Journal of Clinical Virology 59 (2014) 208–211

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short Communication

Epidemiology of respiratory viruses in bronchoalveolar lavage samples in a tertiary hospital

Stefanie Drieghe[a], Inge Ryckaert[a], Kurt Beuselinck[b], Katrien Lagrou[b], Elizaveta Padalko[a,c,*]

[a] Department of Clinical Chemistry, Microbiology and Immunology, University Hospital Ghent, De Pintelaan 185, 9000 Ghent, Belgium
[b] Department of Microbiology & Immunology, KU Leuven and Clinical Department Laboratory Medicine University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium
[c] School of Life Sciences, Hasselt University, Agoralaan Building D, 3590 Diepenbeek, Belgium

ARTICLE INFO

Article history:
Received 13 July 2013
Received in revised form 28 October 2013
Accepted 23 December 2013

Keywords:
Bronchoalveolar lavage
Epidemiology
Multiplex PCR
Respiratory infection
Laboratory diagnosis

ABSTRACT

Background: The prevalence of respiratory viruses in adults is largely underexplored, as most studies focus on children. Additionally, in severely ill or immunocompromised adults, where respiratory infections are mostly attributed to bacteria and fungi; respiratory viruses can lead to severe complications.
Objectives: To evaluate the epidemiology of respiratory viruses in bronchoalveolar lavage fluid (BAL) specimens from patients with lower respiratory tract disease. The study population consisted of different groups including immunocompetent patients (control patients), solid organ transplant recipients, patients with haematological malignancies and other immunocompromised adults.
Study design: A total of 134 BAL fluid specimens collected during 2009–2011 were retrospectively assessed with the new commercial multiplex real-time PCR FTD Respiratory 21 Plus®, targeting 18 different viruses and 2 atypical bacterial pathogens.
Results: Viral or atypical bacterial pathogens were detected in 29.1% of BAL fluid specimens. Coronaviruses were most prevalent (13.4%), followed by rhinoviruses (5.2%), RSV (4.5%) and bocaviruses (3.7%). Comparing the total number of viruses detected, a statistically significant difference was observed between the control group and patients with haematological malignancies (27.5% vs. 57.1%, $p < 0.05$).
Conclusion: In conclusion, our study highlights the high prevalence of respiratory viruses in BAL fluid specimens from adult patients with lower respiratory tract disease. The methods to be used should be sensitive and cover a wide range of potential pathogens. The specific patient population can also influence the detection rates of respiratory viruses.

1. Background

Respiratory syncytial virus (RSV), influenza (Flu), adenoviruses (AdV), human metapneumovirus (hMPV), parainfluenza viruses and human rhinoviruses (hRV) are considered to be important pathogens in the aetiology of respiratory infections [1–4]. During the past decade, improvements in detection techniques have contributed to an increase in sensitivity and discovery of new respiratory viruses, such as hMPV, novel strains of coronaviruses (SARS-hCoV, hCoV-NL63 and hCoV-HKUI, MERS-virus), human bocavirus (hBoV) and novel polyomaviruses (WU and KI) [1,5–7].

However, the prevalence of respiratory viruses in adults is still largely underexplored, as most studies focus on children, while in severely ill or immunocompromised adults respiratory viruses can also lead to severe complications.

2. Objectives

In the present study, we evaluated the epidemiology of respiratory viruses in bronchoalveolar lavage fluid (BAL) specimens from patients with lower respiratory tract disease (in- and outpatients) using a new commercial qualitative multiplex real-time PCR FTD Respiratory 21 Plus® (Fast-track Diagnostics, Junglinster, Luxembourg), targeting 18 different viruses and 2 atypical bacterial pathogens. In addition, we assessed the epidemiology

Abbreviations: PCR, polymerase chain reaction; BAL, bronchoalveolar lavage fluids; hRV, rhinoviruses; RSV, respiratory syncytial virus; hBoV, bocavirus; hCoV, coronaviruses; AdV, adenoviruses; Flu, influenza; hMPV, human metapneumovirus; PIV, parainfluenza; hEV, enteroviruses; hPeV, parechoviruses; *Mpp*, *Mycoplasma pneumoniae*; *Cpp*, *Chlamydophila pneumoniae*; Ct, cycle threshold; RT, reverse-transcriptase; QCMD, Quality Control for Molecular Diagnostics.

* Corresponding author at: Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, De Pintelaan 185 (2P8), B-9000 Ghent, Belgium. Tel.: +32 9 332 21 08; fax: +32 9 332 49 85.
E-mail address: elizaveta.padalko@uzgent.be (E. Padalko).

1386-6532/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jcv.2013.12.008

of respiratory viruses in different patient populations at high risk for complications, including solid organ transplant recipients, patients with haematological malignancies and other immunocompromised conditions.

3. Study design

3.1. Clinical specimens

A total of 134 BAL fluid specimens from 129 patients admitted to the University Hospital of Ghent with lower respiratory tract infections, during three consecutive respiratory seasons (2009–2011), were analysed. Bronchoscopy was performed by a team of pulmonologists following a standardised protocol: 20 mL sterile saline solution was instilled 5 times into the distal bronchial tree with a maximal recovery of the instilled volume. Gram staining was performed to evaluate sample quality (magnification 10×) and for direct identification of bacteria and fungi. All samples were stored at −70 °C and retrospectively analysed in the spring of 2012 with the commercial multiplex real-time PCR FTD Respiratory 21 Plus®.

The subjects were enrolled in different patient populations according to underlying conditions. Six groups were defined: (i) no immunosuppressive conditions (control group), (ii) acute myeloid leukaemia (AML), (iii) haematopoietic stem cell transplant recipients, (iv) other haematological malignancies, (v) solid organ transplant recipients and (vi) other immunosuppressive conditions. For detailed composition of disease groups, see Table 2. Patient ages ranged between 22 and 83 years; with 57% of the subjects being between 51 and 70 years, 18% were between 31 and 50 years, 17% were older than 70 years, and only 7% were adults between 22 and 30 years.

3.2. FTD Respiratory 21 Plus®

FTD Respiratory 21 Plus® was used according to manufacturer's instructions (Fast-track Diagnostics, Junglinster, Luxembourg) following total nucleic acid extraction performed by NucliSens EasyMAG™ (BioMérieux, Lyon, France); allowing simultaneous detection and identification of the following respiratory viruses: Flu A (separate detection of Influenza A/H1N1) and Flu B (Flu), hRV, hCoV 229E, NL63, HKU1 and OC43, PIV 1, 2, 3 and 4, hMPV, hBoV, AdV, RSV, Enteroviruses (hEV), Parechoviruses (hPeV), *Chlamydophila pneumoniae* (*Cpp*) and *Mycoplasma pneumoniae* (*Mpp*).

Evaluation of the FTD Respiratory 21 Plus® assay with description of the performance characteristics is added in Supplementary File 1.

3.3. Statistical analysis

Data were analysed using MedCalc® (MedCalc Software, Mariakerke, Belgium). Comparison of proportions (Chi-square) was used to compare detection rates between the different populations; results with a $p < 0.05$ were considered significant.

4. Results

Viral or atypical bacterial pathogens were detected in 39/134 BAL fluid specimens (29.1%), ranging from 23.2% to 37.0% for the different respiratory seasons (2009–2011). Single pathogens were found in 30/39 (76.9%) of the samples, whereas infection with multiple pathogens was less frequently observed (9/39 samples, 23.1%). In 7/9 (77.8%) patients, two different viruses were detected concomitantly, whereas three viruses were detected in 2/9 (22.2%) patients. On the totality of BAL fluid specimens, the viral distribution at genus level was as follows: hCoV (43, 229, 63 and HKU) (13.4%) and hRV (5.2%) were most frequently encountered, followed by RSV (4.5%) and hBoV (3.7%). Flu (A, A/H1N1, B) (2.2%), AdV (2.2%), PIV (1, 2, 3 and 4) (1.5%), hMPV (1.5%), hEV (1.5%) and hPeV (1.5%) were detected in only a limited number of samples (≤3.0%) (Table 1).

Table 1
Epidemiology and prevalence of respiratory viruses in BAL fluid specimens (2009–2011).

Year	2009	2010	2011	2009–2011
Number of BAL tested	69	38	27	134
Total positives	16 (23.2%)	13 (34.2%)	10 (37.0%)	39 (29.1%)
Single infections	15 (93.8%)	11 (84.6%)	4 (40.0%)	30 (76.9%)
Co-infections	1 (6.3%)	2 (15.4%)	6 (60.0%)	9 (23.1%)
hRV	3 (4.3%)	3 (7.9%)	1 (3.7%)	7 (5.2%)
RSV	2 (2.9%)	1 (2.6%)	3 (11.1%)	6 (4.5%)
hBoV	1 (1.4%)	1 (2.6%)	3 (11.1%)	5 (3.7%)
AdV	1 (1.4%)	0 (0.0%)	2 (7.4%)	3 (2.2%)
hCoV	6 (8.7%)	7 (18.4%)	5 (18.5%)	18 (13.4%)
Flu	3 (4.3%)	0 (0.0%)	0 (0.0%)	3 (2.2%)
hMPV	0 (0.0%)	2 (5.3%)	0 (0.0%)	2 (1.5%)
PIV	1 (1.4%)	0 (0.0%)	1 (3.7%)	2 (1.5%)
hEV	0 (0.0%)	1 (2.6%)	1 (3.7%)	2 (1.5%)
hPeV	0 (0.0%)	0 (0.0%)	2 (7.4%)	2 (1.5%)
Mpp	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cpp	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

BAL, bronchoalveolar lavage fluids; hRV, rhinoviruses; RSV, respiratory syncytial virus; hBoV, bocavirus; hCoV, coronaviruses; AdV, adenoviruses; Flu, influenza; hMPV, human metapneumovirus; PIV, parainfluenza; hEV, enteroviruses; hPeV, parechoviruses; *Mpp*, *Mycoplasma pneumoniae*; *Cpp*, *Chlamydophila pneumoniae*.

The epidemiology of respiratory viruses in BAL fluid specimens in different patients groups is presented in Table 2. Viral pathogens were detected in 23.5% of the BAL fluid specimens for the control group compared with 32.5% for the total disease group (not statistically significant). Comparing the proportion of positive BAL samples between the control group and the different patient populations, a statistically significant difference was observed for patients with other haematological malignancies (23.5% vs. 50.0%, $p < 0.05$). Single infections were more frequent observed in the control group compared with the disease group (83.3% vs. 74.1%, not statistically significant). In addition, when comparing the total number of viruses detected between the control group and the different patient populations, a statistically significant difference was observed for patients with other haematological malignancies (27.5% vs. 58.3%, $p < 0.05$) and for all haematological malignancies (27.5% vs. 57.1%, $p < 0.05$).

5. Discussion

The prevalence of respiratory viruses in adults is largely underexplored, as most studies focus on infants and children. In the present study, respiratory viruses were recovered in 29.1% of the BAL fluid specimens, ranging from 23.2% to 37.0% for the different years. The reported detection rates of respiratory viral infections using molecular assays range from 3.6% to 42.2%, what is in line with our findings [3,4,8–17]. Differences can be explained by the heterogeneity of the included population, the specimen type, the number of viruses simultaneously tested and the method used.

The importance of the specimen type is highlighted in several studies. In BAL specimens a diagnostic yield ranging from 3.6% to 32.0% was reported [3,4,10]. Soccal et al. evaluated paired nasopharyngeal and BAL fluid specimens and observed an overall viral positivity rate of 29.3% in the upper respiratory tract specimens and 17.2% in the BAL samples ($p < 0.001$) [11].

Composition of study population has major influence on the observed detection rates [10–17]. Garbino et al. assessed the prevalence of respiratory viruses in different groups of hospitalised