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Clinical decision making is improved by BioFire Pneumonia Plus in suspected lower respiratory tract infection after lung transplantation: Results of the prospective DBATE-IT* study

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*DNA-based testing of BAL in suspected lower respiratory tract infection after lung transplantation.

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Abstract

Background: Lower respiratory tract infections (LRTIs) are a significant cause of morbidity and mortality in lung transplant (LTx) recipients. Timely and precise pathogen detection is vital to successful treatment. Multiplex PCR kits with short turnover times like the BioFire Pneumonia Plus (BFPPp) (manufactured by bioMérieux) may be a valuable addition to conventional tests.

Methods: We performed a prospective observational cohort study in 60 LTx recipients with suspected LRTI. All patients received BFPPp testing of bronchoalveolar lavage fluid in addition to conventional tests including microbiological cultures and conventional diagnostics for respiratory viruses. Primary outcome was time-to-test-result; secondary outcomes included time-to-clinical-decision and BFPPp test accuracy compared to conventional tests.

Results: BFPPp provided results faster than conventional tests (2.3 h [2–2.8] vs. 23.4 h [21–62], $p < 0.001$), allowing for faster clinical decisions (2.8 [2.2–44] vs. virology 28.1 h [23.1–70.6] and microbiology 32.6 h [4.6–70.9], both $p < 0.001$). Based on all available diagnostic modalities, 26 (43%) patients were diagnosed with viral LRTI, nine (15 %) with non-viral LRTI, and five (8 %) with combined viral and non-viral LRTI. These diagnoses were established by BFPPp in 92%, 78%, and 100%, respectively. The remaining 20 patients (33 %) received a diagnosis other than LRTI. Preliminary therapies based on BFPPp results were upheld in 90% of cases. There were six treatment modifications based on pathogen-isolation by conventional testing missed by BFPPp, including three due to fungal pathogens not covered by the BFPPp.

Conclusion: BFPPp offered faster test results compared to conventional tests with good concordance. The absence of fungal pathogens from the panel is a potential weakness in a severely immunosuppressed population.

Abbreviations: BAL, bronchoalveolar lavage; BFPP, BioFire Pneumonia panel multiplex PCR; BFPPp, BioFire Pneumonia Plus panel multiplex PCR; CLAD, chronic lung allograft dysfunction; CRP, C-reactive protein; FEV1, forced expiratory volume in 1 second; hMPV, human Metapneumovirus; IFT, immunofluorescence test; LRTI, lower respiratory tract infection; LTx, lung transplantation; PCR, polymerase chain reaction; RSV, respiratory syncytial virus

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KEYWORDS
bronchoalveolar lavage, lower respiratory tract infection, lung transplantation, microbiology, multiplex polymerase chain reaction, point of care systems

1 | INTRODUCTION

Since the first lung transplantation (LTx) in 1963,[1] LTx has become a widespread therapy in end-stage lung disease, and long-term outcomes have improved.[2] Infections, particularly lower respiratory tract infections (LRTI), remain a major cause of morbidity and mortality after LTx.[3] Opportunistic bacterial and fungal pathogens are common and are often inadequately covered by empiric antibiotic regimens.[4,5] Furthermore, the course of viral LRTI is more severe in transplant recipients and increases the risk of chronic lung allograft dysfunction (CLAD), the main driver of post-LTx mortality.[6–8] Therefore, timely and accurate pathogen identification is of vital importance in suspected LRTI in LTx patients.

Bronchoscopy with bronchoalveolar lavage (BAL) is a key procedure in the work-up of suspected LRTI after LTx.[9,10] For the majority of bacteria and fungi, microbiological culture from BAL remains the gold standard for diagnosis. Cultures are limited by long turnaround time and decreased sensitivity in patients that have received empiric anti-infective therapy.[11] In hard-to-culture and slow growing microorganisms like atypical bacteria, mycobacteria, and certain fungi, molecular methods of pathogen detection like polymerase chain reaction (PCR) are a valuable diagnostic tool.[12–15] PCR-based tests have become the method of choice in viral pathogen detection in addition to immune-fluorescence testing (IFT).[3] Short turn-over multiplex PCR with a pre-defined group of pathogens allows for swift detection of both viral and bacterial pathogens and is suitable for point of care testing.[16] One such test system is the BioFire Pneumonia Plus panel (BFPPp), manufactured by bioMérieux.[17] BFPPp was demonstrated to be accurate for pathogen detection in both individual samples and contrived aggregate samples simulating a polymicrobial infection.[17] Recent studies demonstrate that BFPPp and the earlier BioFire Pneumonia panel (BFPP) perform well in both sputum and BAL samples in a variety of settings outside the context of LTx.[16–30] Furthermore, the older BFPP was recently shown to allow for faster clinical decisions while retaining good overall concordance with conventional diagnostics in LTx recipients.[31] Notably, the majority of patients in this study underwent bronchoscopy for routine surveillance, not due to suspected LRTI.[31] We therefore conducted a prospective study comparing the expanded BFPPp to conventional testing in LTx patients with suspected LRTI to evaluate its potential benefits in improving the time to pathogen detection and clinical decision making.

2 | METHODS

2.1 | Study design

Between October 2019 and August 2020, 60 LTx recipients with suspected LRTI in the LTx outpatient clinic of a large university hospital were included in an open-label longitudinal, observational cohort study. The time to clinical decision and test accuracy of the BFPPp BioFire “Pneumonia Plus” panel (bioMérieux, France, see Table 1) were compared to routine microbiological (culture-based) and virological (IFT and/or PCR) diagnostic tests. The study was approved by the internal Ethics Review Board of Hannover Medical School (#8690_MPG_23b_2019) and registered in the German Clinical Trials Register (DRKS00019225). All patients provided written informed consent.

2.2 | Eligibility criteria

All LTx recipients with suspected LRTI presenting to the outpatient clinic were eligible. Suspicion of LRTI was defined as at least two of six of the following criteria: new or progressive pulmonary opacities, hypoxemia (SpO_2 or $SaO_2 < 92\%$ or need for oxygen), temperature of 38°C or above within the last 7 days, new-onset or progressive productive cough, reduction of forced expiratory volume in 1 second (FEV_1) by 10% or more compared to last outpatient spirometry measurement and C-reactive protein (CRP) of ≥5 mg/l.[32] Patients were included consecutively. Patients unwilling to participate or unable to undergo bronchoscopy with BAL were ineligible for study participation.

2.3 | Study procedure

Following study inclusion, patients underwent bronchoscopy including BAL with a total volume of 100 ml of normal saline, as previously described.[33] Lavage was performed in the lingula or middle lobe or targeted to opacities on chest x-ray. BAL fluid was pooled and examined in parallel by BFPPp, conventional microbiological culture, and conventional virological testing using IFT ± PCR if IFT returned negative but clinical suspicion of a viral LRTI persisted. In select cases with high clinical suspicion of viral LRTI, conventional virological PCR was used as the initial conventional virological test in first line. Test results were immediately sent to the LTx outpatient clinic. As per our standard clinical procedure, transbronchial biopsy to rule out acute allograft rejection was performed if endobronchial findings were incompatible with infection.

Digital time stamps were set in our clinical database (FileMaker Pro; Claris International Inc, Santa Clara, CA, USA) upon sample retrieval as well as arrival of each diagnostic result, as well as upon clinical decision based on each result. If the first diagnostic results did not reveal a relevant pathogen, the clinical decision was deferred until the all LRTI-specific test results became available, or an alternative clinical diagnosis was made.

TABLE 1 BFPPp (BioFire Pneumonia Plus) pathogen spectrum according to manufacturer

Bacteria (semi-quantitative)	Virus (qualitative)	Atypical Bacteria (qualitative)	Antibiotic resistance genes
Acinetobacter calcoaceticus-baumannii complex	Influenza A	*Legionella pneumophila*	**Carbapenemases**
Enterobacter cloacae	Influenza B	Mycoplasma pneumoniae	KPC
Escherichia coli	Adenovirus	Chlamydia pneumoniae	NDM
Haemophilus influenzae	Coronavirus		Oxa48-like
Klebsiella aerogenes	Parainfluenza virus		VIM
Klebsiella oxytoca	Respiratory syncytial virus		IMP
Klebsiella pneumoniae group	Human rhinovirus/enterovirus		**ESBL**
Moraxella catarrhalis	Human metapneumovirus		CTX-M
Proteus spp.	Middle East Respiratory Syndrome Coronavirus		**Methicilin resistance**
Pseudomonas aeruginosa			mecA/mecC and MREJ
Serratia marcescens			
Staphylococcus aureus			
Streptococcus agalactiae			
Streptococcus pneumoniae			
Streptococcus pyogenes			

Abbreviations: CTX-M, cefotaximase-Munich; IMP, imipenemase metallo-beta-lactamase; KPC, Klebsiella pneumoniae carbapenemase; mecA/mecC, resistance genes encoding the penicillin-binding protein 2A; MREJ, mec right extremity junction; NDM, New Delhi metallo-β-lactamase; Oxa48-like, oxacillinase-48-like carbapenemase; VIM, Verona integron-encoded metallo-β -lactamase.

2.4 | BioFire Pneumonia Plus PCR-assay

Information on the BFPPp is available at the manufacturer's website (https://www.biomerieux-diagnostics.com/biofire-filmarray-pneumonia-panel; accessed on OCT-28-2020). Panel coverage is summarized in Table 1. BFPPp analysis time was 70 min.

2.5 | Conventional diagnostics

As per our standard protocol, BAL samples were initially assessed by antigen testing for common viral causes of LRTI using IFT. In IFT-negative patients, conventional viral multiplex PCR testing as outlined below was added in case of clinical suspicion for viral LRTI. Analysis time of IFT was 2–3 h, with some variability as parts of the method require manual steps. In selected patients with strong suspicion of a viral LRTI due to clinical presentation and endobronchial findings, conventional viral multiplex PCR was performed without prior IFT (as per clinician's judgement).

The conventional virology IFT and PCR panels both included adenovirus, human metapneumovirus (hMPV), influenzavirus type A and B, parainfluenzavirus type 1–4, and respiratory syncytial virus (RSV). Conventional virology PCR additionally included herpes simplex virus and rhinovirus (RV). Real-time PCRs were performed on the ABI 7500 system (ABI) for influenzavirus type A and B and adenovirus utilizing previously described laboratory developed tests[34,35] or the R-Gene multiplex PCR kits for detection of RSV/hMPV (RSV/hMPV R-GENE), RV (Rhino and EV/Cc R-GENE bioMérieux), Coronavirus and Parainfluenza (HCoV/HParaflu R-GENE, bioMérieux). Conventional viral PCR required 4 h. Coronavirus (subtypes NL63, 229E, HKU1 and OC43) was part of the conventional PCR panel until March 2020. SARS-CoV-2 PCR testing was performed based on clinical suspicion during the last week of the study during the pandemic. Antigen testing for phosphoprotein 65 in purified blood leukocytes was performed in all patients to check for cytomegalovirus infection.

For microbiological testing, we used direct microscopy and quantitative culture techniques. Direct microscopy was performed using BAL Gram and Auramin stain. Standard bacterial culture media included Columbia blood agar (BD BBL), chocolate agar (BD BBL), MacConkey Agar (MAST), Thayer-Martin agar (Oxoid), Mannit-NaCl-Agar (BD), and tryptic soy broth as enrichment medium (Oxoid). For mycological cultures, we used malt extract agar (Oxoid). Mycobacterial testing was performed using Löwenstein-Jensen-Medium (bioMérieux), Stonebrink Medium (Oxoid), and MGIT liquid medium (BD). We used MALDI-TOF (bioMérieux Vitek-MS with Saramis database) or Vitek identification cards (Vitek2-XL, bioMérieux) for bacterial and yeast identification. Molds were identified via microscopy or sequencing of the internal transcribed spacer region. Antibiotic susceptibility was tested with the Vitek-2 XL system (bioMérieux) or Merlin MICRONAUT microdilution system (Merlin Diagnostika).

2.6 | Outcome

The primary endpoint of this study was the time to test result of the BFPPp compared to conventional diagnostics, measured from time of

bronchoscopy. The time to clinical decision, sensitivity, specificity, and positive/negative agreement (based on Cohen's Kappa) of pathogen isolation by BFPPp-based testing versus standard diagnostic procedures were key secondary endpoints. We used conventional microbiological culture as the gold standard for bacterial pathogen detection based on previous studies.[32] Since both BFPPp and our conventional virology IFT and multiplex PCR are certified in accordance to EU in vitro diagnostic testing standards (CE-IVD), we chose to weigh both tests equally. Virology outcomes are expressed as positive and negative test agreement between BFPPp and conventional diagnostics rather than sensitivity and specificity to reflect this.[36] Diagnosis was established based on the results of all diagnostic tests, including BFPPp, conventional microbiological culture, conventional virology testing, and additional work-up for non-infectious diseases.

Viral or non-viral LRTI were diagnosed if either BFPPp, conventional diagnostics, or both modalities detected a probable causative pathogen. Organisms belonging to the normal lower respiratory tract flora or without lung-pathogenicity were disregarded as positive results. Facultative pathogens such as *Achromobacter* species, *Stenotrophomonas maltophilia*, and *Penicillium* species were only considered if they were isolated for the first time, had a significant value (>10^2) of colony forming units, and if no other, more likely pathogen was isolated in the same sample. Additional testing for atypical bacteria (i.e., *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila)* was reserved for cases with a high pre-test probability based on the clinical presentation. If no LRTI-typical pathogen was detected, further diagnostic work-up was initiated, and the final diagnosis was deferred until all necessary diagnostic steps were completed.

2.7 | Statistical analysis

Statistical analysis was performed using SPSS v27 (IBM SPSS Statistics, Armonk, NY, USA) and R Studio v1.4.1103 (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables are presented as numbers (*n*) or percentages (%), and continuous variables as median and 25% and 75% quartiles or percentiles. Agreement between PCR and conventional testing was assessed using Cohen's κ. Differences between time to test results and clinical decision were assessed by Wilcoxon signed-rank test.

For sample size calculation, a clinically relevant additional benefit of BFPPp testing in time to test result together with routine diagnostics of 20% would be relevant, since a previous study with a different BFPPp kit yielded no additional benefit as per that definition.[32] We defined receiving identical or additional pathogen identification with BFPPp faster than conventional tests as clinically beneficial. For calculation, we used Fisher's exact method and assumed a distribution of proportions p1 = 100% and p2 = 80%, with an alpha-error of 0.05 and a power (1-ß) of 95%. This yielded a sample size per group of 50 (equal to total sample size since all study participants receive both tests) with an actual power of 0.952 and actual alpha of 0.028. Adjusting for anticipated loss to follow-up, we aimed for a sample size of 60.

TABLE 2 Patient characteristics

Characteristic	Total *n* = 60
Female, *n* (%)	22 (37)
Age (years), median (25, 75 % percentile)	56 (45, 62)
CRP (mg/L), median (25, 75 % percentile)	10.9 (4.3, 43.4)
Empiric antibiotic therapy prior to visit, *n* (%)	30 (50)
Concomitant transbronchial biopsy performed, *n* (%)	20 (33)
Biopsy-proven acute graft rejection, *n* (%)	4 (7)
Transplant type, *n* (%)	
Bilateral	59 (98)
Unilateral	1 (2)
Underlying disease, *n* (%)	
Cystic fibrosis/bronchiectasis	12 (20)
Emphysema incl. alpha1ATD	21 (35)
Fibrosis/interstitial lung disease	17 (28)
Other	10 (17)
Years after transplantation, median (25, 75 % percentile)	2.9 (1.2, 7.1)
Previous airway colonization, *n* (%)	16 (27)
Pseudomonas aeruginosa	11 (69)
Methicillin sensitive Staphylococcus aureus	2 (13)
Other	3 (19)
Inclusion criteria, *n* (%)	
Fever (>38°C)	18 (30)
Hypoxemia	5 (8)
Opacity on chest x-ray (new or progressive)	5 (8)
FEV1 decline ≥10%	49 (82)
CRP ≥5 mg/L	43 (72)
Productive cough (new or progressive)	49 (82)

Abbreviations: Alpha1ATD, alpha-1 antitrypsin deficiency; CRP, C-reactive protein; FEV1, forced expiratory volume in 1 second.

3 | RESULTS

Sixty LTx patients with suspected LRTI were enrolled in this study between November 2019 and August 2020. The median age was 56 years (25% and 75% quartiles 45–62), with a median time posttransplantation of 2.9 years (1.2–7.1). Fifty percent of patients had received prior empiric antibiotic therapy. Patient demographics are shown in Table 2.

3.1 | Primary endpoint – time to test result and clinical decision

The comparisons of times to test result and clinical decision are summarized in Figure 1A,B. The median time to test result was 2.3 h (2–2.8) for the BFPPp. For conventional testing, the median time to test result

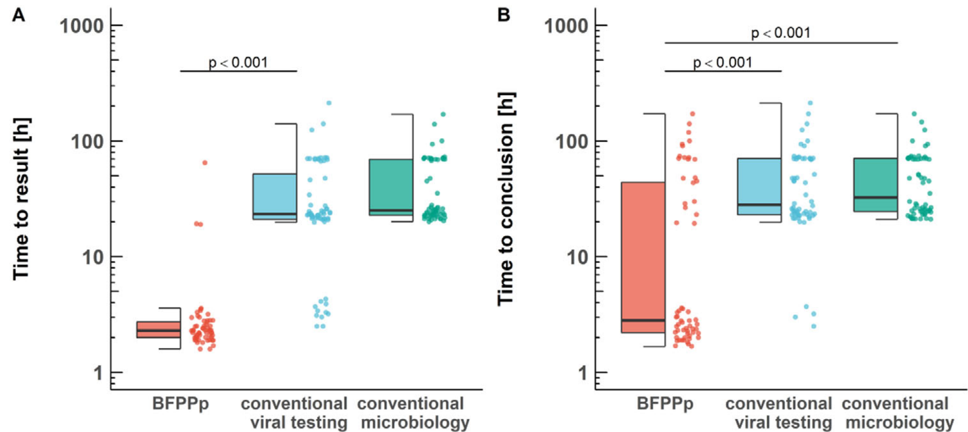

FIGURE 1 Time to test result notification (A) and time to relevant clinical decision (B) for BioFire Pneumonia Plus panel (BFPPp) versus viral and microbiology conventional testing

was 23.4 h (21.1–62, $p < 0.001$) and 25.2 h (22.8–69.5, $p < 0.001$), for virology and microbiology, respectively. Regarding conventional virological diagnostics, 36 patients were tested using IFT only, 21 were tested using IFT plus subsequent conventional virological PCR, and three were tested using conventional virological PCR only. Median time to test result was 23 h (20.1–25.8), 47.6 h (22–70.6), and 21.4 h (19.8–72) in patients tested using IFT, IFT plus subsequent conventional PCR, and conventional PCR only, respectively (both IFT and IFT plus PCR $p < 0.001$ compared to BFPPp).

Time to relevant clinical decision and resulting treatment modification was 2.8 (IQR 2.2–44) for BFPPp versus 28.1 h (23.1–70.6), and 32.6 h (24.6–70.9) for overall conventional virology and microbiology, respectively (both $p < 0.001$). Within conventional virology, median time to clinical decision was 25.4 h (22.9–50.7), 69.4 h (25.1–81.8), and 21.4 h (46.7–71.9) in patients tested with IFT alone, initial IFT plus subsequent conventional PCR and immediate conventional PCR, respectively (IFT-only $p < 0.001$, IFT plus PCR $p = 0.018$ compared to BFPPp).

3.2 | Pathogen detection

In 48 of 60 (80%) patients, a pathogen was detected by either BFPPp, conventional testing or both, with results illustrated in Figure 2. In 18 of 60 (30%) of patients, multiple pathogens were identified in the same patient. In 25 of 48 (59%) patients with pathogen isolation by any method, BFPPp and conventional diagnostics showed a complete match. In 17 of 48 (35%), there was a partial match, that is, agreement between BFPPp and either conventional virology or microbiology, but diverging results between BFPPp and the other conventional modality. We observed one complete mismatch in both (*Haemophilus parainfluenzae* identified by conventional microbiology; coronavirus found by BFPPp in addition to hMPV found by both test modalities). Additionally, there were five of 48 cases with a negative BFPPp and non-viral pathogens detected in conventional tests. In three of five of these false-negative BFPPp, therapy was modified in response to the pathogen isolation by conventional methods.

Pathogen overlap between BFPPp and conventional methods is summarized in Figure 3. BFPPp revealed 38 viral and 19 bacterial pathogens, of whom 36 of 38 (96%) viral and 17 of 19 (90%) bacterial pathogens were judged clinically relevant. Conventional testing identified 32 viral, 28 bacterial, and six fungal pathogens. Of these, 31 of 32 (98%) viral pathogens, 20 of 28 (71%) bacterial, and three of six (50%) fungal pathogens were judged clinically relevant.

3.3 | Antibiotic resistance markers

Aside from pathogen detection, the BFPPp panel also includes seven genetic antibiotic resistance markers (see Table 1). BFPPp detected an antibiotic resistance marker in only one instance (a *Staphylococcus aureus* isolate with mecA/mecC and MREJ marker, which is associated with methicillin-resistance).[37] However, conventional microbiology showed the isolated *Staphylococcus aureus* to be methicillin-sensitive, and the patient improved on a non-methicillin-resistance-specific antibiotic therapy.

3.4 | Positive and negative test agreement

Overall BFPPp positive test agreement was 78.7% for the pathogens included, while overall negative test agreement was 100%, with findings summarized in Table 3 and Table S1. There was no significant difference in sensitivity of either test between patients who had received antibiotic therapy prior to bronchoscopy and those who had not (BFPPp $p = 0.838$, conventional diagnostics $p = 0.710$). BFPPp sensitivity and specificity for included bacteria were 58% and 100% (Cohen's κ 0.564, $p < 0.001$). For viral pathogens, positive and negative test agreement of BFPPp with conventional virology was 95% and 100%, respectively, with an overall test result match compared to conventional virology in 50 of 60 patients, corresponding to a Cohen's κ of 0.693 ($p < 0.001$). Conventional virology showed a positive test agreement of 80% and a negative test agreement of 100% (aggregate value

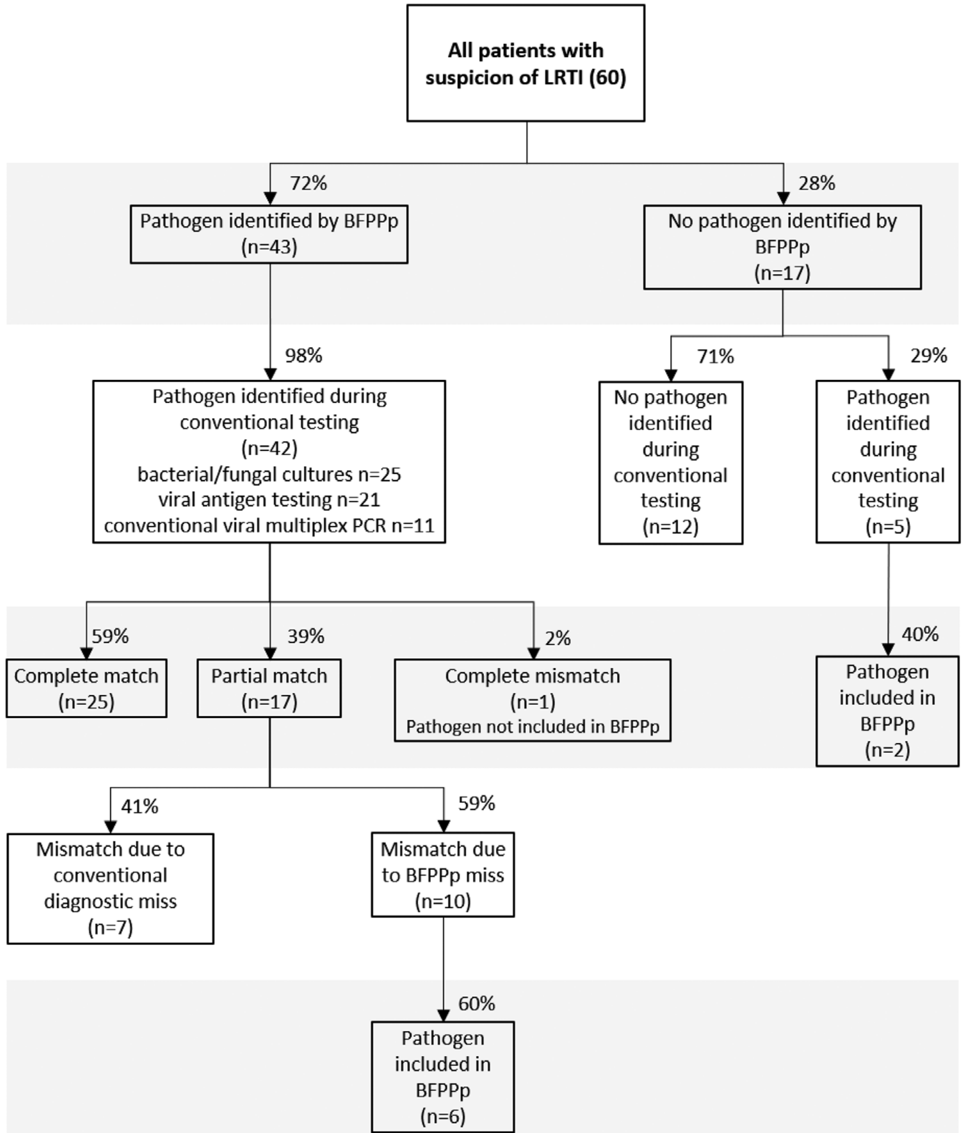

FIGURE 2 BFPPp/conventional diagnostic (mis-)matches. BFPPp, BioFire Pneumonia Plus panel; PCR, polymerase chain reaction

of IFT and PCR). IFT identified 21 of 29 viral pathogens in 36 patients, with a positive test agreement of 72%, while BFPPp identified 29 of 29. By contrast, conventional PCR identified 11 of 11 viral pathogens in the remaining 24 patients. BFPPp identified nine of 11 viral pathogens, achieving 82% positive test agreement in this subgroup. Correlations between BFPPp and conventional virology PCR results are summarized in Table S2.

3.5 | Final diagnoses, hospitalizations, and diagnostic tools

A synopsis of pathogen discovery, final diagnoses, and diagnosis-establishing diagnostic methods is shown in Figure S1. The final diagnoses made were viral LRTI in 26 of 60 (43%), bacterial LRTI in nine of 60 (15%), combined LRTI in five of 60 (8%), and a diagnosis other than LRTI in 20 of 60 (33%). Five (8%) patients were hospitalized subsequent to the study visit, of which three (60%) received an LRTI diagnosis. The majority of LRTI diagnoses (29/40, 72,5%) were made in agreement of both conventional diagnostics and BFPPp. Seven viral LRTIs were diagnosed based solely on BFPPp, while two viral and two non-viral LRTI were diagnosed exclusively based on conventional diagnostics. Therapy was modified from the preliminary BFPPp-based treatment decision in six of 60 (10%) patients, modifications are summarized in Table 4. In two of these, identification of a fungal pathogen led to an escalation of antifungal therapy, although expanded diagnostics identified a diagnosis other than LRTI as the main cause of the patients' symptoms.

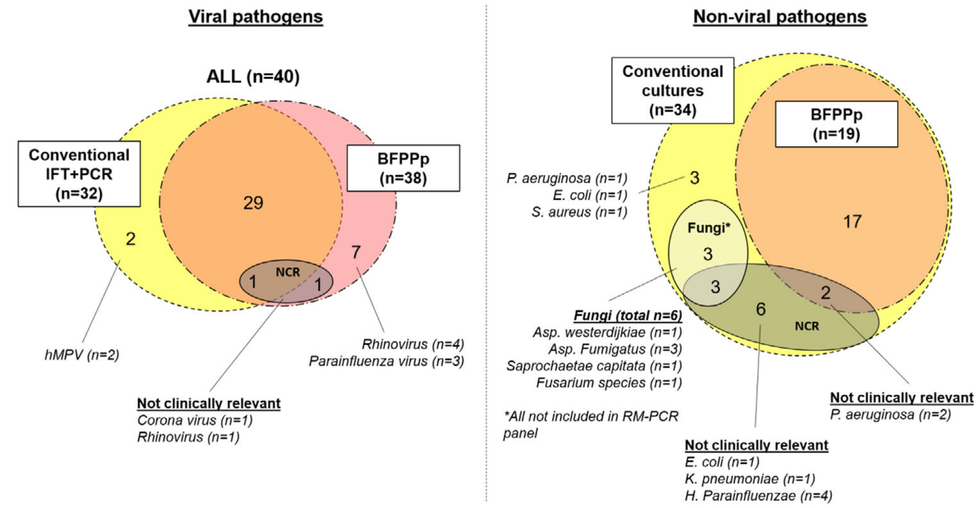

FIGURE 3 Venn diagram illustrating test result overlap for viral pathogens and non-viral pathogens. Asp, Aspergillus; BFPPp, BioFire Pneumonia Plus panel; IFT, immunofluorescence test; PCR, polymerase chain reaction; NCR, not clinically relevant

TABLE 3 Test accuracy and detection rate per pathogen

		Conventional testing			Rapid multiplex PCR				
Pathogen - microbiology	**Total**	***N***	**(PPV; NPV)**	**(Sens; Spec)***	***N***	**(PPV; NPV)**	**(Sens; Spec)***	**Cohen's kappa**	***p* value**
Moraxella catarrhalis	2	2	(100; 100)	(100; 100)	2	(100; 100)	(100; 100)	1.00	<0.001
Pseudomonas aeruginosa	12	12	(100; 100)	(100; 100)	11	(100; 98)	(92; 100)	0.95	<0.001
Staphylococcus aureus	6	6	(100; 100)	(100; 100)	5	(100; 98)	(83; 100)	0.90	<0.001
Streptococcus pneumoniae	1	1	(100; 100)	(100; 100)	1	(100; 100)	(100; 100)	1.00	<0.001
Escherichia coli	2	2	(100; 100)	(100; 100)	0	(-; 97)	(0; 100)	0.00	<0.001
Klebsiella pneumoniae	1	1	(100; 100)	(100; 100)	0	(-; 98)	(0; 100)	0.00	<0.001
Haemophilus parainfluenzae	4	4	(100; 100)	(100; 100)	Not included in BFPPp				
Aspergillus fumigatus	3	3	(100; 100)	(100; 100)	Not included in BFPPp				
Aspergillus westerdijkiae	1	1	(100; 100)	(100; 100)	Not included in BFPPp				
Fusarium species	1	1	(100; 100)	(100; 100)	Not included in BFPPp				
Saprochaetae capitata	1	1	(100; 100)	(100; 100)	Not included in BFPPp				
Pathogen - Virology	**Total**	***N***	**(PPV; NPV)**	**(PosA; NegA)***	***N***	**(PPV; NPV)**	**(PosA; NegA)***	**Cohen's kappa**	***p* value**
Coronavirus	4	3	(100; 98)	(75; 100)	4	(100; 100)	(100; 100)	0.85	<0.001
hMPV	11	11	(100; 100)	(100; 100)	9	(100; 96)	(82; 100)	0.88	<0.001
RSV	3	3	(100; 100)	(100; 100)	3	(100; 100)	(100; 100)	1.00	<0.001
Rhinovirus	8	4	(100; 93)	(50; 100)	8	(100; 100)	(100; 100)	0.63	<0.001
Influenza A	2	2	(100; 100)	(100; 100)	2	(100; 100)	(100; 100)	1.00	<0.001
Parainfluenzavirus	12	9	(100; 94)	(75; 100)	12	(100; 100)	(100; 100)	0.83	<0.001

Abbreviations: BFPPp, BioFire Pneumonia Plus panel multiplex polymerase chain-reaction; hMPV, human metapneumovirus; NegA, negative agreement; NPV - negative predictive value; PCR, polymerase chain reaction; PosA, positive agreement; PPV, positive predictive value; RSV, respiratory syncytial virus; Sens, sensitivity, Spec, specificity.

*Virology results are labeled positive and negative agreement (PosA and NegA) rather than sensitivity and specificity (Sens and Spec) as both modalities were weighed equally.[36]

TABLE 4 Treatment modifications due to additional pathogen identified by conventional diagnostics

Pathogens detected by BFPPp	Initial diagnosis based on BFPPp	Initial therapy based on BFPPp	Additional pathogen detected by conventional diagnostics	Treatment modification	Final diagnosis
Pseudomonas aeruginosa	*Pseudomonas aeruginosa bacterial LRTI*	Antibiotic therapy	*Staphylococcus aureus and Saprochaetae capitata*	Escalate antifungal therapy, no change to antibiotic therapy	Fungal and bacterial LRTI
Pseudomonas aeruginosa (known colonization)	Unclear, further diagnostic steps required	None	hMPV	Reduction of cell cycle inhibitor	Viral LRTI
Rhinovirus	Viral LRTI	Reduction of cell cycle inhibitor	hMPV	Ribavirin therapy	Viral LRTI
None	Suspected LRTI	None	*Escherichia coli and Pseudomonas aeruginosa*	Inhaled and oral antibiotics	Bacterial LRTI
None	Unclear, further diagnostic steps required	None	*Aspergillus westerdijkiae*	Escalate antifungal therapy	ARAD and fungal graft colonization
None	Unclear, further diagnostic steps required	None	*Fusarium species*	Escalate antifungal therapy	Biopsy-proven acute allograft rejection and fungal graft colonization

Abbreviations: ARAD, azithromycin-reversible allograft dysfunction; BFPPp, BioFire Pneumonia Plus panel multiplex polymerase chain-reaction; hMPV, human metapneumovirus; LRTI, lower respiratory tract infection.

4 | DISCUSSION

In this prospective study, BFPPp was superior in time to test result and time to clinical decision while maintaining an acceptable accuracy rate in conventional microbiology and virology testing. In 63% of cases, BFPPp results led to same-day targeted initiation of anti-infective therapy.

The utility of the BFPPp and the previous generation BFPP, featuring a similar panel except for the absence of MERS-CoV, were studied in intensive care unit and other in-patient settings and demonstrated a shortened response time and equal or even better sensitivity compared to conventional diagnostics.[16–30] In LTx patients, PCR-based testing from BAL has previously been described in the context of viral infections, impossible-to-cultivate atypical bacteria, and *Pneumocystis jirovecii*.[12,13,38,39] Recent studies have evaluated PCR-based testing for specific fungal pathogens and *Nocardia*.[14,40] Given the larger number of potential LRTI-causing pathogens in LTx recipients,[3,41] specialized extended-spectrum PCR panels are required. A prior study by our group evaluating another rapid multiplex PCR test (Curetis P55 pneumonia) targeting bacterial pathogens had excellent turnaround time compared to conventional testing but limited sensitivity, which proved problematic in the context of high prevalence of viral LRTI in LTx patients.[32] Recently, the older generation BFPP panel was demonstrated to improve clinical decision making in LTx recipients.[31] Notably, the large majority of bronchoscopies in this study were performed for routine surveillance without suspicion of LRTI.[31] However, there are no data on the use of BFPPp in the context of LRTI in LTx.

BFPPp delivered results significantly faster than conventional diagnostics, allowing for swift preliminary diagnoses and treatment decisions. In all but two cases, BFPPp results were available on the day of bronchoscopy, enabling us to discharge 63% of all patients (90% of patients with LRTI) with a tailored anti-infective therapy. By contrast, conventional diagnostic results usually became available 24 h after bronchoscopy. Prior to the establishment of BFPPp in our outpatient clinic, this delay hindered targeted anti-infective therapy initiation, forcing the patient to obtain a prescription from his general practitioner before therapy is started. Given the current pandemic, the avoidance of a second contact of LTx recipients with the health care system is a meaningful advantage in a population particularly at risk from COVID-19. At the time of this study, which spans from before the pandemic to after the first European wave, SARS-CoV-2 was not included in the testing panel. However, the manufacturer has already released an updated version of the test that includes SARS-CoV-2.[42]

Partially, this advantage was due to organizational factors since extensive same-day diagnostics make test results of BAL samples frequently available only after the regular work-hours of laboratory staff. Thus, the 3- to 4-h run-time of conventional tests is a significant hurdle to same-day initiation of targeted therapy. Notably, our standard operating procedure for BAL infection diagnostics includes IFT testing followed-up by conventional virological PCR if IFT remains negative but viral LRTI suspicion persists, whereas conventional virological PCR was used directly in a few cases as per physician's discretion. BFPPp results were available faster than conventional virology, regardless of whether conventional virology consisted of IFT or IFT and subsequent PCR. A comparison between BFPPp and conventional PCR-only cases was statistically unfeasible on the basis of only three patients directly receiving conventional PCR without IFT.

Fifty-eight percent of the cases in this study involved a viral pathogen while non-viral LRTIs were less common. This may be explained by the outpatient setting of this study, as we provide outpatient LTx follow-up care to patients all over Germany, and patients with very severe symptoms are advised to present to a local emergency department without delay for initial work-up. Therefore, the pre-test probability for severe bacterial LRTI in our patient cohort was low, as reflected by the low prevalence of radiological changes and the low mean CRP. Another aspect to consider is that half our study population had received empiric oral antibiotics prior to visiting our outpatient-clinic, potentially obscuring some cases of bacterial LRTI. Traditionally, PCR-based tests have been described as more resilient to these obscuring effects than conventional culture[43]; however, another group reported negative BFPP/BFPPp results in BAL samples loaded in vitro with ≤log10^3 copies of *Staphylococcus*.[17] Therefore, reduction of bacterial burden by antibiotic pre-treatment might have led to false negative BAL samples in our study as well. We specifically suspected an obscuring of bacterial pathogens due to prolonged empiric antibiotic pre-treatment in at least three patients with BFPPp and culture negative BAL samples and improvement upon further antibiotic therapy. As our study focused on diagnostic tests, these three cases were nonetheless classified as "alternative final diagnoses."

Overall, BFPPp positive agreement with conventional diagnostics was excellent regarding viral pathogens, particularly in comparison to routine virology IFT. Test agreement between BFPPp and conventional virology was substantial ($\varkappa > 0.61$) or even close-to-perfect ($\varkappa > 0.81$) both overall and for IFT and PCR, individually.[44] Specifically, BFPPp missed two cases of hMPV identified by conventional PCR. However, in both cases, hMPV was found in low concentrations (at 42 and 43 PCR cycle turns) with unclear clinical significance. A missed diagnosis of hMPV nonetheless represents a relevant flaw in LTx-recipients, since hMPV increases CLAD risk and is treatable.[45]

BFPPp sensitivity toward bacteria was more limited. In part, this was due to limitations of the BFPPp panel regarding fungal pathogens and facultatively pathogenic bacteria that are of questionable relevance outside severely immunocompromised patients, such as *Haemophilus parainfluenzae*. While none of the cases of *Haemophilus parainfluenzae* found in this study were deemed clinically relevant, it is a rare potential cause of LRTI in the immunosuppressed and was thus included in the analysis.[46] Notably, this flaw was also remarked upon by another study evaluating the use of BFPPp in a setting emphasizing LTx routine surveillance.[31] Antifungal therapy had to be modified in three of six (50%) instances of fungal pathogens identified by conventional microbiology. The absence of fungal pathogens, particularly Aspergillus species and *Pneumocystis jirovecii*, on the BFPPp panel represents a significant weakness in LTx patients and other immunocompromised groups at particular risk from fungal infections, although significant disease requiring treatment is often anticipated by either bronchoscopic or radiological findings. Most mismatches between BFPPp and conventional microbiology and virology were of limited clinical consequence, and therapies established based on BFPPp results were confirmed by conventional diagnostics in 90% of cases. This accuracy regarding the detection of clinically relevant pathogens, combined with the fast return-time, highlights the suitability of the BFPPp for a point-of-care approach in LTx patients.

Financially, at the time, the study was conducted, BFPPp was cost equivalent to conventional virology PCR plus microbiology and 24% more expensive compared to conventional virology IFT plus microbiology. Given the absence of several relevant bacteria and fungi from the BFPPp panel, we would regard it an addition to rather than a substitute for conventional testing.

4.1 | Limitations

While we aimed to provide real-life data on the use of BFPPp in LRTI diagnostics in LTx recipients, the generalizability of our study is limited by a number of factors: Firstly, following our internal standard-of-care guidelines, most patients received IFT testing as the first, and in 60% of patients sole, conventional virology test. We cannot exclude that the exclusive use of IFT for conventional virology led us to underestimate conventional virology sensitivity, nor can we exclude that there may have been viral LRTI missed by both IFT and BFPPp that would have been detected by conventional virology PCR.

Secondly, atypical bacterial pathogens like *Mycoplasma*, *Chlamydia*, *and Legionella spp*. were not part of the routine conventional microbiology test panel except in patients requiring hospitalization. Therefore, these pathogens may have been missed, although none were detected by BFPPp. Thirdly, we chose low threshold inclusion criteria defining suspicion of LRTI, in line with our clinical practice and the outpatient setting of our study. This may have biased our patient selection toward lower morbidity viral LRTI. Furthermore, the paucity of cases of genetic markers of antibiotic resistance detected by BFPPp leaves us unable to evaluate this feature of the BFPPp in clinical practice. Notably, the only instance of such a gene detection was not confirmed by conventional microbiology and did not turn out to be clinically relevant.

In conclusion, our findings match those of other groups evaluating the use of BFPPp in settings other than LRTI in LTx recipients.[16–30] The BFPPp significantly decreased the response time in the treatment of LRTI in an outpatient setting, with same-day results available in all but two cases. While BFPPp was a useful tool in the diagnosis of viral LRTI, accuracy was more limited in bacterial LRTI. The absence of fungal pathogens from the panel is a significant weakness in the context of severely immunosuppressed patients. The BFPPp may be a useful addition to conventional diagnostics in LTx recipients because it enables same-day tailored anti-infective therapy, but cannot replace the broader detection range of conventional microbiological methods.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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AUTHOR CONTRIBUTIONS

Dr. Kayser and Dr. Seeliger are responsible for the content of the manuscript and are both considered first authors.

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SUPPORTING INFORMATION

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