Detection of respiratory viruses and the associated chemokine responses in serious acute respiratory illness

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ABSTRACT

Background A specific diagnosis of a lower respiratory viral infection is often difficult despite frequent clinical suspicion. This low diagnostic yield may be improved by use of sensitive detection methods and biomarkers.

Methods The prevalence, clinical predictors and inflammatory mediator profile of respiratory viral infection in serious acute respiratory illness were investigated. Sequential bronchoalveolar lavage (BAL) fluids from all patients hospitalised with acute respiratory illness over 12 months (n=283) were tested for the presence of 17 respiratory viruses by multiplex PCR assay and for newly discovered respiratory viruses (bocavirus, WU and KI polyomaviruses) by single-target PCR. BAL samples also underwent conventional testing (direct immunoflorescence and viral culture) for respiratory virus at the clinician's discretion. 27 inflammatory mediators were measured in a subset of the patients (n=64) using a multiplex immunoassay.

Results 39 respiratory viruses were detected in 37 (13.1% of total) patients by molecular testing, including rhinovirus (n=13), influenza virus (n=8), respiratory syncytial virus (n=6), human metapneumovirus (n=3), coronavirus NL63 (n=2), parainfluenza virus (n=2), adenovirus (n=1) and newly discovered viruses (n=4). Molecular methods were 3.8-fold more sensitive than conventional methods. Clinical characteristics alone were insufficient to separate patients with and without respiratory virus. The presence of respiratory virus was associated with increased levels of interferon γ-inducible protein 10 (IP-10) ($p<0.001$) and eotaxin-1 ($p=0.017$) in BAL.

Conclusions Respiratory viruses can be found in patients with serious acute respiratory illness by use of PCR assays more frequently than previously appreciated. IP-10 may be a useful biomarker for respiratory viral infection.

INTRODUCTION

Lower respiratory tract infection is a leading cause of hospitalisation.[1 2] Because it can cause high morbidity and mortality, especially in immunocompromised hosts,[3–5] prompt diagnosis is crucial for optimal clinical management and avoidance of unnecessary antibiotic use. Analysis of bronchoalveolar lavage (BAL) fluid is often used to assist the diagnosis in patients with unexplained acute respiratory illness. However, in current practice, an aetiological agent is often not found,[6 7] even in patients whose clinical presentation strongly suggest a viral aetiology. Despite frequent clinical suspicion, a specific viral diagnosis is rarely made. The failure to detect viral aetiologies could have multiple explanations, including difficulty in differentiating respiratory virus infection from other acute respiratory infections or diseases, lack of sensitivity of conventional detection methods (direct immunoflorescence and viral culture) and the existence of unrecognised infectious agents. Moreover, even when respiratory viruses are detected in the respiratory tract, it may be difficult to define their contribution to the patient's illness. Improvement in the diagnosis of acute respiratory viral infection may be achieved by the use of sensitive diagnostic methods or biomarkers associated with respiratory viral infection. Previous studies have shown that molecular-based methods may improve the sensitivity of respiratory virus detection.[8–11] However, some of these studies were retrospective in design, reporting enhanced detection of respiratory viruses in BAL samples, without performing a comprehensive clinical evaluation to assess the accuracy of the diagnosis. Moreover, previous studies did not address simultaneously the potential diagnostic utility of measuring inflammatory mediators in BAL fluid. Accordingly, we hypothesised that respiratory viruses would be found commonly in BAL fluid, and that the profile of inflammatory mediators would also be useful for making a diagnosis of lower respiratory virus infection in hospitalised patients. Here we applied comprehensive molecular testing for respiratory viruses and a multiplex immunoassay to measure multiple inflammatory mediators in BAL fluid from patients hospitalised with acute respiratory illness to establish an improved diagnostic approach.

METHODS

Patients and BAL collection

We included all adult patients hospitalised at Barnes-Jewish Hospital in St Louis, Missouri who underwent bronchoscopy with microbiological assessment of their BAL for the diagnosis of acute respiratory illness from October 2005 to October 2006. A total of 283 sequential BAL samples were prospectively collected. Patients were excluded only for the following reasons: outpatient status; bronchoscopy performed immediately postsurgery or

trauma; and collection of only bronchial wash samples. For patients who underwent a repeat bronchoscopy within 3 weeks of a first procedure, only the earlier sample was included. Fibreoptic bronchoscopy was performed by pulmonary or critical care physicians based on clinical judgement independent of this study. BAL was performed by instilling 100–150 ml of sterile saline into the distal airways at either the site of radiographic abnormality or the right middle lobe. BAL fluid samples were stored at 4°C in the Microbiology Laboratory after routine testing and were aliquoted and stored at −80°C. The study was approved by the Washington University Human Research Protection Office.

Respiratory virus detection

Viral RNA extraction

Viral nucleic acid extraction was performed with the Qiagen BioRobot M48 instrument using the MagAttract Virus Mini M48 kit (QIAGEN, Valencia, California, USA). A 200 μl aliquot of unprocessed BAL fluid was used and eluted to a final volume of 100 μl. A fixed amount of inactivated mouse hepatitis virus was included in each BAL sample during the extraction process to ensure quality of the nucleic acid extraction.

Conventional microbe and viral testing

All BAL samples underwent standard bacterial culture at the hospital Microbiology Laboratory. Selected samples were submitted to the hospital Virology Laboratory for virus detection according to physician judgement independent of this study. Those samples (n=197) underwent conventional viral testing (direct immunofluorescence assay and viral culture), as described previously.[12]

Multiplex PCR for respiratory viruses

The Multicode-PLx Respiratory Virus Panel (Multicode-PLx RVP; EraGen, Madison, Wisconsin, USA) was used for detection of influenza virus (type A and B), respiratory syncytial virus (RSV: type A and B), parainfluenza virus (types 1–4a,b), human metapneumovirus (MPV), adenovirus (groups B, C and E), coronavirus (OC43, NL 63 and 229E) and rhinovirus, as previously described.[13 14]

Single-target quantitative real-time PCR

Single-target quantitative real-time PCR was used for detection of bocavirus, and the newly described human polyomaviruses KI virus (KIV) and WU virus (WUV). Further details are provided in the online supplement.

Multiplex immunoassay for inflammatory mediators

We measured the levels of multiple inflammatory mediators in the 32 samples that were positive for a respiratory virus, excluding three samples positive solely for bocavirus, WUV or KIV, and two samples for which sample quality was suboptimal for this analysis. In addition, we randomly selected 32 BAL samples without a respiratory virus as a control group, using a computer-based random selector (STATA, StataCorpLP, Texas, USA). The demographic characteristics of the patients who were randomly selected were not different from those who were not selected (data not shown). We analysed cell-free supernatants in a multiplex flow cytometry-based assay (BioRad Bio-Plex Human 27 Panel) as previously described.[15 16] Further details are provided in the online supplement.

Statistical analysis

Descriptive data were expressed as mean±SD, or median (IQR) for non-normally distributed data. χ^2 or the Fisher exact test were used to compare categorical variables. Continuous variables were compared using the unpaired t test performed on raw values or on log-transformed data if log transformation produced approximately normal distribution. For non-normally distributed data, the Wilcoxon rank sum test (Mann–Whitney U test) was used to compare two groups. Receiver operating curve (ROC) analysis was performed to evaluate the optimal cut-off level of each inflammatory mediator to differentiate the groups with respiratory virus and without respiratory virus. All statistical tests were two-tailed, and p values of <0.05 were considered statistically significant. These analyses were performed using the STATA version 9 for Macintosh (StataCorp).

RESULTS

Patient characteristics

Demographic and clinical characteristics of 283 patients are shown in table 1. The majority of patients had illness severe enough to require care in an intensive care unit (67.8%) with mechanical ventilation (67.1%), and the overall in-house mortality rate was 25%, reflecting the severity of illness in our study population. Nearly half of the patients were immunosuppressed, defined by the presence of one of the following: organ transplant recipient; on immunosuppressive medications (including systemic corticosteroids in a dose >10 mg of prednisone per day, or other immunosuppressive medications); receipt of chemotherapy within 12 months; or HIV infection. Almost all were treated with antibiotics for the acute respiratory illness.

Table 1 Demographic and clinical characteristics of patients (n=283)

	n (%)
Mean age (years ± SD)	55±15
Male	161 (56.9)
ICU	192 (67.8)
Mechanical ventilation	190 (67.1)
Immunosuppressive state	131 (46.3)
Antibiotic treatment	271 (95.8)
Median hospital length of stay (median, IQR)	19 (10–34)
In-hospital mortality (%)	71 (25.1)
Underlying disease/condition	
Solid organ malignancy	40 (14.1)
Haematological malignancy	39 (13.8)
Lung transplantation	37 (13.1)
Lung disease	34 (12.0)
Other solid organ transplantation	13 (4.6)
Autoimmune disease	13 (4.6)
HIV	11 (3.9)
Others	78 (27.6)
No underlying disease	14 (5.3)
Uncertain	4 (1.4)
Chest radiograph	
Any abnormality	283 (100)
Multifocal infiltrates or consolidation	99 (35.0)
Diffuse infiltrates	77 (27.2)
Respiratory diagnosis	
Pneumonia	153 (54.1)
Interstitial lung diseases	30 (10.6)
ARDS	19 (6.7)
Lung transplant complication/rejection	13 (4.6)
Pulmonary haemorrhage	9 (3.2)
Cardiac decompensation	8 (2.8)
Malignancy	8 (2.8)
Others	43 (15.2)

ARDS, acute respiratory distress syndrome; ICU, intensive care unit.

Most of the patients had underlying conditions: solid organ malignancy was the most common followed by haematological malignancy and lung transplantation. All patients had abnormal chest radiographs, with >60% showing multifocal or diffuse abnormality. The patient's respiratory diagnoses assigned by the clinicians were highly variable, with the most common diagnosis being pneumonia.

Detection of respiratory viruses by molecular testing

Thirty-nine viruses were detected in 37 (13.1%) patients by molecular testing (table 2). There were two cases of dual infection. Rhinovirus was the most frequently found virus, followed by influenza virus, RSV and human MPV. KIV was found in two samples and human bocavirus and WUV were each found in one. Rhinovirus was found year-around, but the other viruses were found mainly during the winter and spring months (February to April) (Figure E1 online).

Yield of respiratory virus detection by molecular testing

To compare molecular and conventional viral testing directly, we analysed BAL samples for which both molecular and conventional methods were performed (n=197). A total of 31 respiratory viruses were found by either type of testing. Molecular testing detected all 31 viruses (100%), while conventional methods detected only 8 viruses (26%), corresponding to a 3.8-fold increase in yield by molecular testing. This increased yield resulted both from the detection of newly discovered viruses not found by conventional testing (MPV, coronaviruses, KIV and WUV), and from enhanced sensitivity for 'classic' respiratory viruses (table 3).

Clinical characteristics of the patients with respiratory virus

To determine if there were clinical features that could identify illnesses associated with a respiratory virus, we compared demographic features, clinical characteristics and disease severity measurements in cases with a respiratory virus (n=34) with those without a respiratory virus (n=246) (table 4). Three BALs with human bocavirus, WUV, and KIV alone were excluded from this analysis since the clinical role of these viruses was not established at the time of submission of this manuscript. The overall proportions of immunosuppressed patients were not different, but there were significantly more lung transplant recipients in the respiratory virus-positive group (23.5% vs 11.4%, p=0.047). Interestingly, the respiratory virus-positive group had multifocal infiltrates or consolidation more frequently (50.0% vs 33.3%) and diffuse infiltrates less frequently (11.8% vs 24.4%). Respiratory viruses were found significantly less frequently during the summer months (8.8% vs 21.9%, p=0.042). No measures of severity were different between the two groups. The proportions of patients receiving antibacterial or antifungal agents were also similar. Overall, there were no clinically useful characteristics that distinguished patients with and without a respiratory virus.

Table 2 Respiratory viruses detected in BAL fluid

Respiratory virus	Number (%)
Rhinovirus	13 (4.6)
Influenza A or B	8 (2.8)
RSV	6 (2.1)
MPV	3 (1.1)
Coronavirus NL63	2 (0.7)
Parainfluenza	2 (0.7)
KI virus*	2 (0.7)
Adenovirus	1 (0.4)
Bocavirus*	1 (0.4)
WU virus*	1 (0.4)
Total	39 (13.1)†

The numbers in parentheses represent the percentage of virus-positive cases in the total study population (n=283).
*Detected by single-target PCR. The rest of the viruses were detected by Multicode-PLX.
†2 cases of dual infection (parainfluenza and influenza B; rhinovirus and KI virus) were included in the total of each virus.
BAL, bronchoalveolar lavage; MPV, metapneumovirus; RSV, respiratory syncytial virus.

Inflammatory mediator profile

Since clinical characteristics did not appear to be sufficient to identify patients with respiratory virus infection, we evaluated the inflammatory mediator profile in BAL fluid. As shown in table 5, measurable levels were present for 20 out of 27 inflammatory mediators. Among these, interferon γ-inducible protein 10 (IP-10) and eotaxin-1 were present at significantly higher levels in patients in whom a respiratory virus was detected (IP-10: p<0.001, eotaxin-1: p=0.017) (figure 1). When examined by virus, no particular virus type was individually associated with high levels of IP-10 or eotaxin-1 (data not shown). A positive correlation was seen between eotaxin-1 and IP-10 concentrations (r=0.56, p<0.0001).

IP-10 and eotaxin-1 as predictors of respiratory virus detection

We next investigated whether the concentration of IP-10 or eotaxin-1 in BAL could be used to differentiate patients with respiratory virus from patients without respiratory virus in BAL in the subpopulation in which the inflammatory mediators were measured (n=64). ROC analysis was used to determine the optimal cut-off values. The optimal cut-off value for IP-10 was 1700 pg/ml, with an area under the ROC curve of 0.76 (95% CI 0.64 to 0.87). Defining an IP-10 level greater than or equal to this value as positive resulted in a sensitivity of 81.3% (95% CI 71.7 to 90.7) and specificity of 59.4% (95% CI 47.3 to 71.4), with a positive likelihood ratio (LR) of 2.00 and a negative LR of 0.32. ROC analysis for eotaxin-1 concentration in BAL revealed that the optimal cut-off value was 5 pg/ml, with an area under the ROC curve of 0.63 (95% CI 0.50 to 0.77). Defining an eotaxin-1 level greater than or equal to this value as positive resulted in a sensitivity of 75.0% (95% CI 64.4 to 85.6) and a specificity of 46.9% (95% CI 34.6 to 59.1), a positive LR of 1.41 and a negative LR of 0.53, indicating that the eotaxin-1 level was less predictive of the presence of a respiratory virus compared with the IP-10 level.

Table 3 Respiratory viruses detected in BAL samples (n=197) tested by both molecular and conventional methods

Virus	Detection method		Total detected
	Molecular	DFA/culture	
Rhinovirus	10	2	10
Influenza virus	5	2	5
RSV	5	3	5
MPV	3	Not tested	3
Coronavirus NL63	2	Not tested	2
Parainfluenza virus	2	1	2
KI virus	2	Not tested	2
Adenovirus	1	0	1
WU virus	1	Not tested	1
Total (yield%)	31 (100%)	8 (26%)	31

BAL, bronchoalveolar lavage; DFA, direct immunoflorescence assay; MPV, human metapneumovirus; RSV, respiratory syncytial virus.

Table 4 Clinical characteristics and disease severity in patients with and without respiratory virus

Clinical characteristics	Respiratory virus (+) n=34	Respiratory virus (−) n=246	p Value
Baseline features			
Mean age	55.7±14.0	55.0±15.1	0.800
Age >65	8 (23.5)	63 (25.6)	0.794
Male, n (%)	20 (58.8)	139 (56.5)	0.798
Immunosuppressive state	20 (58.8)	110 (44.7)	0.122
Lung transplant recipient	8 (23.5)	28 (11.4)	0.047
Haematological malignancy	5 (14.7)	34 (13.8)	0.889
Admitted to medical service	28 (82.4)	182 (74.0)	0.291
Chest radiograph			
Multifocal infiltrates or consolidation	17 (50.0)	82 (33.3)	0.057
Diffuse infiltrates	4 (11.8)	72 (29.3)	0.032
Focal findings	4 (11.8)	23 (9.4)	0.655
BAL testing			
BAL done >7 days after hospitalisation	8 (23.5)	81 (32.9)	0.270
Sample sent for routine viral detection	7 (20.6)	78 (31.7)	0.186
Microbiological findings in BAL fluid			
No microorganism detected	12 (35.3)	107 (43.5)	0.365
Bacterial/fungal pathogen detected	13 (38.2)	84 (34.2)	0.639
Seasonality			
Winter–spring season (November–April)	24 (70.6)	154 (62.6)	0.364
Summer months (June–August)	3 (8.8)	60 (24.4)	0.042
Severity			
In-hospital mortality	7 (20.6)	64 (26.0)	0.495
On mechanical ventilation	21 (61.8)	167 (68.2)	0.456
Requiring oxygen	31 (91.2)	230 (93.9)	0.548
Days of ICU stay (median, IQR)	9 (0–22)	18 (0–24)	0.371
Days of hospitalisation (median, IQR)	16 (99–30)	19.5 (11–35)	0.237
Any use of antibiotics	34 (100)	236 (96.3)	0.256
Days of antibiotic use (median, IQR)	13 (8–28)	15 (7–28)	0.655
Any use of antifungal agents	18 (52.9)	121 (49.2)	0.682

Values are number (percentage of column total), mean±SD or median (IQR) for continuous variables.
BAL, bronchoalveolar lavage.

DISCUSSION

In this study, we have made several important findings with regard to the role of respiratory viruses in adult patients with serious acute respiratory illness: (1) highly sensitive molecular testing detected a respiratory virus in 13% of hospitalised patients who underwent bronchoscopy for acute respiratory illness; (2) these patients could not be accurately identified by their clinical features alone and were usually not identified by conventional viral diagnostic testing; and (3) two inflammatory mediators, IP-10 and eotaxin-1, were present at elevated levels in BAL of patients with respiratory virus.

By using highly sensitive molecular methods, we were able to assess the prevalence of lower respiratory virus infection in the inpatient adult hospital setting in a comprehensive manner. The frequency of respiratory viral infection in the present study was similar to that in a recent study of hospitalised adults in University hospitals in Switzerland, in which respiratory viruses were detected by molecular methods in 17% of BAL samples.[17] In that study, coronavirus was the most common virus detected, followed by rhinovirus and parainfluenza virus. In contrast, rhinovirus was the most common respiratory virus detected in our study and coronaviruses were detected infrequently. Rhinovirus has traditionally been thought to cause mainly upper airway disease, but our frequent recovery of rhinovirus from the lower respiratory tract is in agreement with recent studies that support the fact that rhinovirus is also an important lower respiratory tract pathogen.[18 19] However, it is also important to note that in our population of hospitalised patients with serious acute respiratory illness, the clinical significance of rhinovirus infection appeared to be less clear than that of 'traditional' respiratory viruses such as influenza virus and RSV. The differences in viruses detected between the present study and the Swiss study could be due to geographical or seasonal differences, or to the fact that our study was directed at a hospitalised population with more severe disease. We also did not test for coronavirus HKU1. We did test for other newly described viruses including human bocavirus and the WU and KI polyomaviruses, and found that these viruses were uncommon in adults with serious respiratory illness.

Our careful review of the clinical courses of our patients revealed that clinical characteristics alone did not distinguish patients in whom respiratory virus was detected. Likewise, in the Swiss study, most clinical characteristics, with the exception of lack of infiltrates on chest x-ray and lack of antibiotic treatment response, also did not predict the presence of respiratory virus infection.

This difficulty in predicting the presence of respiratory viral infection based on clinical characteristics led us to investigate the use of biomarkers. Recent advances in multiplex assay technology allowed us simultaneously to quantify 27 inflammatory mediators in an unbiased fashion. Using the BioPlex assay, we found that two mediators, IP-10 (also known as CXCL10) and eotaxin-1, were both associated with the presence of respiratory virus. This is the first study of which we are aware that evaluated the concentration of multiple inflammatory mediators in BAL from patients with respiratory virus infection.

IP-10 is a ligand for the CXCR3 receptor, and acts as a chemoattractant for activated T helper 1 (Th1) cells, and natural killer cells.[20–22] It has been shown to play an important role in the host

Table 5 Inflammatory mediator concentration in BAL fluid

Mediator (pg/ml)	Respiratory virus (+) n=32	Respiratory virus (−) n=32	p Value
IL-1	6.35 (3.09–31.22)	6.58 (1.24–36.65)	0.552
IL-1α	534.55 (292.43–1753.36)	524.84 (326.94–1040.44)	0.501
IL-2	Not detected	Not detected	
IL-4	Not detected	Not detected	
IL-5	Not detected	Not detected	
IL-6	22.79 (4.09–194.46)	32.46 (5.16–310.08)	0.981
IL-7	1.61 (1.19–2.10)	1.66 (1.36–2.78)	0.390
IL-8	360.61 (161.00–1592.96)	204.85 (68.92–1264.6)	0.464
IL-9	7.98 (2.68–14.44)	3.24 (0.63–9.61)	0.085
IL-10	4.00 (2.49, 7.91)	2.945 (1.33–4.64)	0.170
IL-12	5.45 (3.31–9.45)	4.25 (2.04–5.94)	0.065
IL-13	1.856 (1.24–3.18)	1.75 (0.975–2.85)	0.435
IL-15	1.82 (0.07–8.94)	0.915 (0.2–2.88)	0.363
IL-17	6.20 (0–24.48)	Not detected	
Basic FGF	Not detected	Not detected	
Eotaxin-1	6.98 (4.71–25.94)	5.39 (2.44–11.78)	0.017
G-CSF	23.55 (7.85–97.77)	14.26 (6.45–76.03)	0.421
GM-CSF	Not detected	Not detected	
IFNγ	12.21 (2.95–34.84)	9.81 (0.99–19.57)	0.144
IP-10	8902.33 (2059.07–57015.20)	1246.82 (305.73–4811.82)	<0.001
CCL2/MCP-1	259.50 (61.71–1583.27)	240.03 (19.33–578.28)	0.296
CCL3/MIP-1α	Not detected	Not detected	
CCL4/MIP-1β	60.55 (29.16–179.11)	44.00 (27.72–87.79)	0.115
PDGF-BB	12.94 (6.38–20.60)	11.80 (4.81–19.00)	0.838
CCL5/RANTES	66.39 (25.41–119.57)	31.59 (11.23–97.15)	0.385
TNFα	9.98 (0–24.57)	1.63 (0–7.07)	0.108
VEGF	36.42 (15.13–80.55)	35.37 (12.25–70.86)	0.995

Values were expressed as median (IQR).
Not detected indicates the level was beneath the lower limit of detection of the assay.
BAL, bronchoalveolar lavage; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor IFNγ, interferon γ; IL, interleukin; IP-10, interferon γ-inducible protein 10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T cell expressed and secreted; TNFα, tumour necrosis factor α; VEGF, vascular endothelial growth factor.

response to a variety of viral infections including rhinovirus,[23] RSV,[24 25] herpes simplex virus[26] and hepatitis C virus.[27 28] Previous studies have demonstrated that IP-10 is released from cultured human airway epithelial cells in response to rhinovirus[23] and H5N1 influenza infection,[29] and is detected in respiratory samples of patients with rhinovirus upper airway infection[23] and RSV bronchiolitis.[24] Moreover, recent studies suggest that the serum IP-10 level may be an important biomarker for various virus infections. In patients with chronic hepatitis C infection, the serum IP-10 level has been shown to predict treatment response.[27 28 30] A study of patients with asthma exacerbations suggested that the serum IP-10 level could be a useful biomarker in differentiating virus-induced acute asthma from non-virus-induced acute asthma.[31] A recent study in patients with chronic obstructive pulmonary disease (COPD) showed that the serum IP-10 level may be a useful biomarker for rhinovirus-induced COPD exacerbation.[32] Our study extends these observations to its potential use in the diagnosis of respiratory viral infection in adults hospitalised with acute respiratory illness. We found that higher levels of IP-10 in BAL were associated with the presence of a respiratory virus, indicating that measurement of the IP-10 level may be useful to differentiate patients with respiratory virus in

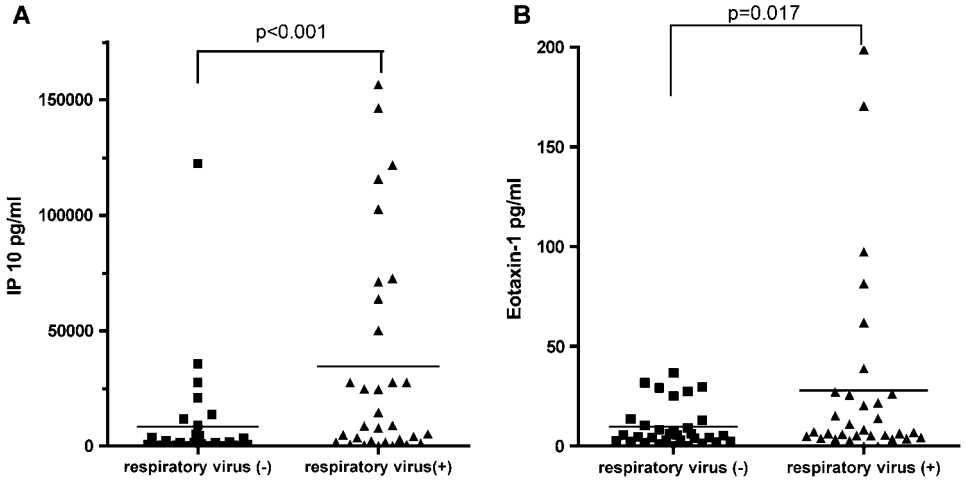

Figure 1 Levels of interferon γ-inducible protein 10 (IP-10) and eotaxin-1 in bronchoalveolar lavage (BAL). IP-10 (A) and eotaxin-1 (B) concentration in BAL samples in patients with respiratory virus (filled squares) and without respiratory virus infection (filled triangles). IP-10 and eotaxin-1 were significantly increased in the respiratory virus-positive group among the 27 inflammatory mediators tested by a multiplex flow cytometry-based assay. Horizontal lines indicate the mean levels in each group.

BAL, especially in a setting when the conventional methods for viral detection are unrevealing.

The other inflammatory mediator that was elevated in patients with respiratory virus infection was eotaxin-1, which was less predictive of the presence of a respiratory virus than was the IP-10 level. Eotaxin-1 is a selective chemoattractant for eosinophils, and is often implicated in allergic responses.[33] Previous studies have demonstrated that eotaxin-1 is produced by bronchial epithelial cells in response to infection by respiratory viruses including influenza virus[34] and rhinovirus,[35] but further investigation is needed to determine the overall role of eotaxin-1 and eosinophils in the host response to viral infection.

The major limitation of this study is that it was not possible to assess the clinical significance of the viruses that were detected. Most of the patients were very ill and had multiple possible explanations for their respiratory illness. Some of the viruses detected, such as influenza A and RSV, appeared to be generally pathogenic. The clinical significance of others, such as rhinovirus and the coronaviruses, was less certain. The inability to assess the clinical significance of the viruses detected also made it difficult to assess whether the levels of IP-10 and eotaxin-1 correlated with the severity of viral infection. In addition, we were able to measure the inflammatory mediators only in a subgroup of the study population. Most of the viral-positive samples were studied (n=32), but we only studied randomly selected samples (n=32) from the virus-negative group of 286. Even though the main clinical characteristics of the randomly selected population were not different from those who were not selected, the finding of the inflammatory mediators may not be generalisable to the whole study population. ROC analysis was also only performed in the subpopulation.

In summary, this study found that comprehensive molecular testing can detect respiratory viruses in BAL fluid frequently and much more effectively than conventional diagnostic methods in patients hospitalised with acute respiratory illness. Measurement of IP-10 may be a useful biomarker for viral infection, although it does not appear that it can be used as a single marker. Further study is needed to confirm our findings and define an appropriate cut-off level for IP-10 in a larger population and also to evaluate its role in assessing the clinical importance in the diagnosis of viral infection.

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