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Rapid Molecular Detection of Airway Pathogens in Lung Transplant Recipients

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Background: Airway infections are difficult to distinguish from acute rejection in lung transplant recipients. Traditional culture techniques take time that may delay treatment. We hypothesized that a rapid multiplex molecular assay could improve time to diagnosis and appropriate clinical decision making.
Methods: In a prospective observational study of recipients undergoing bronchoscopy, we assessed the BioFire® FilmArray® Pneumonia Panel (BFPP) in parallel to standard of care (SOC) diagnostics. Research clinicians performed shadow (research only) clinical decision making in real time. Time to report and interpretation were reported as median and interquartile ranges and compared by Wilcoxon signed-ranked test. Agreement was defined based on detection of any species targeted in the molecular assay.
Results: For the 150 enrolled subjects, BFPP results were available 3.8 hours (IQR 2.8–5.1) following bronchoscopy, compared to 13 hours for viral SOC (IQR 10–34, $P<0.001$) results and 48 hours for bacterial SOC (IQR 46–70, $P<0.001$) results. Positive BFPP were interpreted in 9 hours (IQR 5–20) following bronchoscopy, compared to 74 hours for SOC (IQR 37–110, $P<0.001$). Assays agreed for 138 (92%) of the 150 subjects. Of 22 BFPP diagnoses, 5 (23%) resulted in a shadow antibiotic recommendation. Notable BFPP deficiencies included fungal species and *H. parainfluenzae*, accounting for 15 (27%) and 13 (23%) of the 56 actionable SOC results, respectively.
Conclusions: This molecular diagnostic including bacterial targets has the potential to shorten time to diagnosis and augment current clinical decision making but cannot replace SOC culture methods.
Trial Registration: NCT03933878

Introduction:

Lung transplantation has the potential to improve quantity and quality of life for patients with end-stage lung diseases such as idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF) [1]. However, lung transplant survival outcomes lag all other solid organs [2]. Pulmonary infections are a major issue limiting post-transplant survival [3,4]. Despite the aggressive surveillance and treatments efforts in lung transplant, lung infections are the leading cause of death in the first post-transplant year [5] and a major risk factor for post-transplant complications such as chronic lung allograft dysfunction (CLAD) [6,7]. These infections may be difficult to identify because symptoms of infection post-transplant can be masked by immunosuppression or acclimation to poor lung function pre-transplant. When present, crucial signs and symptoms of infection may overlap with rejection [8]. Despite the acute need to distinguish infection and rejection, diagnosis of bacterial infection and associated antimicrobial sensitivities may require several days using standard techniques.

Molecular assays that detect bacterial organisms by nucleic acid sequences rather than culture have the potential to improve time to diagnosis for airway infections. However, their performance characteristics are unknown in lung transplant recipients, for whom airway bacterial loads have been reported to be significantly higher [9]. Therefore, we examined the speed and accuracy of the BioFire® FilmArray® Pneumonia Panel (BFPP) molecular diagnostic in lung transplant recipients. A previous study in a multicenter, broad

inpatient and outpatient cohort found this panel to have 87% specificity relative to standard of care (SOC) culture results, but that false-positive BFPP results (or false negative cultures) were common [10]. We hypothesized that molecular detection of bacterial and viruses would lead to a faster time to result compared to SOC clinical assays, where only viral molecular testing is performed.

Patients and Methods

Study population

This prospective observational cohort study was approved by the University of California, San Francisco (UCSF) institutional review board under protocol 13-10738 and registered on ClinicalTrials.gov (identifier NCT03933878). The study was performed in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. Lung transplant recipients at UCSF were enrolled who 1) provided written consent for research bronchoalveolar lavage (BAL) collection and 2) had a research BAL sample collected during a scheduled bronchoscopy within the enrollment period April – December 2019. Only the first available BAL sample was included for each enrolled subject to prevent a loss of statistical power from repeat measures within subjects and particularly avoid overrepresentation of chronically colonized patients. BAL samples were excluded if there was incomplete clinical documentation or for technical error running the assay.

Lung transplant recipients received immunosuppression per institutional protocols [11]. Subjects were started on azithromycin 250 mg three times a week for CLAD prophylaxis starting at day 30. Subjects underwent bronchoscopy for surveillance, scheduled at 2, 4, 8, 12, 26, 52, and 78 weeks post-transplant, or for cause. SOC microbial detection was performed in a CLIA-certified microbiology laboratory and included bacterial and fungal culture speciation from the same BAL samples. BAL fluid was cultured on blood, chocolate, and MacConkey agar plates. Cystic fibrosis lung transplant recipient BAL was also cultured on mannitol salt (*Staphylococcus aureus*) and *Burkholderia cepacia* agars. After exclusion of oral flora, species were identified by matrix-assisted laser desorption ionization time of flight (MALDI TOF) mass spectrometry. The NxTAG® Respiratory Pathogen Panel, which is also a molecular diagnostic, was used for SOC viral detection and covers influenza, parainfluenza, and coronavirus (4 strains each), respiratory syncytial virus A&B, rhinovirus, adenovirus, metapneumovirus, bocavirus, *Chlamydophila* and *Mycoplasma pneumoniae* (Luminex Corp, Austin, TX).

Molecular diagnostic assay

Molecular detection was performed using the BioFire® FilmArray® Pneumonia Panel (BFPP, BioFire Diagnostics, Salt Lake City, UT), which assesses a BAL sample for 26 lower airway pathogens, as well as select antibiotic resistance genes, using a multiplex polymerase chain reaction (PCR)-based technology: *Acinetobacter* complex, *E. cloacae, E. coli, H. influenzae, K. aerogenes, K. oxytoca, K. pneumoniae, M. catarrhalis, Proteus spp., P. aeruginosa, S. marascens, S. aureus, S. agalactiae, S. pneumoniae, S. pyogenes, C. pneumoniae, L. pneumophila, M. pneumoniae*, Adenovirus, Coronavirus, Metapneumovirus, Rhinovirus/Enterovirus, Influenza A, Influenza B, Parainfluenza, and RSV. BAL samples were stored at 4° C and processed through the BFPP system as soon as possible. After cleansing the workspace, the BFPP pouch was placed in the loading station. A manufacturer-supplied hydration vial was inserted into the appropriate well. A swab was placed in the BAL fluid and added to the sample injection vial containing sample buffer, before adding to the pouch via the loading station. The pouch was then inserted into the BioFire PN Panel unit and automated processing was initiated. Endpoint melting curve data was analyzed within the FilmArray's internal software to determine the result for each target. For discrepant results, BioFire product specialists reviewed run files, manufacturing, quality control, and other internal records and no system malfunctions were identified. BFPP were not reported to clinicians in the SOC arm.

Outcome measures

The primary outcome was the difference in time to report for SOC and BFPP assays. Clinical SOC reporting time was abstracted from electronic medical records (EMR) for bacterial cultures and viral PCRs. BFPP assay report times, which were

80 minutes after run start times, were abstracted from the FilmArray device.

The secondary outcomes were 1) differences in time to clinical interpretation, 2) agreement between assays, and 3) differences in clinical management decisions based on results. Clinical SOC management decisions for SOC cultures were determined from EMR review. New onset, acute symptoms of fever, cough, dyspnea, fatigue, and flu-like symptoms were assessed based on review of provider notes in the most recent clinic visit and just prior to bronchoscopy. Clinicians indicated their review of follow up results directly in the EMR, and these times were abstracted. A pulmonary and critical care board-certified physician (DRC or JRG), referred to as a shadow clinician, was assigned to review the BFPP diagnostic results and clinical symptoms. The shadow clinician provided a management recommendation that was recorded by research staff with the time of receipt. Shadow decision making was not reported to treating clinicians and did not influence patient care.

Sample size and statistical analysis

The target recipient enrollment was 150 subjects, which was estimated to provide 94% power to identify a time to a clinically relevant difference of at least 48 hours between the SOC and BFPP pathogen diagnosis methods with an alpha of 0.05. This estimate was based on Monti-Carlo simulation using the infection incidence from the prior year of 15%, which is equivalent to 22 positive BFPP results.

Two-tails chi-squared test with Yates correction was used to determine if significant differences existed between the enrolled and excluded subjects. Time to detection results and clinical recommendation for the SOC and BFPP diagnostic methods were compared using Wilcoxon signed-ranked tests, with Holm adjustment for multiple comparisons. Agreement in organism detection between clinical SOC methods and the BFPP method was determined using Gwet's AC1 statistic. Gwet's AC1 statistic was used because it is more robust to skewed data as compared with Cohen's kappa statistic [12]. Statistical Analyses were performed in R (version 3.5.3, The R Foundation for Statistical Computing, Vienna, Austria) using the "stats," "irrCAC," "dplyr," "ggpubr," and "reshape2" packages.

Table 1: Characteristics of study participants

Total subjects	150
Age at BAL, median (IQR)	62 (54 – 67)
Gender, N (%)	
Male	90 (60)
Female	60 (40)
Recipient Ethnicity, N (%)	
White	96 (64)
Hispanic	26 (17)
Asian	15 (10)
Black	9 (6)
Other	4 (3)
Transplant Diagnosis Group, N (%)	
A-Obstructive	21 (14)
B-Pulmonary Vascular	6 (4)
C-Cystic Fibrosis	9 (6)
D-Restrictive	114 (76)
Transplant type, N (%)	
Double	136 (91)
Single	14 (9)
Months post-transplant median (IQR)	12.1 (2 – 25)

Results

Study Population

Of UCSF lung transplant recipients, 97% consented for BAL and medical records collection. Of the 177 eligible subjects, 27 were excluded for one of the following reasons (Supplemental Figure 1): a research bronchoscopy sample was not collected (N=22), SOC result review times were not documented in EMR (N=3), or there was a research sample processing error (N=2). The baseline characteristics of the 150 included subjects, each contributing one BAL sample, are shown in Table 1. The median time post-transplant was 1 year (IQR 2 – 25 months).

Of included subjects, 68% were undergoing routine surveillance bronchoscopy (Table S1). Twenty-five percent of subjects reported acute symptoms, and 14% required clinical follow-up for rejection or infection. The most common symptom at the time of bronchoscopy was cough (19%), followed by dyspnea (13%), fatigue (5%), flu-like symptoms

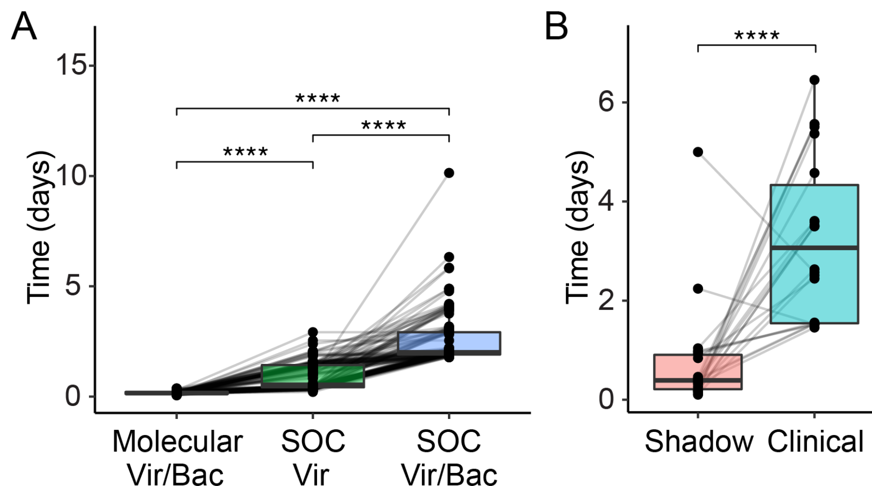

Figure 1: Time to diagnostic result and interpretation. (A) Time to report for the molecular assay for viral (vir) and bacterial (bac) targets, standard of care (SOC) viral results, and SOC viral and bacterial results. Time to report in days are shown as median and interquartile ranges and compared by Kruskal-Wallis test. **(B)** Time to interpretation in days by the shadow clinicians using the BFPP results and the clinical recommendations using SOC results. These results were reported as median and interquartile ranges and compared by the Wilcoxon signed-ranked test.

(2%), and fever (0.5%). The 27 excluded subjects were not substantially different from the 150 enrolled subjects, although there was a statistically significant increase in the number of excluded subjects who received a short course of prednisone for viral infection based on bronchoscopy results (P=0.03, unadjusted for multiple comparisons, See Table S1).

Comparison of Time to Result and Clinical Decision

For the primary endpoint, BFPP results were available 3.8 hours (IQR 2.8–5.1) following bronchoscopy, compared to 13 hours for viral SOC (IQR 10–34, P <0.001) results and 48 hours for bacterial SOC (IQR 46–70, P <0.001) results (Figure 1A). Positive BFPP results were interpreted in 9 hours (IQR 5–20) following bronchoscopy, compared to 74 hours for SOC (IQR 37–110, P <0.001) (Figure 1B).

Agreement between BFPP and Standard of Care Assays

There was high agreement between assays with a Gwet's AC1 of 0.98 (95% CI 0.95 – 1.0, *P* <0.001) for bacteria and 0.92 (95% CI 0.87 – 0.97, *P* <0.001) for viruses. Excluding subjects undergoing surveillance, there was perfect agreement for bacteria (AC1 = 1, *P* = 0), but only 0.84 (95% CI 0.70 – 0.98, *P* <0.001) agreement for viruses. Most patients had 0 or 1 positive BFPP result, while one patient had 2 positive results and another patient had 3 concurrent positive results (Figure 2A). In sum, there were 22 subjects with positive BFPP results and 18 subjects with positive SOC results, limited to species on the BFPP assay. Considering SOC as the reference, the BFPP assay had a sensitivity of 94%, specificity of 78%, positive predictive value of 97%, negative predictive value of 64%.

The most common diagnosis of the BFPP assay was Human Rhinovirus 1A for which 9 cases were identified by both BFPP and SOC testing, while 2 cases were detected by BFPP only and 1 by SOC only (Figure 2B, Table S2). One case of parainfluenza virus was detected by both assays and 1 case was detected by BFPP assay only, although this patient did have a SOC diagnosis of parainfluenza diagnosis from the month prior that was no longer detected in SOC assays. One case of coronavirus NL63 was also detected by the BFPP assay only. One case of metapneumovirus was detected by both assays, while another was detected by the BFPP assay only.

The most common bacterial pathogen detected was *S. aureus*, with 3 cases confirmed by both assays and 1 diagnosis made by SOC only. Of these *S. aureus* cases, 2 cases of methicillin-resistance were detected based on the presence of mecA/C and MREJ resistance genes detected by BFPP assay. SOC methods confirmed methicillin-resistant *S. aureus* in these 2 cases, and methicillin-resistant *S. aureus* was also detected in an additional case detected by SOC only (Table S2). *P. aeruginosa* was the second most common diagnosis with 2 diagnoses confirmed by both assays and 1 diagnosis made by SOC only (Figure 2B, Table S2). One case of *S. pneumoniae* and 1 case of *K. oxytoca* were made by SOC only (Figure 2B, Table S2).

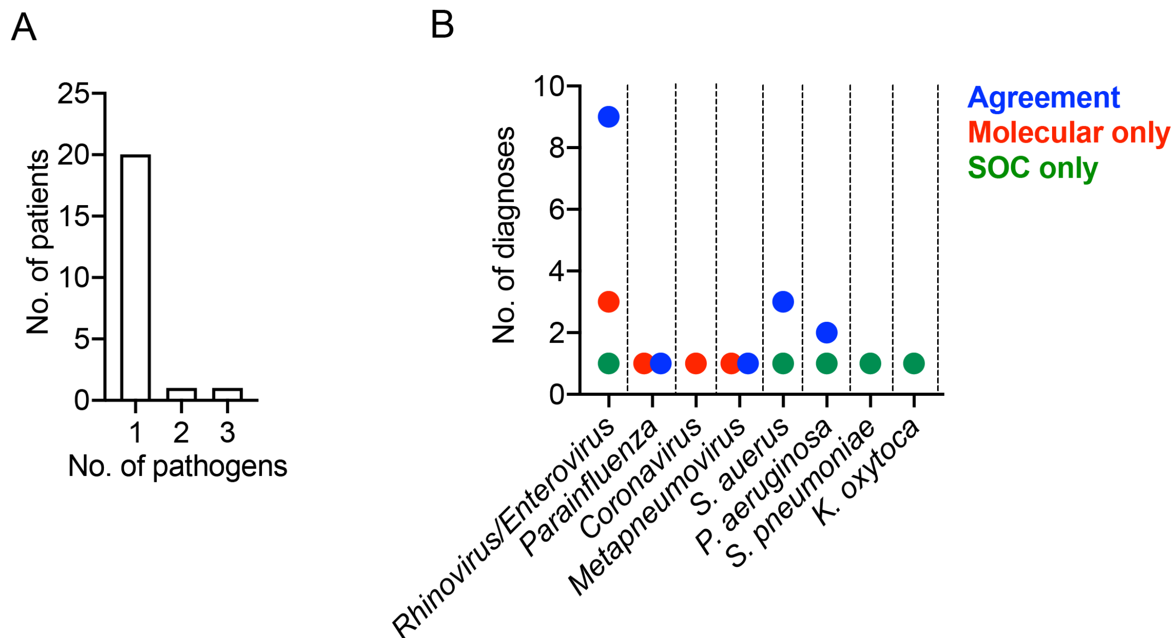

Figure 2: Distribution of diagnoses by BFPP and SOC diagnostics, limited to the 26 BFPP targets. (A) Number of subjects with 1, 2, or 3 positive BFPP diagnostic results. **(B)** Number of diagnoses of the 8 pathogens detected in subjects limited to the BFPP targets. Diagnoses made by both the BFPP and SOC assays are shown in blue, diagnoses made by molecular assay are shown in red, and diagnoses made by the SOC assays only are shown in green.

Agreement between Clinical Recommendations

Of the 33 BFPP targets, 3 cases of *S. aureus* and 2 cases of *P. aeruginosa* were diagnosed by both methods and prompted recommendations for antibiotics from both shadow and treating clinicians (Figures 2 & 3). In one patient, a BFPP diagnosis of Human Rhinovirus 1A prompted a shadow recommendation of a short course of prednisone, but this virus was not detected by SOC. Instead, *Microascus expansus* was found by SOC cultures leading to anti-fungal treatment initiation (Figure 3). Of the 22 subjects with positive BFPP diagnostic results, 16 (72%) had clinical symptoms at the time of bronchoscopy (Figure 3). Limited to the pathogens on the BFPP assay, there were 18 subjects with positive SOC results, of whom 11 (61%) had symptoms.

To understand the potential impact of the BFPP diagnoses on patient care, we examined the 5 patients whose care would have been altered by the BFPP diagnostics and shadow clinical recommendations (Table 2). Of the 5 patients with bacterial infection BFPP diagnoses, 4 of them would have received appropriate antibiotic treatment 4–7 days earlier than with the SOC diagnostics and the other patient had a logistic delay in BFPP results.

Limitations of the BFPP in Lung Transplant recipients

SOC diagnostics led to 56 actionable diagnoses, including 15 cases of initiation of anti-fungal regimens, 21 instances of initiation of antibiotics, and 8 cases with multiple treatment strategies (Figures 4 & 5). Of the patients with SOC diagnoses not included in this BFPP assay, 34 had clinical symptoms at the time of bronchoscopy. 23 of the patients treated for SOC diagnoses not included in the Pneumonia Panel had symptoms while 14 did not. This contrasts with the 7 SOC actionable diagnoses when limited to species present on the BFPP assay (Figures 2 & 3 and Table S1). Notable BFPP assay deficiencies included fungal pathogens and *H. parainfluenzae*, accounting for 15 (27%) and 13 (23%) of the 56 actionable SOC results, respectively (Figure 4, 5).

Discussion

This prospective study assessed the performance characteristics of a molecular diagnostic for bacterial and viral pathogens in a cohort of 150 lung

Table 2: Details of 5 cases in which the molecular diagnostic technique identified a bacterial pathogen.

Case Presentation	SOC Studies	SOC Management	Molecular Studies	Management difference
55 yo man 2 years s/p lung transplant for cystic fibrosis undergoing surveillance bronchoscopy, but with cough, congestion and sputum production. His FEV1 was down 210 ml (5%). CT showed an elliptical opacity within the lingula with adjacent ground glass opacities.	Numerous multiple resistant *S. aureus* (BAL and bronchial wash), Few *Pseudomonas aeruginosa* (multiple resistance), Rare *Aspergillus fumigatus* in bronchial wash	Developed fever 1 day after bronchoscopy and was started on doxycycline. After SOC studies, started Cefuroxine 500 mg BID x 14 days, inhaled tobramycin for 3 alternating month cycles, and Posaconazole 300 mg daily for 3 months, decrease Tacrolimus.	*S. aureus* mecA/C and MREJ, frozen and thawed resulting 6 days after bronchoscopy. Shadow recommendation was gram positive antibiotic treatment.	Shadow clinicians and SOC both treated for *MRSA*. SOC also included treatment for gram negative organisms, fungal organisms and resulted in a decrease in immunosuppression.
43 yo man 12 years s/p bilateral lung transplant for pulmonary hypertension with CLAD on photopheresis underwent bronchoscopy indicated for suspected infection. Reported shortness of breath and congestion. His FEV_1 was down 70 ml (6%) and CT showed diffuse bronchiectasis and centrilobular disease and nodules and ground glass opacities of consolidation.	Parainfluenza 3, Rhinovirus, *Lichtheimia*, *Pseudomonas aeruginosa*, Oronasal flora, *Mycobacterium abscessus complex*	No treatment for viral infections. 6 days after bronchoscopy started on levofloxacin 750mg 1x daily x10 days for *Pseudomonas*, started inhaled amphotericin B for fungal infection.	*Pseudomonas aeruginosa*, Parainfluenzae	Shadow clinicians recommended gram negative antibiotic coverage 30 min after results compared to 6 days. Clinical management also started on anti-fungal regimen.
62 yo man 8 years s/p bilateral lung transplant for idiopathic pulmonary fibrosis underwent bronchoscopy indicated for suspected infection. Reported 2 weeks of new exertional dyspnea and non-productive cough. His FEV_1 was down 180 ml (5%) and CT showed new scattered ground glass opacities throughout both lungs.	*Penicillium*, Rare *aspergillus spp* resembling versicolor, Numerous Nafcillin resistant *S. aureus*	7 days after bronchoscopy start Doxycycline 100mg bid x10days and started inhaled amphotericin B for penicillium and *aspergillus* 19 days after bronchoscopy	*S. aureus* mecA/C and MREJ	Shadow clinicians recommended gram positive coverage 1 hour after results compared to 7 days. Clinical management also started on anti-fungal regimen.

50 yo woman 9 years s/p bilateral lung transplant for cystic fibrosis underwent bronchoscopy indicated for decreasing spirometry concerning for rejection. Reported fatigue and intermittent chest pain. Her FEV_1 was down 150 ml (8%) and CT showed no changes.	*Pseudomonas aeruginosa*, Oronasal flora	5 days after bronchoscopy ciprofl oxacin 500 mg BID for 10 days.	*Pseudomonas aeruginosa*	Shadow clinicians recommended gram negative coverage 1 hour after results compared to 5 days.
32 yo woman 5 years s/p bilateral lung transplant for cystic fibrosis underwent bronchoscopy indicated for suspected infection. Reported a feeling of reduced lung capacity and upper respiratory symptoms. Her FEV_1 was down 260 ml (8%) and CT showed new foci of nodular consolidation and increased right pleural effusion.	*S. auerus*, Oronasal flora	6 days after bronchoscopy Cephalexin 500 mg BID for 10 days	*S. aureus*	Shadow clinicians recommended dicloxacillin treatment 2 hours after results compared to Cephalexin treatment 6 days after bronchoscopy.

Abbreviations: BAL, bronchoalveolar lavage; CT, computed tomography; s/p, status post; SOC, standard of care; yo, years old

transplant recipients. Despite concern for a high false positivity rate given the high bacterial loads in transplant recipients identified by 16S ribosomal sequencing [9], BFPP assay results closely matched SOC studies for the species included in the panel. Indeed, there were no observations of false positive BFPP assay results by comparison to clinical symptoms or culture. In 3% of cases, the BFPP assay led to recommendations for antibiotics, for which the observed decrease in median time to clinical interpretation of 59 hours would likely be clinically meaningful. However, in some cases clinically important pathogens were detected by culture but not by the BFPP assay. Also, some important microbial pathogens were not represented on this panel, most notably *H. parainfluenzae* and fungal pathogens, which accounted for 45% of the standard of care actionable results. Thus, while the inclusion of bacterial molecular diagnostics could have meaningful advantages for select patients, this technology cannot replace SOC culture techniques.

The high rates of infection of about 25% of subjects were consistent with prior data. For example, a study of surveillance bronchoscopy found clinically significant infection in 17% of the cohort [13]. Lung allograft recipients are at high risk for post-operative pulmonary infections, manifesting as tracheobronchitis or pneumonia. In addition to being immunosuppressed, lung transplant recipients have impaired mucociliary clearance and denervation of the lung allograft resulting in impaired cough reflex [14]. Single lung transplant recipients may also be at risk of infection spread from the native lung, IPF recipients appear at particular risk of aspiration [15], and CF lung transplants are at particular risk of recolonization from the untransplanted airway [16].

In contrast to findings outside the transplant setting, we identified more bacterial pathogens by

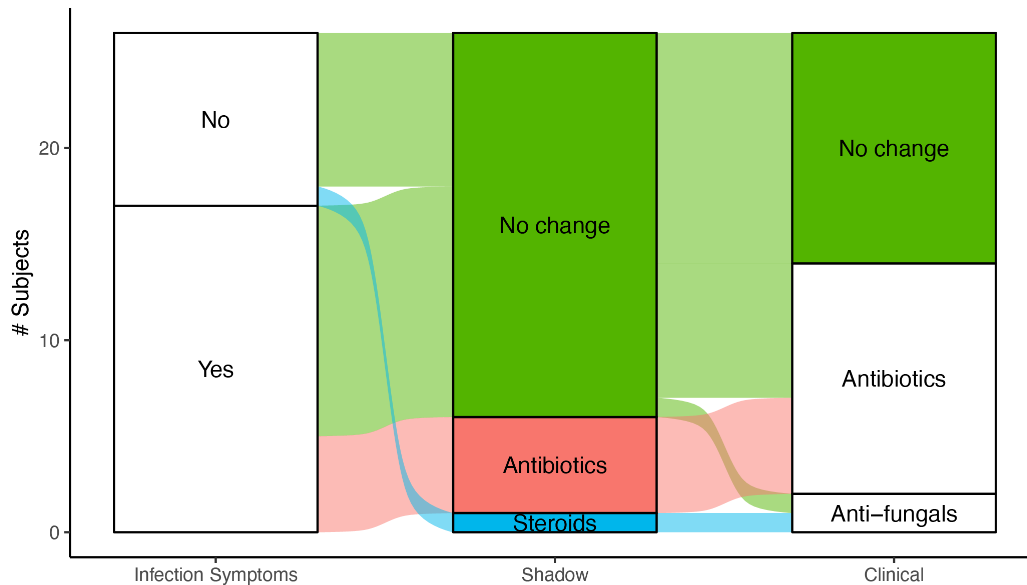

Figure 3: Shadow clinician treatment recommendations in relation to infectious systems and real-world clinical decision making. An alluvial plot shows treatment recommendations by shadow physicians (center column) and the actively-treating clinical physicians (right column) for the 26 patients that had BFPP or SOC results indicating a species included in the BFPP assay. Research clinicians' shadow recommendations are grouped as no change (green), antibiotics targeting bacterial infections (red), or steroids for viral infections (blue). These recommendations were not reported to treating providers. Treatment recommendations are also shown in relation to the patient's presentation with infectious symptoms at the time of bronchoscopy (left column). All shadow recommendations for antibiotics were in patients with symptoms and were corroborated by clinical decision making.

SOC assays compared to the BFPP test[17]. This finding is surprising as molecular assays can detect pathogen nucleotide sequences even from non-viable pathogens. One might have expected an increased detection rate in transplant patients given the decreased symptom burden, increased total microbial loads, and use of routine macrolide prophylaxis. One possible explanation is that differences in microbiome composition might result in a relative decrease in transcripts for BFPP targets because of competition from commensal flora. Of note, the BFPP software does not normalize for total microbial counts. Since many of these discrepant infections were not associated with symptoms, it is hard to determine which assay is more clinically relevant.

Rhinovirus was the most common pathogen detected in BAL by BFPP and SOC assays. There is seasonality to respiratory pathogens, so this finding may have even more pronounced if this study had extended through the North American winter season. Given that Rhinovirus can infect the lower respiratory tract of lung transplant recipients and may be present in patients with CLAD, it may be an important pathogen for clinical care[18,19]. At the same time, Rhinoviral infection is associated with less lung function decline compared to other community acquired respiratory viral infections [20]. Additionally, rapid diagnosis of a Rhinovirus in the setting of acute symptoms could limit unnecessary empiric treatment for bacterial infection.

The most notable deficiencies in the BFPP assay included gram negative rods, *H. parainfluenzae*, and fungal species, most notably *Asperigillus*. There were 13 diagnoses of *H. parainfluenzae* made by SOC assay, all of which were treated with antibiotics. The frequency and outcomes of *H. parainfluenzae* infections in the lower respiratory tract of lung transplant recipients is unknown. One study examining *H. influenzae* infection in lung

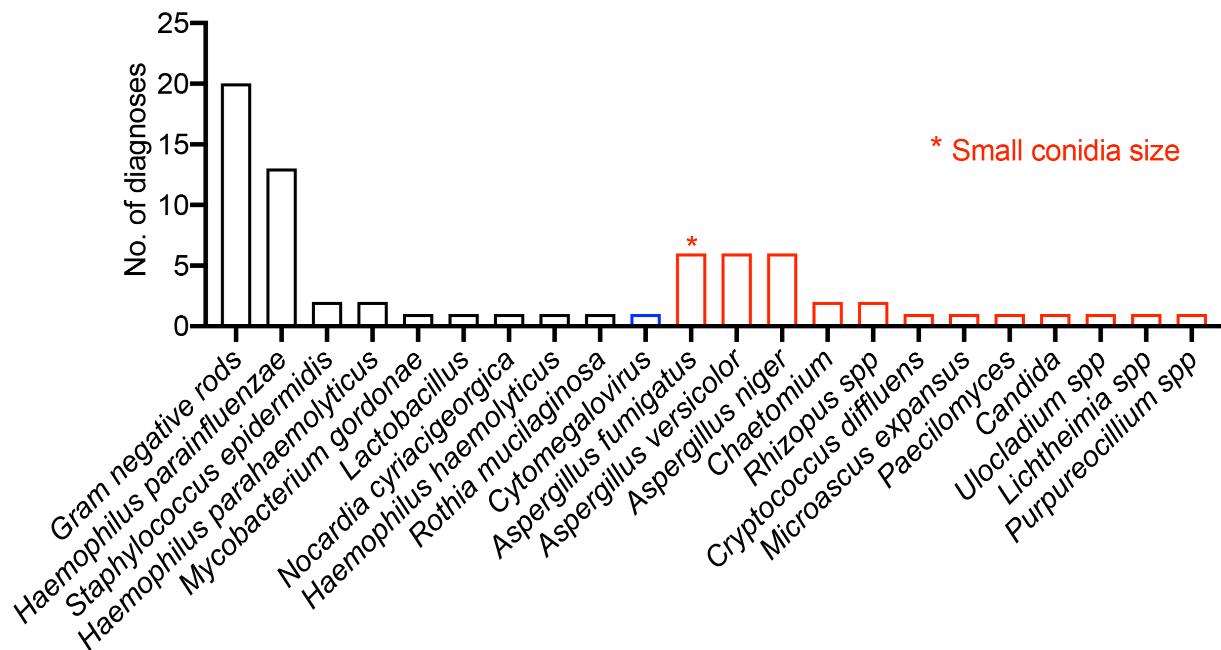

Figure 4: Distribution of diagnoses made by SOC diagnostics of pathogens not present in the BFPP panel. Number of diagnoses made by the SOC method excluding the pathogens that were included in the 26 pathogens targeted by this BFPP diagnostic panel. Bacterial pathogens shown in black, viral pathogens in blue, and fungal pathogens in red. Aspergillus fumigatus is shown with an asterisk to indicate a small conidia size. The 20 cases classified as Gram negative rods were not further speciated.

explants from a study of 49 lung transplant recipients did not detect *H. parainfluenzae*, suggesting that a high frequency of infection in the lower respiratory tract may not be common in other lung transplant cohorts [21]. Our local microbiology data show statistically significant increases in *H. parainfluenza* rates over the past six years (data not shown). Whether this increase is a result of azithromycin prophylaxis is unknown [22]. Immune responses to *H. parainfluenzae* in patients with chronic obstructive lung disease suggest that it could be pathogenic in the transplant setting as well [23]. *Aspergillus spp*. accounted for 21 SOC diagnoses, and some *Aspergillus spp.* have been linked to mortality risk in lung transplant recipients [24,25]. While molecular diagnostics have been developed for fungal pathogens, extracting nucleic acids through fungal cell walls and excluding fungal contamination in reagents can be challenging [26]. Of note, revised guidelines for invasive fungal disease incorporate fungal PCR testing [27]. A panel designed for the lung transplant setting would ideally detect *H. parainfluenzae* and fungal pathogens. Future technologies could capitalize on host molecular signatures to detect rejection, infection, or co-infections [28,29].

This study has several important limitations as a prospective observational trial: It should be noted that the SOC viral assay was also a molecular diagnostic. The NxTAG viral assay requires two steps and is thus slightly slower, while the BioFire assay is more amenable to point-of-care use. Achieving the observed decrease in time to viral result in practice would largely depend on decreased sample handling time though a point of care protocol. Nonetheless, much of the observed decrease in time to result for the viral assays likely reflects differences between research and clinical lab settings that would not be realized in practice or substantially impact clinical care. While there were significant differences in time to detection and clinical interpretation for some bacterial pathogens, it is unclear if the rapid turnaround would have improved long term outcomes. The shadow clinicians' rapid decision making also represented an idealized clinical scenario where abnormal

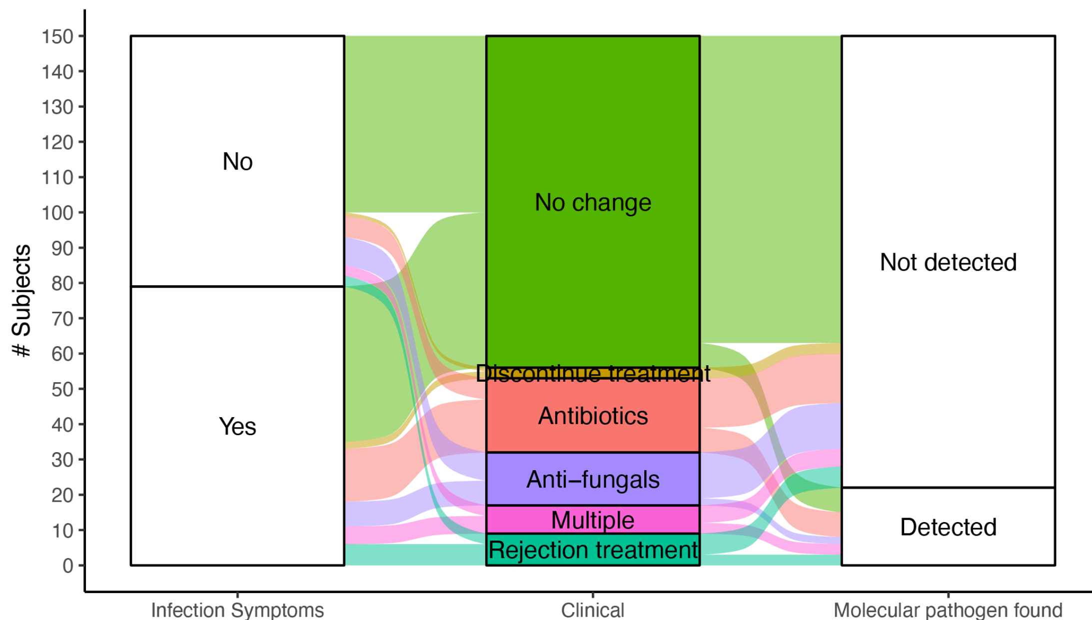

Figure 5: Distribution of clinical treatment recommendations in relationship to infectious systems and BFPP pathogen detection. An alluvial plot shows clinical treatment recommendations for the included 150 subjects. Treatment recommendations are shown in relation to the patient's presentation with infectious symptoms at the time of bronchoscopy and whether a pathogen was detected by the BFPP diagnostic.

microbial results were immediately communicated and reviewed. There are legitimate reasons that might diminish differences between these groups, such as the inclusion of trainees in medical decision making or batch review of study results. The study is also limited to a single center, and the results may be less applicable at centers with different flora, SOC lab approaches, or antimicrobial protocols. Additionally, it is difficult to compare accuracies for less common pathogens. Contamination during bronchoscopy might affect both SOC and BFPP results, although care was taken to avoid suctioning prior to lavage when possible. While we assessed the association of microbial test result with clinical symptoms, it remains difficult to differentiate infection from colonization in many instances. Reducing unnecessary antibiotic exposure with a molecular assay would be a challenge, since with current technology, bacterial culture results would be needed to exclude co-infection. In this study, there was only one case where antibiotics were started prior to culture results (see Table 2).

This study demonstrates the potential utility and limitations of rapid molecular diagnostics for lower respiratory tract infections in lung transplant recipients undergoing bronchoscopies. While this BFPP assay may shorten time to diagnosis and treatment recommendations for several viral and bacterial targets, it cannot replace SOC diagnostics. However, the BFPP assay may be able to improve care for a subset of lung transplant recipients for whom rapid identification of bacterial infections is most critical.

Keywords: Bacterial infection, Pneumonia, Tracheobronchitis, Lung Transplant, BioFire, Molecular Diagnostics

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Author contributions: DRC, JK, and JRC designed the study and obtained funding. SRH, JPS, DRC, and JK contributed to subject enrollment and sample collection protocols. JH, JC,

and FD collected and processed study samples. JRG and DRC functioned as shadow clinicians. EA performed additional chart review. JH, MAM, FD, and JRG performed the analyses. MAM and JRG drafted the manuscript. All authors reviewed and edited the manuscript.

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Conflicts of Interest: BioFire Diagnostics provided funding for the study, met with investigators, reviewed, and provided feedback on the study protocol and manuscript. The funder did not have control over publication decisions or manuscript content. The authors have no other relevant conflicts of interest to disclose.

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