 17%, $P < .001$). Unfavorable outcomes (death or discharge to hospice) trended higher but were not statistically different in patients with a BAL sample that grew *Aspergillus* compared to those that did not (67% vs 45%, $P = .15$). There was no difference in time to inpatient mortality between the 2 groups using time-dependent Cox proportional hazard models (hazard ratio [HR], 0.79 [95% confidence interval {CI}, 0.40–1.59]; $P = .51$). Kaplan-Meier survival curves of inpatient mortality for patients who grew *Aspergillus* or did not grow *Aspergillus* during their hospitalization are shown in

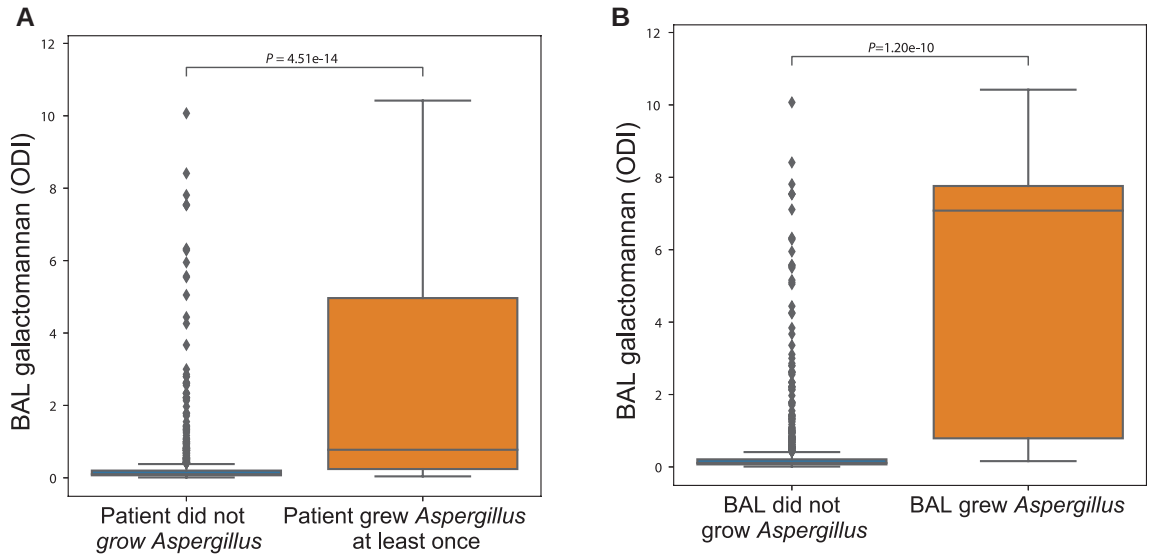

Figure 2. *A*, Bronchoalveolar lavage (BAL) fluid galactomannan (GM) optical density index (ODI) is higher in patients who grow *Aspergillus* at least once during admission. *B*, BAL fluid GM ODI is higher in the BAL samples where *Aspergillus* grew, though sometimes also elevated in BAL samples that did not grow *Aspergillus*.

Supplementary Figure 2. Of the 287 patients who died, 253 (88%) died in the ICU and 25 (8.5%) died outside of the ICU.

The subset of patients with BAL fluid GM ODI >1 but no positive *Aspergillus* culture (n = 42) was closer in age to patients without any elevated BAL fluid GM measurements, with a median age of 63 years (Table 3). Their median intubation days fell between the other 2 categories at 15.5 days, as did ICU days at a median of 22.5 days. Twenty-six of these 42 patients (62%) were treated with antifungal therapy with anti-*Aspergillus* activity, with no difference in outcome based on receipt of antifungal therapy ($P = .86$). There was no statistical significance in a time-dependent Cox model using first elevated BAL GM as the time-dependent variable when evaluating the hazard of composite unfavorable outcome (HR, 1.15 [95% CI, .78–1.69]; $P = .49$). There was no difference in time to inpatient mortality between the 3 groups ($P = .32$ by multivariate log-rank test) (Supplementary Figure 3 for Kaplan-Meier curves). Results were similar for the subset of patients with elevated BAL GM ODI >0.5 (Supplementary Table 3) and BAL GM ODI >0.8 (Supplementary Table 4). *Aspergillus* first grew a median (Q1, Q3) of 9 (3, 22.5) days after ICU admission at our hospital, and on day 9 (2.5, 22.5) of mechanical ventilation (Supplementary Table 5). The median (Q1, Q3) days of antibiotics before positive culture was 8 (3, 13.5) days, with cumulative NAT score of 4 (−6.5, 12.5). Six patients had already received antifungal therapy before a positive *Aspergillus* culture; all 6 of these patients received antifungal therapy with anti-*Aspergillus* coverage. The median (Q1, Q3) hydrocortisone anti-inflammatory equivalent steroid dose was 750 (520, 1375) mg in the ICU days prior to growth.

Among the 53 patients who had BAL fluid GM ODI >1 on their first sample, the sample was obtained on a median (Q1, Q3) ICU day 2 (1, 14) and ventilation day 2 (1, 10). The median (Q1, Q3) days of antibiotic exposure while in the ICU before elevated BAL GM was 3 (0, 9) days, with cumulative NAT score of 3 (0, 9). Thirteen patients had already received antifungal therapy before an elevated BAL GM, with 7 receiving antifungal therapy with anti-*Aspergillus* coverage. The median (Q1, Q3) hydrocortisone anti-inflammatory equivalent dose was 350 (0, 1300) mg before the first elevated GM.

DISCUSSION

These results from a large collection of BAL samples from a cohort of mechanically ventilated patients undergoing BAL for suspected pneumonia, approximately 30% of whom were immunocompromised, demonstrate that growth of *Aspergillus* is rare among a general MICU population. Patients whose BAL fluid grew *Aspergillus* and patients with elevated BAL fluid GM levels, compared with patients with neither of these, had more ventilation days, ICU days, antibiotic days, and higher cumulative steroid dose. However, no statistically significant difference in mortality was found, as has been reported in other studies of *Aspergillus* superinfection, especially those involving patients with COVID-19–associated IPA [32–34]. The SCRIPT study cohort is biased toward a more severely ill patient population compared to typical ICU patients, many of whom do not require mechanical ventilation. Even so, despite >1700 BALs being performed, only 18 BAL samples with *Aspergillus* growth were recovered, indicating that this is a rare occurrence. The

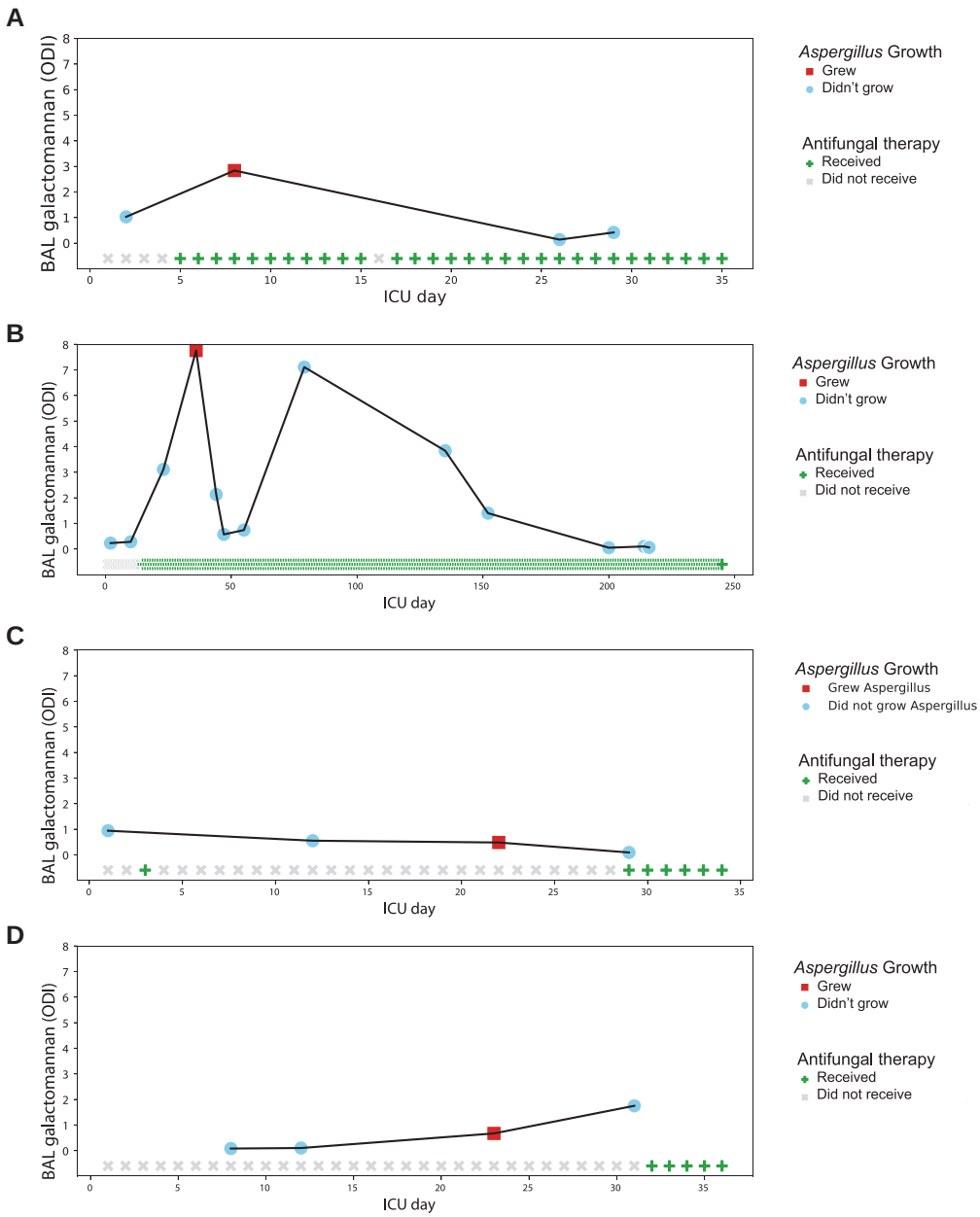

Figure 3. Example timelines of four patients (*A, B, C, D*) who grew *Aspergillus* on bronchoalveolar lavage (BAL) fluid culture (red square indicates *Aspergillus* growth, blue circle indicates no *Aspergillus* growth) and BAL fluid galactomannan (GM) optical density index (ODI) levels (y-axis). Patients who grew *Aspergillus* did not grow it consistently, even on BAL samples that had elevated BAL fluid GM ODIs. The bottom line of each graph shows antifungal therapy with anti-*Aspergillus* activity by intensive care unit (ICU) day (gray x marker, no antifungal therapy; green plus marker, antifungal therapy administered).

majority of BALs were obtained bronchoscopically, due in part to institutional protocol that patients with COVID-19 be sampled bronchoscopically with an aerosol-minimizing protocol, but approximately one-third were performed non-bronchoscopically, though our non-bronchoscopic BAL protocol obtains alveolar sampling similar to a bronchoscopic BAL [18]. One of the strengths of our study is that only 23% of our cohort did not undergo BAL GM testing [35], thus minimizing the potential for occult IPA in patients without high clinical risk and allowing for exposure of potential false-positive results. Testing was performed at the treating team's discretion; in cases without BAL GM sent, it was likely that the clinical team had low suspicion for *Aspergillus* infection. The 528 patients who did have testing sent on 1146 BALs provide, to our knowledge, the largest collection of BAL GM data in critically ill patients in the literature.

Table 2. Key Demographics and Outcomes, Grouped by Whether Patients Grew *Aspergillus*

Characteristic	Grew *Aspergillus* During Admission	Did Not Grow *Aspergillus* During Admission	*P* Value
No. of patients	15	673	
Age, y	71.0 (67.0, 74.0)	62.0 (51.0, 71.0)	.023
Admitted with COVID-19	11 (73.3)	198 (29.4)	.001
Admitted with influenza	…	15 (2.2)	1
Immunocompromised	4 (26.7)	204 (30.3)	1
Solid organ transplant	2 (13.3)	37 (5.5)	.207
Stem cell transplant	…	36 (5.3)	1
Leukemia	…	30 (4.5)	1
Chemotherapy	…	43 (6.4)	.616
Neutropenic during admission	2 (13.3)	60 (8.9)	.637
Received tocilizumab	1 (6.7)	18 (2.7)	.346
PMNs over admission	10.5 (7.2, 12.9)	8.4 (5.3, 12.2)	.226
Cumulative ICU days	34.0 (24.0, 37.5)	15.0 (8.0, 27.0)	.002
Cumulative intubation days	29.0 (23.5, 36.5)	11.0 (5.0, 23.0)	.002
Received tracheostomy	8 (53.3)	170 (25.3)	.031
Steroid dose over ICU admission, hydrocortisone equivalents	1660.0 (1325.0, 2475.0)	780.0 (0.0, 2150.0)	.01
Treated with antifungals	13 (86.7)	249 (37.0)	<.001
Days of antifungal therapy	8.0 (4.5, 35.0)	0.0 (0.0, 5.0)	<.001
Treated with antifungals with anti-*Aspergillus* activity	13 (86.7)	117 (17.4)	<.001
Days of antifungal therapy with anti-*Aspergillus* activity	8.0 (4.5, 35.0)	0.0 (0.0, 0.0)	<.001
Summed NAT score	17.0 (−5.5, 60.0)	6.0 (−4.0, 18.0)	.34
Days of antibiotic therapy	19.0 (16.0, 33.5)	11.0 (6.0, 21.0)	.001
Discharge disposition			.3
Died	9 (60.0)	278 (41.3)	
Home	…	147 (21.8)	
Hospice	1 (6.7)	23 (3.4)	
LTACH	2 (13.3)	76 (11.3)	
Rehabilitation facility	3 (20.0)	108 (16.0)	
Skilled nursing facility	…	41 (6.1)	
Unfavorable outcome	10 (66.7)	301 (44.7)	.154

Numerical values are reported as median (quartile 1, quartile 3) and compared by Mann-Whitney *U* tests. Categorical variables are reported as No. (%) and compared by χ^2 tests. Medication days/doses are summed across ICU days. Unfavorable outcome is defined by in-hospital mortality, requiring lung transplant during hospitalization, or discharge to hospice.

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; LTACH, long-term acute care hospital; NAT, Narrow Antibiotic Treatment; PMN, polymorphonuclear neutrophils.

Our study began enrolling in 2018, and this study includes patient data until 2023; a significant portion of our cohort includes patients with COVID-19, who have been known to have longer duration of intubation, ICU days, and hospital stay [36]. Our rate of COVID-19 patients (n = 209) who either grew *Aspergillus* (n = 11) or had elevated BAL GM (n = 10) is within the range of published rates, albeit on the lower side [10, 37]. In contrast to other studies, we almost universally sampled recently intubated patients with severe viral pneumonia [20]. This rate is lower compared to the analysis of Feys et al, which found higher rates of IPA in studies that performed BAL for IPA diagnosis in a greater proportion of cases [35]. However, direct comparisons are difficult across studies and case series due to differences in diagnostic criteria or cutoffs, patient populations, antibiotic exposure, and environmental prevalence and exposure to *Aspergillus* [38]. *Aspergillus* is found in a variety of environmental locations and has various strains across the globe [39], possibly explaining the variability in infectious rates reported across different hospitals from different geographic locations. Many patients received antifungal therapy before sample collection, potentially altering test and culture results. Other limitations include the imperfect diagnostic tests used and inclusion of patients without underlying immunocompromise, who thus have a lower risk of invasive aspergillosis.

A range of optimal cutoff values and algorithms have been proposed to evaluate critically ill patients for IPA [7]. Hence, we presented our data categorized at various proposed ranges of BAL GM cutoffs to facilitate comparison. We found similar results to previous studies in that patients with positive *Aspergillus* cultures had higher BAL fluid GM compared to those who did not grow *Aspergillus* [40]. In contrast, elevated GM may be valuable to distinguish between colonization and active infection in samples that grow *Aspergillus* on culture. Use of BAL in our study likely minimized the detection of *Aspergillus* airway colonization, in contrast to tracheal aspirates, with only 1 positive culture not associated with an elevated BAL GM level. Determining true IPA versus simple colonization based on a single BAL GM level is difficult, and the decision to treat by the clinical team was often based on judgment or patient illness; many patients were treated with

Table 3. Key Demographics and Outcomes

Characteristic	Grew *Aspergillus* at Least Once During Admission	Had Elevated BAL GM >1 but Did Not Grow *Aspergillus*	Did Not Have Elevated BAL GM	*P* Value
No.	15	42	631	
Age, y	71.0 (67.0, 74.0)	63.5 (55.2, 70.8)	62.0 (50.5, 71.0)	.061
Admitted with COVID-19	11 (73.3)	10 (23.8)	188 (29.8)	.001
Admitted with influenza	…	1 (2.4)	14 (2.2)	.841
Immunocompromised	4 (26.7)	13 (31.0)	191 (30.3)	.951
Solid organ transplant	2 (13.3)	3 (7.1)	34 (5.4)	.385
Stem cell transplant	…	1 (2.4)	35 (5.5)	.44
Leukemia	…	4 (9.5)	26 (4.1)	.178
Chemotherapy	…	4 (9.5)	39 (6.2)	.412
Neutropenic during admission	2 (13.3)	7 (16.7)	53 (8.4)	.163
Received tocilizumab	1 (6.7)	…	18 (2.9)	.356
PMNs over admission	10.5 (7.2, 12.9)	8.0 (3.6, 10.6)	8.5 (5.4, 12.5)	.153
Cumulative ICU days	34.0 (24.0, 37.5)	22.5 (9.2, 44.8)	15.0 (8.0, 27.0)	<.001
Cumulative intubation days	29.0 (23.5, 36.5)	15.5 (9.2, 37.5)	11.0 (5.0, 22.5)	<.001
Received tracheostomy	8 (53.3)	17 (40.5)	153 (24.2)	.003
Steroid dose over ICU admission, hydrocortisone equivalents	1660.0 (1325.0, 2475.0)	1350.0 (500.0, 2145.0)	750.0 (0.0, 2125.0)	.004
Treated with antifungals	13 (86.7)	27 (64.3)	222 (35.2)	<.001
Days of antifungal therapy	8.0 (4.5, 35.0)	5.0 (0.0, 21.8)	0.0 (0.0, 4.0)	<.001
Treated with antifungals with anti-*Aspergillus* activity	13 (86.7)	26 (61.9)	91 (14.4)	<.001
Days of antifungal therapy with anti-*Aspergillus* activity	8.0 (4.5, 35.0)	5.0 (0.0, 21.0)	0.0 (0.0, 0.0)	<.001
Summed NAT score	17.0 (–5.5, 60.0)	8.0 (–1.2, 23.8)	6.0 (–4.0, 17.0)	.298
Days of antibiotics therapy	19.0 (16.0, 33.5)	18.5 (7.2, 32.5)	11.0 (6.0, 20.0)	<.001
Discharge disposition				.24
Died	9 (60.0)	18 (42.9)	260 (41.2)	
Home	…	4 (9.5)	143 (22.7)	
Hospice	1 (6.7)	1 (2.4)	22 (3.5)	
LTACH	2 (13.3)	8 (19.0)	68 (10.8)	
Rehabilitation facility	3 (20.0)	9 (21.4)	99 (15.7)	
Skilled nursing facility	…	2 (4.8)	39 (6.2)	
Unfavorable outcome	10 (66.7)	19 (45.2)	282 (44.7)	.24

Numerical values are reported as median (quartile 1, quartile 3) and compared by Kruskal-Wallis tests. Categorical variables are reported as No. (%) and compared by χ^2 tests.

Abbreviations: BAL, bronchoalveolar lavage; COVID-19, coronavirus disease 2019; GM, galactomannan; ICU, intensive care unit; LTACH, long-term acute care hospital; NAT, Narrow Antibiotic Treatment; PMN, polymorphonuclear neutrophils.

antifungal therapy despite no growth on fungal cultures. The optimal threshold of BAL GM can be difficult to determine, as there is frequently lack of gold standard in critically ill patients, who often cannot tolerate biopsy. Studies have furthermore shown that cases that did not meet consensus definitions for invasive aspergillosis have similar mortality to those who meet definitions [41]. We found that serum BDG was elevated more consistently than serum GM in patients who grew *Aspergillus*, in line with previous reports of the higher sensitivity of BDG compared with serum GM [42, 43].

Another strength of our study is the availability of serial BAL information on many patients. *Aspergillus* often does not always grow on cultures, as shown in the multiple elevated BAL fluid GM levels in patients who only grew *Aspergillus* on 1 sample or did not grow it at all. Patients with only elevated BAL fluid GM have clinical features (such as age and duration of intubation) that fall between the values of those with positive cultures and patients with neither positive culture nor GM elevation, suggesting that many patients with elevated BAL fluid GM levels are actively infected. Conversely, a single BAL fluid GM elevation in serial samples may simply represent a false-positive result (reported false-positive rates of 13%–42%) [44, 45]. Overall low yield has been reported for repeat fungal PCR assays on blood and BAL when repeated within 4 weeks of a negative PCR result, as reported by Wang et al [46].

Patients with severe viral pneumonia, such as from SARS-CoV-2, may lack traditional immunocompromised risk factors and still grow *Aspergillus*. Numerous mechanisms for IPA complicating acute viral pneumonia have been hypothesized [47]. Severe direct lung damage from the primary viral infection may predispose patients to secondary fungal

(and bacterial) infections [48], viral infections can cause suppression of cellular immunity, and hypothesized cytoskeletal changes may mediate internalization of *Aspergillus* [49]. Immunomodulatory therapies, such as corticosteroids and interleukin 6 receptor inhibitors, used to treat patients with COVID-19 may have a role in IFIs [50]. In addition, clinical suspicion of bacterial superinfection in patients with severe viral pneumonia results in a high frequency of antibiotic use, including broad-spectrum agents [51, 52]. Prolonged exposure to broad-spectrum antibiotics promotes susceptibility to IFIs [53]. We demonstrate differences in both steroid exposure and duration of antibiotic exposure correlated with evidence of *Aspergillus* in our cohort.

This study has several limitations. The study was conducted at a single quaternary care center with a well-established antibiotic stewardship emphasis (using rapid PCR pathogen testing to guide the narrowing antibiotics) [20] and high use of BAL sampling compared to other centers, although recent calls for more aggressive BAL sampling in severe viral pneumonia suggest this may be appropriate [35]. Given the relatively low number of patients with elevated BAL fluid GM or growth of *Aspergillus*, our study may have been underpowered to detect other important risk factors or differences in outcome. BAL GM is sent routinely by our clinical teams, irrespective of pretest probability, compared to other centers that may only send it when clinical suspicion is higher. These results and sampling practices may not reflect the population of hospitals with different antibiotic management strategies, and thus our results may not generalize to other sites. We had only 15 cases of influenza pneumonia in our cohort, thus limiting conclusions in this cohort known to be at increased risk for IPA. Our medication administration data was limited to doses given while in our ICU; some patients may have spent time on the hospital floor or an external ICU before being transferred, and medications given in those locations were not captured. Furthermore, it can be sometimes unclear when antifungal agents are being used for treatment intention or prophylaxis, and our code did not differentiate between these. Numerous confounders affect test and culture results, such as prior antifungal therapy, variability in patients' immune status, differences in environmental exposure, and biases in selecting patients for testing [54]. A proportion of BALs sent for GM testing were performed after antifungal therapy was administered, which has been reported to decrease the yield of BAL GM. We did not routinely collect tracheobronchitis data and biopsy and autopsy are infrequent, although we reviewed the charts for the subset of patients who grew *Aspergillus* and reported the results above. We do not have information collected on duration of symptoms prior to hospital admission. We did not have details about triazole resistance genotype/phenotypes. We did not always have detailed species information on all samples. We did not have *Aspergillus* PCR available at our institution. Given its promising test characteristics, it may have utility in future research for critically ill patients [55]. Furthermore, other GM assay formats, such as detection via lateral flow assay formats, could have improved sensitivity in BAL samples, but needs further study and wider verification [56]. Blood BDG can be positive in other invasive mycosis infections besides *Aspergillus* and can have false positives from cross-reactivity with some antimicrobials or even hemodialysis filters, and so must have its results interpreted carefully within the clinical context [57].

Accurate radiographic studies such as CT scans to demonstrate cavitation, which suggests invasive *Aspergillus*, were not prospectively recorded or coded in our database. However, all patients enrolled in our study had abnormal imaging that prompted a BAL for suspected pneumonia, and we reviewed the chest imaging from patients who grew *Aspergillus* and found that most had only ground glass and consolidative opacities, with a minority having true cavitary disease. Imaging in patients with IPA without the traditional significant immunosuppression history, such as patients with COVID-19–associated pulmonary aspergillosis, lack the traditional findings described with IPA (halo sign, air-crescent sign, etc) [58]. In addition, lung abscess in ICU patients is caused by *Aspergillus* in only 8.8% of cases in which a cavity is detected, again emphasizing the nonspecific nature of these findings to IPA [5]. Given that the decision to treat with antifungals was up to the treating team, factors considered in diagnosing IPA, and in cases with unfavorable outcomes, whether IPA treatment impacted outcome is difficult to clearly abstract from the medical record.

In our large cohort of critically ill patients requiring mechanical ventilation, we found that *Aspergillus* growth was rare while elevated BAL fluid GM was slightly more common and that both correlated with unfavorable outcomes, though the extent to which this is due directly to suspected IPA is unclear. Classifying IPA in critically ill patients remains challenging, despite a variety of proposed guidelines and algorithms, and we did not undertake formal classification in this analysis, due to the challenges highlighted above. Instead, we present our large cohort of rich data at a variety of cutoffs. Overall, in our cohort with frequent BAL practices, detection of *Aspergillus* by culture or GM positivity was rare. These data suggest that the pretest probability of IPA should be considered low in a general ICU population undergoing BAL evaluation to define the etiology of pneumonia, even among patients with immunocompromise and those with viral pneumonia. BAL GM and fungal culture results must be interpreted with careful consideration of the clinical context. Improved scoring systems would be beneficial to enhance pretest probability for diagnostic test stewardship purposes.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the

posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors greatly thank Dr Chao Qi for providing details surrounding the mycology laboratory practices. Large language models were used to help coding, with all output reviewed by authors, who take responsibility for the final product.

Author contributions. C. A. G., B. D. S., and R. G. W. conceived and designed the study. A. P. and M. K. performed data acquisition. C. A. G. and N. S. M. performed data processing and cleaning. C. A. G. performed chart review, analysis, and visualization. C. A. G. drafted the manuscript. All authors read and approved the final draft of the manuscript. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Data availability. A significant portion of these data has been already made available through PhysioNet at https://physionet.org/content/script-carpediem-dataset/1.1.0/ [59]; a future update will include the new patients enrolled since the publication of the original dataset. Code is available at https://github.com/NUSCRIPT/gao_Aspergillus_2024.

Disclaimer. The funding sources did not have a role in the design, execution, or prior review of the study or in the data presented in this manuscript. Opinions expressed in this work do not necessarily reflect those of the funding sources.

Patient consent. Student participants or their surrogates provided informed consent. This study was approved by the Northwestern University Institutional Review Board with study ID STU00204868.

Financial support. SCRIPT is supported by the National Institute of Allergy and Infectious Diseases (grant number U19AI135964). C. A. G. is supported by the National Heart, Lung, and Blood Institute (award number K23HL169815), a Parker B. Francis Opportunity Award, and an American Thoracic Society unrestricted grant. B. D. S. is supported by the National Institutes of Health (grant numbers R01HL149883, R01HL153122, P01HL154998, P01AG049665, and U19AI135964). R. G. W. is supported by the National Institutes of Health (grant numbers U19AI135964, U01TR003528, P01HL154998, R01HL149883, and R01LM013337).

Potential conflicts of interest. B. D. S. holds US patent 10,905,706, "Compositions and methods to accelerate resolution of acute lung inflammation," and serves on the scientific advisory board of Zoe Biosciences, for which he holds stock options. All other authors report no potential conflicts of interest.

References

1. Lin S-J, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis **2001**; 32:358–66.
2. Baddley JW, Stephens JM, Ji X, Gao X, Schlamm HT, Tarallo M. Aspergillosis in intensive care unit (ICU) patients: epidemiology and economic outcomes. BMC Infect Dis **2013**; 13:29.
3. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis **2020**; 71:1367–76.
4. Zoumot Z, Bonilla MF, Wahla AS, et al. Pulmonary cavitation: an underrecognized late complication of severe COVID-19 lung disease. BMC Pulm Med **2021**; 21:24.
5. Montméat V, Bonny V, Urbina T, et al. Epidemiology and clinical patterns of lung abscesses in ICU: a French multicenter retrospective study. Chest **2023**; 165: 48–57.
6. Alexander BD, Lamoth F, Heussel CP, et al. Guidance on imaging for invasive pulmonary aspergillosis and mucormycosis: from the imaging working group for the revision and update of the consensus definitions of fungal disease from the EORTC/MSGERC. Clin Infect Dis **2021**; 72(Suppl 2):S79–88.
7. Blot SI, Taccone FS, Van den Abeele A-M, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med **2012**; 186:56–64.
8. Hamam J, Navellou J-C, Bellanger A-P, et al. New clinical algorithm including fungal biomarkers to better diagnose probable invasive pulmonary aspergillosis in ICU. Ann Intensive Care **2021**; 11:41.
9. Verweij PE, Rijnders BJA, Brüggemann RJM, et al. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. Intensive Care Med **2020**; 46:1524–35.
10. Gioia F, Walti LN, Orchanian-Cheff A, Husain S. Risk factors for COVID-19-associated pulmonary aspergillosis: a systematic review and meta-analysis. Lancet Respir Med **2024**; 12:207–16.
11. Fekkar A, Lampros A, Mayaux J, et al. Occurrence of invasive pulmonary fungal infections in patients with severe COVID-19 admitted to the ICU. Am J Respir Crit Care Med **2021**; 203:307–17.
12. Meersseman W, Lagrou K, Maertens J, et al. Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. Am J Respir Crit Care Med **2008**; 177:27–34.
13. D'Haese J, Theunissen K, Vermeulen E, et al. Detection of galactomannan in bronchoalveolar lavage fluid samples of patients at risk for invasive pulmonary aspergillosis: analytical and clinical validity. J Clin Microbiol **2012**; 50:1258–63.
14. Mercier T, Castagnola E, Marr KA, Wheat LJ, Verweij PE, Maertens JA. Defining galactomannan positivity in the updated EORTC/MSGERC consensus definitions of invasive fungal diseases. Clin Infect Dis **2021**; 72(Suppl 2):S89–94.
15. Gao CA, Markov NS, Stoeger T, et al. Machine learning links unresolving secondary pneumonia to mortality in patients with severe pneumonia, including COVID-19. J Clin Invest **2023**; 133:e170682.
16. Grant RA, Morales-Nebreda L, Markov NS, et al. Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia. Nature **2021**; 590:635–41.
17. Walter JM, Ren Z, Yacoub T, et al. Multidimensional assessment of the host response in mechanically ventilated patients with suspected pneumonia. Am J Respir Crit Care Med **2019**; 199:1225–37.
18. Walter JM, Helmin KA, Abdala-Valencia H, Wunderink RG, Singer BD. Multidimensional assessment of alveolar T cells in critically ill patients. JCI Insight **2018**; 3:e123287.
19. Gao CA, Bailey JI, Walter JM, et al. Bronchoscopy on intubated patients with COVID-19 is associated with low infectious risk to operators. Ann Am Thorac Soc **2021**; 18:1243–6.
20. Pickens CO, Gao CA, Cuttica MJ, et al. Bacterial superinfection pneumonia in patients mechanically ventilated for COVID-19 pneumonia. Am J Respir Crit Care Med **2021**; 204:921–32.
21. Starren JB, Winter AQ, Lloyd-Jones DM. Enabling a learning health system through a unified enterprise data warehouse: the experience of the Northwestern University Clinical and Translational Sciences (NUCATS) Institute. Clin Transl Sci **2015**; 8:269–71.
22. Pickens CI, Gao CA, Bodner J, et al. An adjudication protocol for severe pneumonia. Open Forum Infect Dis **2023**; 10:ofad336.
23. Wang AA, Pickens CO, He H, et al. The narrow-spectrum antibiotic treatment score: a novel quantitative tool for assessing broad- and narrow-spectrum antibiotic use in severe community-acquired pneumonia. In: B28 host and microbial clinical studies in lung infections and lung diseases. American Thoracic Society, **2020**:A2929.
24. Kane SP. Corticosteroid conversion calculator. **2015**. Available at: https://clincalc.com/Corticosteroids/. Accessed 26 October 2024.
25. Waskom M. Seaborn: statistical data visualization. J Open Source Softw **2021**; 6: 3021.
26. Hunter JD. Matplotlib: a 2D graphics environment. Comput Sci Eng **2007**; 9:90–5.
27. Pedregosa F, Varoquaux G, Gramfort A, et al. Scikit-learn: machine learning in Python. J Mach Learn Res **2011**; 12:2825–30.
28. Virtanen P, Gommers R, Oliphant TE, et al. Scipy 1.0: fundamental algorithms for scientific computing in Python. Nat Methods **2020**; 17:261–72.
29. Pollard TJ, Johnson AEW, Raffa JD, Mark RG. Tableone: an open source Python package for producing summary statistics for research papers. JAMIA Open **2018**; 1:26–31.
30. Lifelines. Lifelines 0.30.0 documentation. Available at: https://lifelines.readthedocs.io/en/latest/index.html. Accessed 31 January 2025.
31. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Int J Surg **2014**; 12:1495–9.
32. Lahmer T, Kriescher S, Herner A, et al. Invasive pulmonary aspergillosis in critically ill patients with severe COVID-19 pneumonia: results from the prospective AspCOVID-19 study. PLoS One **2021**; 16:e0238825.
33. Vélez Pintado M, Camiro-Zúñiga A, Aguilar Soto M, et al. COVID-19–associated invasive pulmonary aspergillosis in a tertiary care center in Mexico City. Med Mycol **2021**; 59:828–33.
34. Chong WH, Saha BK, Neu KP. Comparing the clinical characteristics and outcomes of COVID-19–associated pulmonary aspergillosis (CAPA): a systematic review and meta-analysis. Infection **2022**; 50:43–56.

35. Feys S, Hoenigl M, Gangneux J-P, Verweij PE, Wauters J. Fungal fog in viral storms: necessity for rigor in aspergillosis diagnosis and research. Am J Respir Crit Care Med **2024**; 209:631–3.
36. Cobb NL, Sathe NA, Duan KI, et al. Comparison of clinical features and outcomes in critically ill patients hospitalized with COVID-19 versus influenza. Ann Am Thorac Soc **2021**; 18:632–40.
37. Kariyawasam RM, Dingle TC, Kula BE, Vandermeer B, Sligl WI, Schwartz IS. Defining COVID-19–associated pulmonary aspergillosis: systematic review and meta-analysis. Clin Microbiol Infect **2022**; 28:920–7.
38. Prattes J, Giacobbe DR, Bassetti M, Gangneux JP, Hoenigl M; POSACOVID Study Group. Antifungal prophylaxis of COVID-19 associated pulmonary aspergillosis in ventilated patients: one solution does not fit all. Intensive Care Med **2024**; 50:1375–77.
39. Ashu EE, Hagen F, Chowdhary A, Meis JF, Xu J. Global population genetic analysis of *Aspergillus fumigatus*. mSphere **2017**; 2:e00019-17.
40. Schroeder M, Simon M, Katchanov J, et al. Does galactomannan testing increase diagnostic accuracy for IPA in the ICU? A prospective observational study. Crit Care **2016**; 20:139.
41. Tio SY, Chen SCA, Heath CH, et al. Identifying gaps in the international consensus case definitions for invasive aspergillosis: a review of clinical cases not meeting these definitions. Open Forum Infect Dis **2024**; 11:ofae594.
42. Dichtl K, Forster J, Ormanns S, et al. Comparison of β-D-glucan and galactomannan in serum for detection of invasive aspergillosis: retrospective analysis with focus on early diagnosis. J Fungi (Basel) **2020**; 6:253.
43. Sulahian A, Porcher R, Bergeron A, et al. Use and limits of (1–3)-β-D-glucan assay (Fungitell), compared to galactomannan determination (Platelia *Aspergillus*), for diagnosis of invasive aspergillosis. J Clin Microbiol **2014**; 52:2328–33.
44. Farmakiotis D, Le A, Weiss Z, Ismail N, Kubiak DW, Koo S. False positive bronchoalveolar lavage galactomannan: effect of host and cut-off value. Mycoses **2019**; 62:204–13.
45. Meersseman W, Lagrou K, Maertens J, et al. Galactomannan in bronchoalveolar lavage fluid. Am J Respir Crit Care Med **2008**; 177:27–34.
46. Wang T, Park B, Anderson G, Shaller B, Budvytiene I, Banaei N. Application of diagnostic stewardship to fungal polymerase chain reaction: low yield of follow-up testing on plasma and bronchoalveolar lavage after a negative result. Clin Infect Dis **2024**; 79:944–52.
47. Thompson GR III, Cornely OA, Pappas PG, et al. Invasive aspergillosis as an under-recognized superinfection in COVID-19. Open Forum Infect Dis **2020**; 7:ofaa242.
48. Gill JR, Sheng Z-M, Ely SF, et al. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Arch Pathol Lab Med **2010**; 134:235–43.
49. Salazar F, Bignell E, Brown GD, Cook PC, Warris A. Pathogenesis of respiratory viral and fungal coinfections. Clin Microbiol Rev **2022**; 35:e0009421.
50. Lu LY, Lee HM, Burke A, et al. Prevalence, risk factors, clinical features, and outcome of influenza-associated pulmonary aspergillosis in critically ill patients: a systematic review and meta-analysis. Chest **2024**; 165:540–58.
51. Shiley KT, Lautenbach E, Lee I. The use of antimicrobial agents after diagnosis of viral respiratory tract infections in hospitalized adults: antibiotics or anxiolytics? Infect Control Hosp Epidemiol **2010**; 31:1177–83.
52. Wunderink RG, Srinivasan A, Barie PS, et al. Antibiotic stewardship in the intensive care unit. An official American Thoracic Society workshop report in collaboration with the AACN, CHEST, CDC, and SCCM. Ann Am Thorac Soc **2020**; 17: 531–40.
53. Drummond RA, Desai JV, Ricotta EE, et al. Long-term antibiotic exposure promotes mortality after systemic fungal infection by driving lymphocyte dysfunction and systemic escape of commensal bacteria. Cell Host Microbe **2022**; 30: 1020–33.e6.
54. Racil Z, Kocmanova I, Toskova M, et al. Galactomannan detection in bronchoalveolar lavage fluid for the diagnosis of invasive aspergillosis in patients with hematological diseases—the role of factors affecting assay performance. Int J Infect Dis **2011**; 15:e874–81.
55. White PL, Wingard JR, Bretagne S, et al. Aspergillus polymerase chain reaction: systematic review of evidence for clinical use in comparison with antigen testing. Clin Infect Dis **2015**; 61:1293–303.
56. Guo J, Xiao C, Tian W, et al. Performance of the *Aspergillus* galactomannan lateral flow assay with a digital reader for the diagnosis of invasive aspergillosis: a multicenter study. Eur J Clin Microbiol Infect Dis **2024**; 43:249–57.
57. Theel ES, Doern CD. β-D-glucan testing is important for diagnosis of invasive fungal infections. J Clin Microbiol **2013**; 51:3478–83.
58. Verweij PE, Brüggemann RJM, Azoulay E, et al. Taskforce report on the diagnosis and clinical management of COVID-19 associated pulmonary aspergillosis. Intensive Care Med **2021**; 47:819–34.
59. Markov N, Gao CA, Stoeger T, et al. SCRIPT CarpeDiem dataset: demographics, outcomes, and per-day clinical parameters for critically ill patients with suspected pneumonia. PhysioNet **2023**.