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Comparison of Nasopharyngeal Specimens and Bronchoalveolar Lavage Specimens of Immunocompromised Adult Patients Using the Genmark DX Esensor Respiratory Viral Panel

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Abstract

Purpose: To determine if the multiplex real-time polymerase chain reaction respiratory viral panel (RVP) provides the same results when performed on nasal wash versus bronchoalveolar lavage from the same patient within 5 days of each other.

Methods: A retrospective chart review was performed on all adult immunocompromised patients who underwent bronchoalveolar lavage (BAL) with a respiratory viral panel (RVP) obtained from the BAL fluid from February 2011 to July 2012. All patients who also had a nasal wash RVP performed within 5 days of the BAL assay were included in this study.

Results: There was exact concordance between BAL and NPW specimens in 45 of 58 patients: 26 cases in which both specimens were negative and 19 cases in which the exact same viruses were present in each specimen. In 8 cases, a virus was detected in BAL fluid that was not detected in NPW fluid; in 5 cases, a virus was found in NPW fluid but not BAL.

Conclusions: There was good correlation between the two assays when performed within 5 days of each other from the 2 separate specimen sources. For optimal diagnostic detection, it may be useful to repeat the assay in both locations when clinically indicated.

Keywords: Respiratory Infection; Bronchoscopy; Immunosuppressed; Respiratory Viral Panel

Introduction

Viruses are responsible for an estimated 200 million cases of community-acquired pneumonia each year [1]. Community-acquired respiratory viruses are a common etiology of infectious pneumonia in immunocompromised patients, and may be associated with higher mortality in these patients than those with an intact immune system [**2**]. Detecting these viruses in immunocompromised patients is important to establish a specific diagnosis, to identify patients who should be placed on contact or droplet isolation, and to avoid procedural and treatment related morbidity associated with prolonged empiric treatments.

Historically, respiratory viruses were detected by either direct fluorescence antibody (DFA) or viral culture. The advent of real-time polymerase chain reaction (PCR) has allowed for detection of respiratory viruses with greater sensitivity [**3**]. The GenMark DX eSensor respiratory viral panel (RVP) was developed to screen for multiple respiratory viruses with a single reaction by detecting amplified products on the eSensor XT-8 instrument. The eSensor RVP provides a similar result to real-time PCR in detecting respiratory viruses in children [**4**]. This assay has a sensitivity and specificity similar to other available molecular respiratory viral panels, including the BioFire Diagnostics FilmArray RVP, Luminex xTAG RVPv1 and Luminex xTAG RVP FAST [**5**].

In previously published studies, the respiratory specimens used to perform multiplex real-time PCR were either nasopharyngeal swabs or washes (NPW) [**5-7**], bronchoalveolar lavage fluid (BAL) [**8**], or a combination of both types of specimens [**3**,**4**,**9-11**]. NPW has been shown to be superior to nasopharyngeal swabs and oropharyngeal swabs in the detection of respiratory viruses using older methods [**12-14**]. In one study, sputum and NPW were shown to be equally superior to nasopharyngeal swabs and oropharyngeal swabs in the detection of influenza virus [**15**]. However, BAL and NPW specimens have never been studied comparatively to determine their individual effectiveness in detecting community–acquired respiratory viruses.

We sought to determine the correlation of the RVP assay between specimens from BAL and NPW and to examine if there is an additive effect to performing the assay on both specimens in immunocompromised patients with pulmonary infiltrates.

Materials and Methods

A retrospective review of all patients at the University of Kansas Medical Center who underwent FOB with BAL from February 1, 2011 through July 1, 2012 was performed. The patients' medical records were screened for the presence of conditions associated with a

compromised immune system. Such conditions included the history of a hematogenous or solid organ malignancy for which the patient had received chemotherapy within 14 days of bronchoscopy, history of a hematopoietic stem cell transplantation, history of a solid organ transplantation, infection with human immunodeficiency virus (HIV) and CD4 <200, neutropenia, or diagnosis of an autoimmune disorder for which the patient was being treated with immune suppressants.

All BAL fluid was collected via fiberoptic bronchoscopy (FOB) performed by members of the Division of Pulmonary and Critical Care Medicine at the University of Kansas Medical Center. All patients or their surrogate decision maker signed informed consent prior to procedural initiation. The patients had a new finding of pulmonary infiltrates, and were selected for FOB with BAL at the discretion of the attending physician based on patient symptoms, medical history and differential diagnosis. FOB with BAL was performed per accepted guidelines [**16**]. FOB occurred in either an endoscopy suite or the intensive care unit, achieving sedation with a combination of midazolam, fentanyl or diphenhydramamine. The bronchoscope was introduced through either the oropharynx or an endotracheal or tracheostomy tube when present. After introduction of the bronchoscope to the bronchial tree, a visual inspection was performed, followed by advancement of the bronchoscope to the lobe of lung to be lavaged. BAL was performed in the lung lobe deemed to be the most afflicted based on radiographic analysis of the chest and the opinion of the performing physician. BAL involved the instillation of 3 – 50 ml aliquots of normal saline through the suction channel of the bronchoscope, with lavage fluid recovered through suction. There may have been slight variability in the procedural details as determined by individual patient tolerance.

Nasopharyngeal wash was performed by standard techniques by a licensed respiratory therapist employed by the University of Kansas Medical Center. The patient was placed sitting upright with their head bent forward over a specimen cup. An aliquot of 3 ml sterile saline was then irrigated into one nare and after retention of the saline for ten seconds, the saline was allowed to flow by gravity into the specimen collecting cup. The procedure was then repeated in the opposite nare to produce the NPW specimen.

All patients included in this study had a respiratory viral panel (GenMark DX eSensor, GenMark Diagnostics, INC, La Place Court, CA), obtained on both specimens from an NPW and BAL within 5 days of each other. The RVP was performed by trained laboratory technicians as per manufacturer's instructions. Purified DNA/RNA was isolated from the patient specimen according to laboratory procedures, and the extracted nucleic acid was reverse transcribed and amplified using specific viral primers with RT-PCR enzyme mix. The amplified DNA was converted to single-stranded DNA using exonuclease digestion. This was then combined with a signal buffer containing ferrocene-labeled signal probes that were specific for different viral targets. The mixture of amplified sample and signal buffer was loaded onto a cartridge containing single-stranded oligonucleotide capture probes bound to gold-plated electrodes. The cartridge was inserted into the XT-8 instrument where the single-stranded targets hybridize to the complementary sequences of the capture probes and signal probes. The presence of each target was determined by voltammetry, which generated specific electrical signals from the ferrocene-labeled signal probe. Viruses that were detected by the GenMark DX eSensor RVP included influenza virus A, B, A H1N1, A sub H1, A sub H3; human rhinovirus; adenovirus B, C, E; coronavirus; human metapneumovirus; parainfluenza virus 1,2,3 and 4;and respiratory syncytial virus (RSV) A and B . The GenMark DX eSensor respiratory viral panel has been shown to have similar sensitivity and specificity as other commercially available multiplex panels [**5**].

Additionally, every patient included in this study had shell vial cultures performed on their BAL fluid. Viral cultures were performed by experienced personnel in the University of Kansas Medical Center microbiology department. R-Mix shell vials were used for the diagnosis of viral respiratory infections. R-Mix shell vials are an engineered tissue monolayer which provides equivocal data in the diagnosis of viral respiratory infections as conventional tissue culture and respiratory shell vial rhesus monkey cultures [**17**,**18**]. In this process, the R-Mix shell vials were warmed to 37°C for 6 hours, followed by adding 1 ml of room temperature R-Mix reefed media to the shell vials. Then 0.2 ml of patient specimen was added to each shell vial and the shell vials were centrifuged at 2000 RPM for 55 minutes. Following this process, the shell vials were placed in 37°C incubator for 48 hours. Shell vial monolayers were then spotted onto slides, fixed with acetone, stained with specific monoclonal antibodies and read under fluorescence microscopy. A positive result was defined by the presence of green fluorescence in 2 or more cells cytoplasm. Shell vial cultures were stained with specific antibodies to detect influenza virus A or B, RSV, adenovirus, and parainfluenzavirus 1, 2, and 3.

Other data collected included patient background information, past medical history and results of other diagnostic tests performed on BAL. All data was collected with the approval of the University of Kansas Medical Center institutional review board, project #12949.

Results

Fifty-eight immunocompromised patients were identified who met inclusion criteria for the study. All of the patients were tested with a BAL RVP and NPW RVP within 5 days of each other. The mean age of patients was 42.4 years (± 13.2 years) and there were 32 females and 26 males included. Thirty of the patients included in this study had undergone hematopoietic stem cell transplantation, while twenty-three were actively receiving chemotherapy. For full patient details (Table 1).

Age	42.4 Years (±13.2 years)
Male (Female)	26 (32)
History of Hematopoietic Stem Cell Transplantation	30
Actively Receiving Chemotherapy	23
History of Solid Organ Transplantation	3
HIV Infection	2
Neutropenic	27
BAL performed in Right Upper Lobe	13
BAL performed in Right Middle Lobe	22
BAL performed in Right Lower Lobe	7
BAL performed in Left Upper Lobe	9
BAL performed in Left Lower Lobe	7

Table 1: Patient Background Information

There was exact concordance between the BAL RVP and NPW RVP in 45 of the 58 cases (77.6%). In 26 of these cases, both panels were negative; whereas in 19 of the patients, both panels were positive for the exact same viruses. Six of these patients had parainfluenza virus and four patients each had rhinovirus and influenza virus. For full details of the viruses isolated (Table 2).

Virus		Number of specimens (BAL result/NPW result)		
		Positive/Positive	Positive/Negative	Negative/Positive
Single Detection	Adenovirus	0	1	2
	Coronavirus	1	0	1
	Influenza virus	4	1	0
	Metapneumovirus	0	0	0
	Parainfluenza virus	6	1	0
	RSV	2	1	0
	Rhinovirus	4	2	0
Multiple Detection	Adenovirus/Influenza	0	1	0
	Adenovirus/ Coronavirus/ RSV	0	0	1
	Coronavirus/ Metapneumovirus	1	1	0
	Metapneumovirus/RSV	0	0	1
	Parainfluenzavirus/RSV	1	0	0

Table 2: Viruses Detected by Respiratory Viral Panel

Viral cultures were collected on all patients who underwent both NPW RVP and BAL RVP. In total, only 6 respiratory viruses were detected by shell vial cultures: 3 patients had influenza virus detected, 2 had parainfluenza virus detected, and 1 patient had RSV detected. All of the respiratory viruses detected by viral culture were also present on both NPW and BAL RVP assay, as shown in Table 3.

Virus	Number Present on NPW RVP	Number Present on BAL RVP	Number Present on Shell Vial Culture
Adenovirus	3	2	0
Coronavirus	4	3	NA
Influenza Virus	4	7	3
Metapneumovirus	4	4	NA
Parainfluenza Virus	7	8	2
Respiratory Syncytial Virus	4	3	1
Rhinovirus	5	7	NA

Table 3: Respiratory viruses detected by viral culture on both NPW RVP and BAL RVP assay

Discussion

In this manuscript, we have identified that respiratory viral panels from NPW and BAL fluid reveal the exact same result in 77.6% of cases. In nearly one out of four cases, there was a discrepancy between the results returned from different respiratory specimens in the same patient.

The optimal specimen to be obtained to diagnose community-acquired respiratory viral infection is unclear from present data. BAL fluid is obtained from the lower respiratory tract and may be indicative of a viral presence in the alveoli and terminal airways, though obtaining BAL fluid may be associated with potential patient morbidity [**19**]. In contrast, NPW samples can be obtained safely in most patients. Whereas one study has shown an association between RSV upper respiratory tract infection and subsequent development of RSV pneumonia [**2**], this association has not been made with other viruses

All of the patients in this study were immunocompromised and being evaluated for an undiagnosed pulmonary disorder involving a new infiltrate on radiographic studies. Identifying the causative pathogen in these patients can help to direct specific therapies and to prevent excessive antimicrobial use. In the case of RSV, influenza and adenovirus infection, specific therapies with anti-viral drugs may be indicated once a specific diagnosis is made [**20-22**].

Another key finding of our study is the heightened sensitivity for detecting viral presence with the nucleic acid based RVP in comparison to the shell vial cultures. In our study, only 6 patients had viruses detected using the R-Mix shell vial cultures, as compared to 32 patients who had viruses detected via the RVP of either BAL or NPW fluid. Shell vial cultures have similar sensitivity as conventional cell culture, but significantly improve the time to diagnosis [**17**]. Our data supports previous works showing multiplex PCR to have greater sensitivity for diagnosing respiratory viral infection than shell vial cultures [**23**,**24**].

One area of uncertainty in this study is the influence of the time interval between obtaining specimens from NPW and BAL on the detection of viral presence. A previous study showed a median duration of viral shedding of 14 days for influenza virus, 16 days for metapneumovirus, 11 days for parainfluenza virus and 16 days for RSV [**25**]. This study utilized quantitative real-time PCR from NPW samples in recipients of hematopoietic stem cell transplantation. Another study of nasal secretions of children with expiratory wheezing showed that half of the patients with rhinovirus detected by PCR had persistent viral shedding when tested 2 weeks after the initial positive test [**26**]. We arbitrarily selected a 5 day interval between the 2 specimens being collected as a cutoff to be included in our study, though these studies suggest that viral shedding should still be occurring within this time period.

While viral shedding may last between 11 and 16 days on average, the ability to detect respiratory viruses by real-time PCR is significantly higher when performed within 6 days of symptom onset [**27**]. In an ideal situation, both NPW and BAL specimens would be obtained at the same time, within 6 days of symptom onset. This was not feasible in our population, as 95% of the patients underwent a NPW first, and only underwent fiberoptic bronchoscopy to obtain BAL specimens if the initial non-invasive testing was non-diagnostic, or if there was a concern for a second pathogen being present.

Limitations

Potential weaknesses in this study are related to its retrospective nature. Although all FOB with BAL and NPW collection processes were obtained in a protocoled manner, the procedures were performed by a variety of personnel. The procedures were not performed in a prospective manner to ensure exact compliance to protocol. Also, the lack of a true "gold standard" in the diagnoses of respiratory viral infection makes interpreting the positive results from the multiplex PCR assay difficulty: does a positive assay equate to a viral infection? Previous studies have shown that patients may be asymptomatically shedding both parainfluenza virus and rhinovirus [**25**,**26**]. Whether any of our patients included in this study happened to be incidentally shedding a virus whilst another pathogen caused their symptoms is difficult to judge from the currently available information.

Conclusion

We have shown a correlation rate of 77.6% between RVP assays of respiratory specimens obtained from BAL and NPW fluid in immunocompromised patients with evidence of lower respiratory tract disease. RVP was noted to have much better ability to detected respiratory viruses than the shell vial cultures. Performing RVP assays on respiratory specimens from both locations improves sensitivity in detecting respiratory viral presence.

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