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FilmArray Respiratory Panel Assay: Comparison of Nasopharyngeal Swabs and Bronchoalveolar Lavage Samples

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The FilmArray respiratory panel (FARP) reliably and rapidly identifies 17 viruses and 3 bacterial pathogens. A nasopharyngeal swab FARP (NP FARP) is performed for many patients with respiratory symptoms. For patients who are acutely ill or immunocompromised or fail to improve, a bronchoalveolar lavage sample FARP (BAL FARP) is performed in addition to the NP FARP. To date, no studies have compared the yield of a BAL FARP with that of an NP FARP. We retrospectively studied all patients who had a BAL FARP within 7 days after an NP FARP between June 2013 and May 2014. Demographic information, comorbidities, FARP results, and all microbiologic data from BAL fluid were collected. Eighty-six patients had a BAL FARP performed within 7 days (mean, 1.6; median, 1) after an NP FARP. Of these, 66 (77%) had concordant BAL and NP FARP results: 15 (23%) had the same pathogen identified from the NP and BAL FARPs, and 51 (77%) had concordant negative FARP results. In 18 of the 86 patients (21%), a pathogen was detected from the NP FARP; of these, 15 (83%) had a concordant match on a subsequent BAL FARP, and the remaining 3 had negative BAL FARPs. In 17 of the 86 patients (20%), pathogens were identified from the BAL FARPs that were not detected by the NP FARPs; of these, 16 (94%) had initial negative NP FARPs. The data suggest that once a pathogen is identified by an NP FARP, a subsequent BAL FARP is unlikely to add new microbiologic information. However, a BAL FARP may provide new, useful microbiologic information when performed within 7 days after a negative NP FARP.

In recent years, advances in PCR techniques have aided in the rapid and accurate detection of common respiratory pathogens from patient specimens. Multiplex PCR can identify and differentiate a large panel of viral and bacterial targets simultaneously. Published studies have shown that multiplex PCR panels are more rapid and more sensitive methods of virus detection than cultures or antigen detection (1, 2). One such method, the FilmArray respiratory panel (FARP) (BioFire Diagnostics, Inc.), is a multiplex, nested PCR technique that can detect 17 common respiratory viruses and 3 bacterial targets in a single reaction in just over 1 h (3). Published studies have shown that for both immunocompetent and immunocompromised patients, the FARP identifies significantly more viral pathogens in both bronchoalveolar lavage (BAL) fluid and nasopharyngeal (NP) samples than viral cultures and direct fluorescent antibody staining and that the FARP is among the most sensitive of the available multiplex assays (1, 4–10). In addition, the FARP has a low hands-on time and very fast turnaround time. Since the FARP is associated with a significant cost to the laboratory and the patient, its judicious use is necessary.

Choosing the least invasive, highest yield, and most cost-effective investigations in a stepwise manner has always been central to the practice of medicine. Patients who present with symptoms of a respiratory tract infection often undergo testing for respiratory viruses. The initial testing at our center may involve collection of an NP sample for influenza A and B and respiratory syncytial viruses. The comprehensive FARP is obtained for patients with complex conditions or those who are immunocompromised and have symptoms of upper or lower respiratory tract infections. In the presence of concurrent pulmonary infiltrates, fever, and hypoxia, patients (especially if immunocompromised) may then undergo a bronchoscopy with BAL with repeat FARP testing on the BAL sample. To date, no studies have compared the yield of FARP on BAL samples with the yield on NP samples. Whether additional microbiologic information is obtained from FARP testing on a BAL sample after testing on a NP swab is not known. This retrospective case-control study evaluates the concordance between FARP testing on NP and BAL samples.

(Part of this research was presented as a poster at the American Thoracic Society International Conference, Denver, CO, 15 to 20 May 2015.)

MATERIALS AND METHODS

We retrospectively reviewed the electronic medical records of all patients evaluated at the Mayo Clinic in Arizona between 1 June 2013, and 31 May 2014, who had FARP testing on both NP and BAL samples. All patients who were included had a BAL sample FARP (BAL FARP) performed within 7 days after an NP swab FARP (NP FARP) during the same hospitalization or illness episode. FARP results obtained on tracheal aspirates were excluded.

Patient electronic medical records were reviewed, and the following information was obtained: demographics (age and sex) and the presence of immunosuppression, which included patients who were recipients of systemic steroids, disease-modifying agents (e.g., inhibitors of tumor necrosis factor), chemotherapy, or calcineurin inhibitors or patients who had positivity for human immunodeficiency virus, a history of organ transplant, or any lymphoproliferative or myelodysplastic syndrome. Dates of and indications for BAL and NP sample collections and all non-

Received 4 June 2015 **Returned for modification** 13 July 2015
Accepted 14 September 2015

Accepted manuscript posted online 16 September 2015

Citation Azadeh N, Sakata KK, Brighton AM, Vikram HR, Grys TE. 2015. FilmArray respiratory panel assay: comparison of nasopharyngeal swabs and bronchoalveolar lavage samples. J Clin Microbiol 53:3784–3787. doi:10.1128/JCM.01516-15.

Editor: Y.-W. Tang

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TABLE 1 Summary data for concordant and discordant results of NP and BAL FARPs in 86 patients

NP and BAL FARP results[a]	No. (%) of patients
Concordant	
NP FARP and BAL FARP both negative	51 (59)
NP FARP and BAL FARP both positive	15 (17)
Total	66 (77)
Discordant	
NP FARP positive, BAL FARP negative	3 (4)
NP FARP negative, BAL FARP positive[b]	17 (20)
Total	20 (23)

[a] Abbreviations: NP FARP, nasopharyngeal swab FilmArray respiratory panel; BAL FARP, bronchoalveolar lavage sample FARP.
[b] These 17 organisms were influenza virus ($n = 4$), parainfluenza virus ($n = 3$), respiratory syncytial virus ($n = 3$), *Coronavirus* ($n = 3$), *Rhinovirus-Enterovirus* ($n = 3$), adenovirus ($n = 1$), and *Mycoplasma pneumoniae* ($n = 1$).

TABLE 2 Data for 28 patients in whom BAL yielded additional pathogens not tested by FARP[a]

	BAL FARP result	
NP FARP result	Positive	Negative
No. positive	15	3
Additional yield (no. [%])	5 (33)[b]	3 (100)[c]
Immunocompromised (no. [%])	5/5 (100)[d]	2/3 (66)
Tx recipient (no. [%])	5/5 (100)[e]	1/3 (33)
No. negative	17	51
Additional yield (no. [%])	4/17 (24)[f]	15/51 (29)[g]
Immunocompromised (no. [%])	2/4 (50)[d]	10/16 (67)[d]
Tx recipient (no. [%])	2/2 (100)[e]	5/10 (50)[e]

[a] Abbreviations: BAL FARP, bronchoalveolar lavage sample FilmArray respiratory panel; NP FARP, nasopharyngeal swab FARP; Tx, transplant.
[b] *Corynebacterium*, *Stenotrophomonas*, methicillin-resistant *Staphylococcus aureus*, cytomegalovirus, and herpes simplex virus.
[c] *Pseudomonas aeruginosa*, *Pseudallescheria boydii*, *Candida parapsilosis*, methicillin-susceptible *Staphylococcus aureus*, *Pneumocystis jiroveci*, and *Aspergillus*.
[d] Patients with metastatic solid tumor, chronic lymphocytic leukemia, acute myeloblastic leukemia, myelodysplastic syndrome, myeloma, lymphoma, stem cell transplant, or solid organ transplant or patients who had received chronic systemic steroids.
[e] Includes stem cell and solid organ transplants.
[f] *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, *Mycobacterium avium-intracellulare*, *Nocardia*, *Saccharomyces cerevisiae*, and beta-hemolytic *Streptococcus*.
[g] *Nocardia*, *Pseudomonas*, *Enterobacter*, methicillin-susceptible *Staphylococcus aureus*, *Klebsiella*, *Pneumocystis jiroveci*, *Coccidioides*, and *Mycobacterium avium-intracellulare*.

FARP microbiologic data from the BAL specimens were recorded. This study was approved by the Mayo Clinic Institutional Review Board.

Concordant results were defined as results that were the same for all targets of the panel (i.e., concordant negative means the same set of negative results, and concordant positive means the same set of positive results). The 2 × 2 table was statistically analyzed with the McNemar test of proportions (http://www.graphpad.com/quickcalcs/mcnemar1.cfm).

NP specimens were collected with a flocked swab and UTM transport medium (Copan Diagnostics). BAL specimens were transported to the laboratory at room temperature and mixed by vortexing. Samples (0.3 ml) of the UTM (NP swab) or BAL fluid were loaded into the FilmArray v1.6 pouch with the FilmArray loading device. Aside from the specimen source, the rest of the manufacturer's instructions were followed. Pouches were run on 1 of 2 FilmArray 1.5 instruments. Before the study, a full validation had been completed for using the BAL specimen as a source for the FARP (T. E. Grys, unpublished data).

Traditional culture methods included plating on aerobic bacterial plates, fungal plates (inhibitory mold agar, potato flake agar, and bovine heart infusion-3 agar), and mycobacterial medium (7H11 agar and MGIT broth [BD]). Bacteria and yeast were identified primarily with identification panels on the BD Phoenix system.

RESULTS

A total of 86 patients met the inclusion criteria. Their average age was 60 years; 50 patients (58%) were male. Twenty-six (30%) patients required intermediate or intensive care. A majority of the patients, 66 of 86 (77%), had concordant BAL and NP FARP results, and the remaining 20 patients (23%) had discordant results. Sixty-one of the 86 patients (71%) were immunosuppressed: 50 of the 66 patients (76%) in the concordant FARP group and 11 of the 20 patients (55%) in the discordant FARP group. The majority of the immunosuppression (39 of the 61 patients, 64%) was due to solid organ or stem cell transplants or acute leukemias.

All 86 patients had BAL FARPs performed 7 days or less (mean, 1.6 days; median, 1 day; range, 0 to 6 days) after NP FARPs. Only 8 patients (9%) had an interval of 4 days or more, and of these only 1 had an interval of 6 days. The documented indications for NP or BAL FARP included upper or lower respiratory tract illness or symptoms (including cough, wheeze, and shortness of breath), systemic inflammatory response syndrome or sepsis, abnormal findings on chest radiography, hemoptysis, and respiratory failure or insufficiency.

Of the 20 patients (23%) with discordant results for the NP and BAL FARPs, 17 had a pathogen identified by the BAL FARP that was not detected by the NP FARP (Table 1). Sixteen of these 17 patients (94%) had a completely negative result on the NP FARP. Among the 17 patients who had a discordant positive BAL FARP, 16 new viruses and 1 new bacterium were detected. The bacterium identified was *Mycoplasma pneumoniae*. Ten of the 16 viruses were influenza, parainfluenza, and respiratory syncytial viruses. Conversely, 3 of the 20 patients with discordant results had positive NP FARPs and subsequently had negative BAL FARPs (Table 1).

The remaining 66 of the 86 patients (77%) had concordant results for the NP and BAL FARPs. Fifty-one of these 66 patients had concordant negative results, and 15 had concordant pathogen recoveries between the NP and BAL FARPs (Table 1).

In 18 patients, a pathogen was identified with the NP FARP. Fifteen of these had a concordant match on the BAL FARP. The remaining 3 patients had negative results on the BAL FARPs.

A noteworthy proportion of patients, 28 of the 86 (33%), had additional microorganisms detected, which were considered true pathogens detected by non-FARP testing on their BAL sample. Of these, 17 (61%) were immunocompromised. Even in the 15 patients who had both NP and BAL FARP results positive for the same pathogen, there was an additional yield with cultures or PCRs in 5 (33%) of them (Table 2). Of the 66 patients with concordant results, 21 (32%) had additional microorganisms detected, which were considered true pathogens detected by non-FARP testing on their BAL sample (Table 2). Among the 20 patients with discordant NP and BAL FARPs, 7 (35%) had additional findings from the BAL sample. As outlined in Table 2, a substantial proportion of patients had additional yield on the BAL sample culture, many of these patients were immunocompromised, and the majority of the immunocompromised patients were transplant recipients (solid organ or stem cell). Among the 86 patients, 25 had additional pathogens of limited clinical signif-

icance (e.g., *Candida* sp., coagulase-negative staphylococcus, and Epstein-Barr virus) found on BAL sample cultures or PCRs (not tested for by the FARP).

DISCUSSION

Advances in PCR technology, such as FARP, have allowed rapid and accurate detection of pathogens. Studies have shown their utility for the rapid identification of pathogens, which is especially useful for achieving a prompt diagnosis in complex cases, such as those in immunocompromised hosts. These multiplex panels are typically approved by the U.S. Food and Drug Administration for limited sources, such as nasal wash or NP swab, but laboratories often validate the assays for various other specimens such as BAL fluid and tracheal secretions. The large multiplex panels do have substantial costs for both reagents and instrumentation and high charges to the patient. Whether the microbiologic yield justifies FARPs on more than 1 respiratory sample is unknown. Many patients who are initially evaluated with an NP FARP may also undergo bronchoscopy with BAL and repeat FARP testing of a BAL sample is of unknown value in terms of unique additional diagnostic information.

Our data showed that the FARP had a 37% positivity rate from BAL specimens, slightly higher than that in a study by Hammond et al. (2), which, among the 56 BAL specimens tested, included some specimens collected to check for rejection rather than infection. In a study of BAL specimens from cancer patients, Ruggiero et al. (9) compared FARP with traditional methods and another molecular method, and the positivity rate in the BAL specimens was around 50%. Thus, our data are in line with those of other studies. The question we sought to answer, however, was whether the results for an NP swab would be indicative of the findings on a subsequent BAL specimen.

Although the majority of our patients, 66 of 86 (77%), had a concordant match between the results of the NP and BAL FARPs, 17 of the 86 (20%) had a BAL FARP that provided new microbiologic information compared with that for the NP FARP (Table 1). Thus, our data indicate that a BAL FARP may provide new, useful microbiologic information when performed 7 days or less after a negative NF FARP.

Among the 17 patients for whom additional microbiologic information was obtained from a BAL FARP (16 new viruses and 1 new bacterium), 16 had an initial negative NP FARP. The bacterium identified was *M. pneumoniae*, and 10 of the 16 viruses were influenza, parainfluenza, and respiratory syncytial viruses, for which antiviral therapy can be considered in patients with lower respiratory tract infections. Although the remaining viruses (rhinovirus, coronavirus, enterovirus, metapneumovirus) are considered self-limited without any available antiviral therapy, establishing an accurate and early diagnosis to account for the patient's ongoing respiratory symptoms may prevent further diagnostic testing and administration of unnecessary antibacterial therapies. Receipt of antibiotics for viral respiratory tract infections increases the risk of antibiotic-related complications and adverse events, emergence of multidrug-resistant pathogens, and increased health care costs (11). Some studies are beginning to support the theory that rapid multiplex testing for respiratory pathogens decreases the length of stay and time in isolation while reducing the duration of antimicrobial use (12).

Of the 17 patients who had additional yield from the BAL FARPs, the majority (12 patients) were immunosuppressed (including patients with stem cell or solid organ transplants or lymphoproliferative or myelodysplastic disorders and receiving chemotherapy). The 5 patients who were not considered immunosuppressed either needed care in an intensive care unit (3 patients) or had a significant history of lung disease (including cystic fibrosis and previous acute respiratory distress syndrome). Nine of the 17 patients required care in an intensive care unit.

Of the 28 patients who had additional yield from the BAL specimen by non-FARP methods, 19 (68%) were immunocompromised. Even when both the NP and BAL FARPs were positive for the same pathogen, there was an additional yield with cultures in 33% (5/15) of the patients, all of whom were immunocompromised (Table 2). In particular, bacteria, fungi, and mycobacteria may be commonly found in BAL specimens from immunocompromised patients. Thus, the FARP should not supplant those methods in the evaluation of BAL specimens for pathogens. Although all of the microbes identified from the BAL specimens are listed in Table 2, the pathogenic significance of *Corynebacterium*, *Candida parapsilosis*, and *Saccharomyces cerevisiae* is unknown; they might represent colonization.

Interestingly, 18 of the 86 patients (21%) had a pathogen identified from their NP FARPs, 15 (83%) of whom had a concordant match on a subsequent BAL FARP. The remaining 3 patients (17%) had negative BAL FARPs. Once a positive result has been obtained on an NP FARP, performance of a BAL FARP can be avoided because our data suggest that no additional information is gained by repeating the FARP on a BAL sample within 7 days after a positive NP FARP. Other microbiologic analyses, such as cultures and staining, should still be performed to increase the chances of finding other pathogens. Additional data are required to determine the specific pathogens that are more likely to be discordant when BAL and NP FARPs are compared.

In our study, the microbiologic yield was significantly higher with a BAL FARP (32 of 86) than with an NP FARP (18 of 86) ($P = 0.004$, 2-tailed using a McNemar test). Some potential explanations include the following: (i) BAL obtains a sampling of a large surface area in the lungs, (ii) most patients who underwent BAL had symptoms consistent with lower respiratory tract infections with the implicated pathogen, and (iii) oropharyngeal intubation for BAL might have resulted in potential contamination by upper respiratory tract secretions, and thus the BAL specimen was in essence both an upper and a lower airway combined sample. The NP swab samples were most often obtained by nurses, and thus the possible variability in obtaining a true NP sample could have resulted in false-negative NP FARPs. Furthermore, empirical antibiotic therapy can certainly be a factor that reduces the yield of bacterial FARP results; however, 78 of the 86 patients (90%) had the BAL FARP within 3 days or less of the NP FARP, and thus residual nucleic acid likely would have triggered a positive result anyway.

This study is inherently limited by its retrospective design and small sample size. Additionally, variations in the NP swab collection technique among health care providers may have confounded our results. Moreover, our selection criteria resulted in a high proportion of immunocompromised patients because such patients are likely to undergo both NP FARPs and subsequent bronchoscopy with BAL FARPs. However, there was no obvious difference between the 2 groups, and our patients had a relatively short duration between the NP and BAL FARPs (mean, 1.6; median, 1 day), which is a strength of our study.

In conclusion, in patients with a lack of improvement after routine clinical care for respiratory tract infections, performance of a BAL FARP after a negative NP FARP may provide useful additional microbiologic information. However, if a pathogen was already identified with an NP FARP, performance of a FARP on a BAL specimen is unlikely to provide additional information.

ACKNOWLEDGMENTS

Nothing in this article implies endorsement of the FilmArray respiratory panel.

We declare no conflicts of interest.

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