Respiratory viruses in bronchoalveolar lavage: a hospital-based cohort study in adults

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ABSTRACT

Background: The epidemiology of respiratory viruses and their potential clinical impact when recovered in lower respiratory specimens has not been established in the hospital setting. A study was performed to investigate the association between positive viral detection and respiratory infection in an at-risk population.

Methods: 299 adult patients who underwent bronchoalveolar lavage (BAL) procedures were enrolled in a hospital-based prospective cohort study. Descriptive epidemiology is presented of 17 different respiratory viruses detected by reverse transcription-polymerase chain reaction assays in BAL fluid specimens. Multivariate analysis was conducted to identify the clinical characteristics independently associated with the presence of virus.

Results: Of 522 BAL fluid specimens analysed, 81% were collected in adult transplant recipients or other immunocompromised patients. Overall, PCR assays identified viral nucleic acid in 91 BAL fluid samples (17.4%). Similar rates of virus-positive BAL fluid were found in the different subpopulations studied ($p = 0.113$). Coronaviruses were the most frequent (32.3%), followed by rhinovirus (22.6%), parainfluenza (19.5%), influenza (9.7%), respiratory synctial virus (8.6%), human metapneumovirus (4.2%) and bocavirus (3.1%). Multivariate analysis using mixed models showed that respiratory viral infections were associated with a lack of antibiotic treatment response (OR 2.2, 95% CI 1.2 to 4.1) and the absence of radiological infiltrate (OR 0.3, 95% CI 0.2 to 0.8). In lung transplant recipients in whom a respiratory infection was suspected, the respiratory viral detection rate was 24.4% compared with 13.8% overall in other patients ($p = 0.02$).

Conclusions: In this cohort of hospitalised adults, respiratory viruses detected in BAL fluid specimens were associated with respiratory symptoms, absence of radiological infiltrates and a poor response to antibiotic therapy.

Most respiratory tract infections acquired in the community are of viral aetiology. In healthy adults the disease is generally self-limited and restricted to the upper respiratory tract. However, respiratory viruses also cause lower respiratory tract illnesses and even the so-called "common cold" viruses like rhinovirus and coronavirus can infect the lower respiratory airways.[1–4] These viral infections are well known to promote complications such as bacterial infections or an exacerbation of a pre-existing chronic lung disease such as asthma.[5]

Lower respiratory tract infections are among the most frequent causes of hospitalisation and, although a significant number of cases are presumably linked to an initial viral infection acquired in the community, the viral aetiology is rarely evidenced.[6–8] This is mainly related to the complexity and the costs of nucleic acid detection procedures, as well as the need for appropriate lower respiratory tract specimens.[9] In particular, the role of recently discovered viruses such as human metapneumovirus (HMpV), coronavirus NL 63 (HCoV-NL63) and HKU1 (HCoV-HKUI) as well as bocavirus has not yet been completely assessed in the hospital setting.[10–13]

In this study, patients who underwent a bronchoalveolar lavage (BAL) procedure were screened by using sensitive nucleic acid detection assays targeting 17 different viruses to assess the potential impact of respiratory virus in hospitalised subjects with lower respiratory tract disease. By definition, this investigation in hospitalised patients will select a population at high risk for complications such as transplant recipients, immunocompromised hosts, or those with co-morbidities and chronic lung diseases. If respiratory viruses do play any significant role, this needs to be assessed first in this at-risk population.

METHODS

Study population and procedures

A hospital-based prospective cohort study was conducted in 299 patients over a 27-month period. All patients who underwent a BAL procedure were eligible, irrespective of the reason(s) leading to the procedure. The study was conducted at the University Hospitals of Geneva (Geneva, Switzerland) and also included lung transplant recipients at the University Hospital of Lausanne (Lausanne, Switzerland) which is 60 km from Geneva and an integral part of our transplantation network.

Bronchoscopy was performed by pulmonary physicians based on guidelines which recommend a BAL procedure for immunocompromised hosts with a persistent chest radiographic infiltrate and/or respiratory symptoms (cough with or without sputum, dyspnoea exacerbation, chest radiographic infiltrates, unexplained hypoxaemia) despite broad-spectrum antibiotic treatment; HIV-positive patients with a low CD4 cell count and a suspected pulmonary opportunistic infection; cases of suspected pulmonary tuberculosis and negative sputum analysis; diffuse persistent interstitial infiltrates of unknown origin; exacerbation of respiratory symptoms in patients with chronic interstitial diseases; or nosocomial pneumonia (including intubated patients) without response to broad-spectrum antibiotic therapy. As the indication for the BAL procedure in lung transplant

recipients ranged from routine surveillance to respiratory signs and symptoms with or without abnormal radiological findings, these patients could have several BAL procedures during the study follow-up period. Patients suspected of having primary lung cancer were submitted to bronchial brushing but not to BAL.

BAL was performed following a standardised protocol (technique, sampling and procedure): 30–50 ml sterile saline solution was instilled 2–3 times into the distal bronchial tree, either at the site of the radiographic abnormality or in the middle lobe in the absence of radiographic abnormalities.

Data collection

A baseline case report form was completed shortly before the BAL procedure. Underlying diseases, fever, respiratory symptoms, response to any antimicrobial therapy, reason/s for the BAL procedure and the presumed diagnosis based on the available evidence and clinical judgment at that time were recorded. Follow-up was conducted at days 3 and 30 after the BAL procedure.

Since one clinical episode could lead to several BAL procedures within a short period of time, we defined an exclusion strategy to avoid reporting multiple BAL procedures linked to a unique event as independent episodes. Any BAL performed within a 4-week period of the previous procedure and yielding the same viral result was excluded from the analysis, but any BAL episode providing a positive result after a negative one was considered, independent of the time elapsed between the two episodes.

Sample processing

BAL fluid specimens were pooled and then aliquoted. Appropriate staining was carried out for direct identification of bacteria, mycobacteria, fungi and parasites. Cultures for bacterial identification were inoculated under standard aerobic conditions on four different media as well as on specific media for *Mycobacterium* detection when indicated. For potentially colonising bacteria, infection was considered as proven only when quantitative culture yielded $>10^3$ cfu/ml of specimen.

Reverse transcription polymerase chain reaction

BAL fluid specimens were inoculated on four different cell lines for viral detection.[9] An aliquot was frozen at −80°C and subsequently used for viral nucleic acid identification. Real-time TaqMan reverse transcription polymerase chain reaction (RT-PCR) assays for the detection of influenza A, B and C, respiratory syncytial virus (RSV) A and B, parainfluenza virus (PIV) 1, 2, 3 and 4, human rhinovirus (HRV), enterovirus, HmPV, coronaviruses OC43, 229E, NL63 and HKU1, and bocavirus were performed as previously described.[9 12 14–16] In brief, RNA was extracted in duplicate using 200 µl of the original specimen. The final precipitate extract of each procedure was eluted in 35 µl and pooled to obtain a final 70 µl volume of RNA. Twenty-five µl were used for the retro-transcription step leading to 100 µl of cDNA. Each PCR assay was then conducted with 5 µl of cDNA. All the different steps of the extraction and reverse transcription were conducted in parallel with known positive supernatant of HRV-2-infected cells and appropriate negative controls.

Compared with the previously cited references, the primers and probes targeting HmPV were updated according to Maertzdorf *et al*.[17] In addition, we completed the panel by adding RT-PCR assays targeting influenza C (NS gene) and PIV4 (phosphorprotein gene of A and B subtypes). The forward primers, reverse primers and probes were as follows: forward 5′-GGC TAC CGA TGA AAT CTC TCT CA-3′, reverse 5′-TCT GGT GTT TCA TTT CCC CAA T-3′, probe 5′-JOEE-TCAAATCAGGAGCCCAGCTCGATCC-TAMRA-3′ for influenza C; forward 5′-TGA CAC TCA ACA AAT YAA AGG TTC A-3′, reverse 5′-ACT CCA GGR TCC ATT ATT TTC ATT G-3′, probe 5′-FAM-TTG CMA CAA TTG AGG GCC TAA TCA C-TAMRA-3′ for PIV4. The analytical sensitivity of the PIV4 was 10 copies/µl. Influenza C analytical sensitivity was assayed using serial dilution of an influenza C/34/WKD virus cell culture supernatant with a detection limit of 10^{-2} dilutions.

Statistical analysis

Data are presented for the entire study group and stratified by clinical conditions to assess potential differences in patient characteristics regarding the frequency of respiratory virus in hospitalised patients. Due to varying numbers of repeated measures per patient, mixed models were used to account for the non-independence of samples. Multivariate mixed logistic regression was performed to identify the clinical variables which were independently associated with the presence of virus. Those variables collected immediately before the BAL procedures were included a priori based on their clinical relevance. As lung transplant recipients also underwent surveillance BAL procedures, supplementary analyses were performed for this group stratified according to the presence/absence of new symptoms in order to verify how symptoms may predict a virus positivity rate in patients with a low probability of respiratory infection. Analyses were performed using GraphPad Prism 3 (GraphPad Software Inc, San Diego, California, USA) and Stata version 10.0 (Stata Corp, College Station, Texas, USA).

RESULTS

Patients

Of 1242 BAL procedures performed during a 27-month period (November 2003 to March 2006), 522 BAL fluid specimens from 299 patients were analysed. Exclusion criteria from the analysis included the inability to obtain informed consent, multiple BAL procedures for the same clinical episode and, in most cases, the lack of BAL fluid after routine investigations since no additional bolus of saline solution was instilled only for the purpose of the study. Baseline characteristics of the clinical conditions and symptoms are summarised in table 1. The mean age of the study subjects was 52 years and 61% of BAL episodes occurred in male patients.

On average, 1.8 BAL procedures were performed in each case, but this number was significantly higher in lung transplant recipients. Of the 323 (61.9%) episodes in transplant recipients, 285 (54.6%) were in lung transplant recipients and 38 (7.3%) in solid organ or haematopoietic stem cell recipients. One hundred and ninety-nine episodes (38.1%) occurred in non-transplanted patients, 60 (11.5%) in the presence of a non-HIV immunosuppressive condition, 41 (7.8%) in HIV-infected patients (median CD4 cell count, 77 cells per mm^3, range 2–600) and 98 (18.8%) in the absence of immunosuppressive condition. Four hundred and twenty-eight episodes (82%) occurred in hospitalised patients, 118 (27.6%) of whom were admitted to the intensive care unit (93% on ventilation support). The presence of radiological infiltrates was evidenced in 46.2% of all episodes. As expected, we observed significant differences between each patient subgroup. Of note, in lung transplant recipients a suspected respiratory tract infection and/or opportunistic infection or the

Table 1 Baseline clinical conditions and symptoms leading to the bronchoalveolar lavage procedure in the different patient groups

	No immunosuppressive conditions (n = 98)	HIV-infected (n = 41)	Other immunosuppressive conditions (n = 60)	Lung transplant recipients (n = 285)	Other transplant recipients (n = 38)	All (n = 522)
No of BAL procedures included in the study						
Mean (SD)	1.1 (0.2)	1.1 (0.3)	1.2 (0.3)	3.6 (1.1)	1.3 (0.9)	1.8 (1.9)
(Median, maximum)	(1, 3)	(1, 2)	(1, 9)	(3, 14)	(1, 4)	(1, 14)
Respiratory tract infection suspected	71.5%	95.1%	86.7%	27.7%	92.1%	52.7%
Respiratory opportunistic infection suspected	20.4%	73.2%	51.7%	8.8%	60.5%	24.7%
Tuberculosis suspected	19.4%	73.2%	16.7%	0%	10.5%	12.1%
Fever	51.0%	70.7%	48.3%	10.9%	94.7%	33.5%
Influenza-like illness	11.2%	21.9%	23.3%	10.5%	10.5%	13.0%
Respiratory symptoms						
Cough	49.0%	70.7%	55.0%	31.6%	57.9%	42.5%
Dyspnoea	55.1%	56.1%	68.3%	29.5%	63.2%	43.3%
Sputum	48.0%	41.5%	43.3%	21.1%	31.6%	31.0%
Rhinopharyngitis	7.1%	22.0%	21.7%	15.1%	15.8%	14.9%
Any respiratory symptoms	75.5	85.4	81.7	47.0	79.0	61.7
New radiological infiltrates	84.7%	82.9%	85.0%	14.7%	81.6%	46.2%
Antibiotic treatment targeting a respiratory tract infection	39.8%	34.2%	43.3%	26.0%	43.4%	32.8%
Respiratory virus-positive, n (%)	12 (12.3)	7 (17.1)	12 (20.0)	48 (16.8)	12 (31.6)	91 (17.4)

BAL, bronchoalveolar lavage.

presence of a radiological infiltrate was significantly less frequent ($p<0.001$).

Microbiological analysis

Ninety-one of 522 episodes (17.4%) were positive for at least one respiratory virus with a positivity rate ranging from 12.3% to 31.6% according to the group studied (table 1). These differences were not statistically significant. The distribution of the different viral genera and species recovered as well as their relative contribution and seasonality is shown in fig 1. The viral distribution at the genus level was as follows: human coronavirus, 32.3% (n = 30); HRV, 22.6% (n = 22); PIV, 19.5% (n = 19); influenza, 9.7% (n = 9); RSV, 8.6% (n = 8); HMpV, 4.2% (n = 4); and bocavirus, 3.1% (n = 3, fig 1A). In five episodes, two different viruses were detected concomitantly.

The proportion of documented bacterial infection was 23.7% in virus-negative cases and 13.2% in virus-positive cases (table 2). The median (interquartile range) total cell count in the BAL fluid was similar in the virus-negative and virus-positive groups (20 (24) and 23 (34)$\times 10^6$ cells/100 ml, respectively).

Four hundred and fifty-six BAL specimens (87%) were sent for viral culture. Eleven (2.4%) were inconclusive because of bacterial contamination, 363 (79.6%) remained negative and 82 (18%) were positive (cytomegalovirus (CMV), 52 cases (63.4%); HSV, 11 (13.4%); PIV, 10 (12.1%); RSV, 5 (6.1%); HRV, 3 (3.6%); and influenza A, 1 (1.2%)). Of the 19 episodes with a positive respiratory virus culture, four had a negative PCR result. In contrast, among all episodes that were PCR-positive for a respiratory virus, only 18 (19.8%) had a concomitant positive culture. CMV was found in 12.1% of virus-negative cases and in 9.9% of virus-positive cases and herpes simplex virus (HSV) in 1.1% and 2.2%, respectively.

Clinical features according to virological results

At the time of the BAL procedure, the physician in charge who was blinded to the virological and microbiological results suspected a respiratory tract infection in 71 of 91 (78.0%) episodes that were found to be respiratory virus-positive compared with 219 of 431 (50.8%) of those that remained negative (odds ratio (OR) 2.3, 95% confidence interval (CI) 1.4 to 3.8). A respiratory opportunistic infection was suspected in 32 of 91 (35.1%) respiratory virus-positive episodes compared with 97 of 431 (22.5%) negative episodes (OR 2.0, 95% CI 1.2 to 3.2). Fifty-one (56%) of subsequently virus-positive episodes had been treated with antibiotics before the BAL procedure compared with 124 (28.8%) of those that remained negative (OR 1.7, 95% CI 1.1 to 2.9). In multivariate analysis, controlling for baseline condition, signs or symptoms available immediately before the BAL procedure without knowledge of the microbiological results, the recent introduction of an antibiotic treatment (OR 2.2, 95% CI 1.2 to 4.1) and the absence of a new radiological infiltrate (OR 0.3, 95% CI 0.2 to 0.8) were significantly associated with the presence of respiratory viruses (table 3). Patients under mechanical ventilation were as likely as others in the intensive care unit to be virus-positive (OR 0.3, 95% CI 0.1 to 1.5). Forty of 299 patients (13.4%) died within 1 month of the BAL procedure. Of these, 8 had a virus-positive BAL compared with 32 with a virus-negative BAL (OR 1.3, 95% CI 0.5 to 3.6).

We also separately analysed lung transplant recipients who represented the larger subgroup of patients enrolled. This analysis showed that the overall incidence of any respiratory symptoms (defined by the presence of at least one of the following: cough; sputum; dyspnoea; fever; or radiological infiltrate) among patients with a respiratory infection suspected before the BAL procedure was 73.7% compared with 38.6% of those without any previous suspicion ($p<0.001$). Importantly, in the group with a suspicion of respiratory infection, the respiratory viral detection rate was 24.4% compared with 13.8% in other cases ($p = 0.02$). These two observations confirmed an association between the presence of symptoms and positive viral detection.

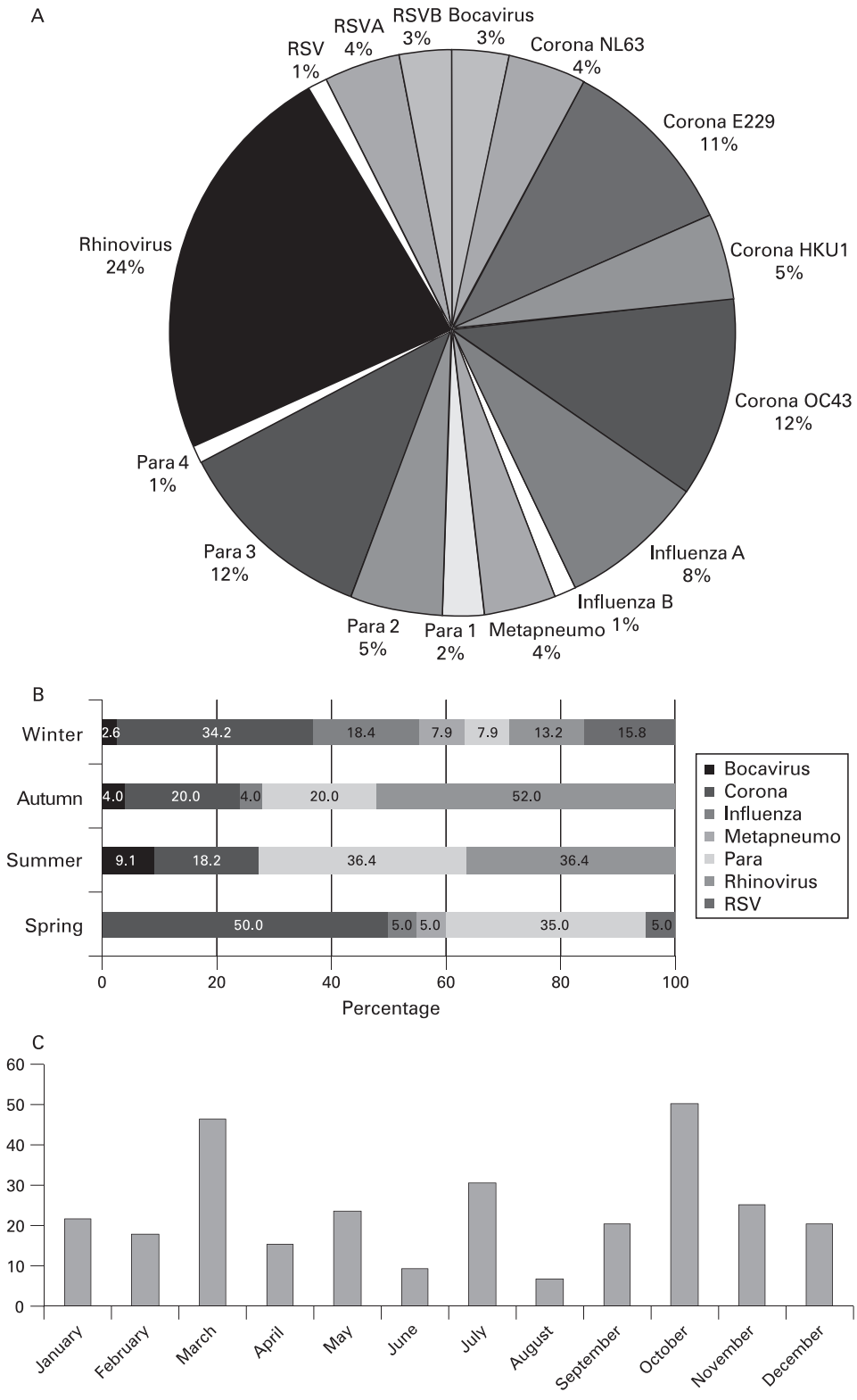

Figure 1 Distribution and seasonality of respiratory viruses among 91 virus-positive bronchoalveolar lavage fluid specimens collected over a period of 27 months in a tertiary care hospital. (A) Distribution of the different viral genera and species. In one case RSV was not typable. (B) Relative contribution of each viral genus according to the season. (C) Monthly rate of respiratory virus positivity. Corona, coronavirus; Metapneumo, metapneumovirus; Para, parainfluenza virus; RSV, respiratory syncytial virus.

Table 2 Microbiological findings associated with the presence of respiratory virus

	Virus-negative (n = 431)	Virus-positive (n = 91)
No microorganisms or colonisation, n (%)	237 (55.0)	76 (83.5)
Bacterial infection, n (%)	124 (23.7)	12 (13.2)
Mycobacterium tuberculosis or atypical mycobacteria, n (%)*	7* (1.6)	2 (2.2)
Pneumocystis jiroveci, n (%)	14 (3.2)	1 (1.1)

*Atypical *Mycobacterium* was identified in two cases (one lung transplant recipient and one HIV-infected subject).

Table 3 Clinical predictors of respiratory virus at the time of the bronchoalveolar lavage procedure*

Predictors	OR (95% CI)	p Value
Baseline conditions		
No immunosuppressive conditions	Reference group	
HIV	1.0 (0.3 to 3.2)	0.98
Other immunosuppressive condition	1.6 (0.6 to 4.2)	0.39
Lung transplantation	1.6 (0.6 to 4.0)	0.32
Other transplantation	2.4 (0.8 to 7.0)	0.10
Signs or symptoms		
New radiological infiltrate	0.3 (0.2 to 0.8)	<0.01
Treated with antibiotics†	2.2 (1.2 to 4.1)	0.01
Sputum	1.9 (1.0 to 3.7)	0.07
Opportunistic infection suspected	1.8 (0.9 to 3.6)	0.10
Cough	1.5 (0.8 to 3.0)	0.25
Respiratory infection suspected	1.5 (0.7 to 3.2)	0.26
Dyspnoea	0.7 (0.4 to 1.3)	0.29
Fever	1.5 (0.7 to 3.3)	0.26
Flu-like illness	1.2 (0.6 to 2.7)	0.57
Rhinopharyngitis	0.9 (0.5 to 2.0)	0.89

*Mixed logistic regression model, clustered on patient.
†Antibiotic treatment targeting a respiratory tract infection.

DISCUSSION

This study shows that, by using sensitive molecular assays, respiratory viruses are recovered in up to 17.4% of BAL fluid specimens performed in a tertiary care hospital. Coronaviruses and rhinoviruses are the most frequent of all viruses screened. A series of analyses of associated clinical conditions indubitably and consistently showed that these viral infections were associated with respiratory symptoms and complications. In particular, in the subgroup of lung transplant recipients we were able to demonstrate that the detection of viral nucleic acid was significantly associated with respiratory symptoms, and this corroborates its role in symptom production. The completeness of the molecular assays, the use of highly standardised lower respiratory specimens and the fact that cases were not selected on predefined clinical conditions strengthen the accuracy of our findings.

Subjects enrolled were those who underwent a bronchoscopic procedure as part of their clinical investigation to identify the cause of a potential respiratory infection. Although this approach limited the spectrum of respiratory diseases studied, this had the advantage of providing high quality lower respiratory tract specimens and to focus on hospitalised patients, those most at risk of complications. The viral detection rate was relatively similar across all subgroups studied. The temporal association between symptoms and the presence of a respiratory viral infection defined by a positive PCR assay was carefully assessed. Univariate analysis using the associated clinical, radiological or microbiological findings revealed a significant association between the presence of an acute respiratory disease and a positive PCR. In multivariate analysis, the non-response to antibiotic treatment targeting a respiratory tract infection and the absence of radiological infiltrates were significantly associated with the presence of positive viral detection. Analysis of the subgroup of lung transplant recipients in whom the BAL procedure is often performed as part of routine follow-up further confirmed the association between symptoms and viral infections. The viral infections were consistently concentrated in lung transplant recipients in whom respiratory symptoms were exacerbated and a respiratory infection suspected. This is also consistent with previous studies.[6 18]

By targeting 17 different viruses using similar sensitive nucleic detection assays applied on the most standardised lower respiratory tract specimens and by avoiding the selection of cases for specific symptoms, our study is likely to provide a representative epidemiological pattern of lower respiratory tract viral infection in hospitalised adults. In this regard, the so-called ''common cold'' viruses (rhinovirus and coronavirus) are the most frequently encountered and represented 23% and 32% of our positive cases, respectively. This mirrors the epidemiology of the adult population living in the community. The ability of these viruses to cause lower respiratory tract diseases has been reported previously,[6 9 13 19 20] and HRV is known to cause complications in patients with asthma and chronic obstructive pulmonary disease as well as in lung transplant recipients. Coronavirus infections are also associated with lower respiratory diseases and pneumonia.[10 11 19 21–23] Taken together, this highlights the significant impact of rhinoviruses and coronaviruses compared with other more classical viruses such as influenza, RSV and PIV, the latter three being the only target of many routine diagnostic procedures. Some viruses such as HMpV, influenza C, enterovirus and PIV4 were very infrequent or absent in this adult population, perhaps reflecting a previous protective immunity or other seasonal aspects. Similarly, relatively few cases of bocavirus infections (3%) were diagnosed. Most studies on bocavirus have focused on children and only a few reports have studied adults in whom the incidence seems significantly lower.[8 24 25] The use of new molecular tools targeting all respiratory viruses represents an opportunity to revisit the respective role of each of these agents. Nevertheless, any conclusion drawn from this type of study needs to take into account the seasonality and the potential inability to detect new viral subtypes. Of note, our detection assays target viral RNA or DNA which does not itself prove the presence of fully competent and replicating virus. Although this could be considered a limitation, it must be kept in mind that most of these viruses are not cultivable and that viral RNA is not equipped to survive in respiratory secretions without facing degradation or clearance by daily mucus production. Thus, a positive RT-PCR assay is most likely proof of an ongoing or a recent viral infection. Viral shedding has been shown to persist for days and even weeks in very young children and other immunocompromised patients.[3 16 26]

It is noteworthy that viruses such as influenza or—to a lesser extent—RSV, for which an antiviral treatment might be available, accounted for only one-fifth of all positive cases in this adult population. This emphasises the potential for therapy targeting other respiratory viruses for which no specific treatment is currently available.

Our study shows that respiratory viruses are detected in approximately one in five hospitalised adults presenting with lower respiratory tract complications leading to a BAL procedure.

When sensitive nucleic acid detection assays are used in this population, rhinoviruses and coronaviruses are the leading agent detected. Our analysis supports a relationship between a positive viral detection and the presence of lower respiratory symptoms, as well as a poor response to antibiotic therapy.

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Competing interests: None.

Ethics approval: Ethical approval for this study was obtained from the ethics committees of the University Hospitals of Geneva and the University Hospital of Lausanne and signed informed consent was obtained from all patients.

PMS and J-DA contributed equally to the study.

JG participated in the development of the study design, implemented the study, and was in charge of data management and analysis and the writing and revision of the manuscript. PMS assisted in the study design, supervised the study in Geneva, collected data and participated in the analysis and writing of the manuscript. J-DA assisted in the study design, supervised the study in Lausanne, collected data, participated in the analysis of the data and the writing of the manuscript. TR participated in the study design, helped to oversee the implementation, supported the recruitment of the patients and assisted with manuscript preparation. PM helped to process the samples and to implement the study in Lausanne and assisted with manuscript preparation. YT was in charge of the development of the molecular assays used, supervised the laboratory analysis and assisted with manuscript preparation. CT was in charge of the development of the molecular assays used, supervised the laboratory analysis and assisted with manuscript preparation. POB was in charge of the data management, analysis and the statistical methods, and participated actively in the writing and revision of the manuscript. LK (guarantor) had the initial idea for the study, designed the protocol, supervised the laboratory analysis and was the recipient of the funding. He participated in the data analysis, wrote the manuscript and revised article drafts for publication. LK takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have seen and approved the final version.

REFERENCES

1. **Papadopoulos NG,** Bates PJ, Bardin PG, *et al*. Rhinoviruses infect the lower airways. *J Infect Dis* 2000;**181**:1875–84.
2. **Malmstrom K,** Pitkaranta A, Carpen O, *et al*. Human rhinovirus in bronchial epithelium of infants with recurrent respiratory symptoms. *J Allergy Clin Immunol* 2006;**118**:591–6.
3. **Kaiser L,** Aubert JD, Pache JC, *et al*. Chronic rhinoviral infection in lung transplant recipients. *Am J Respir Crit Care Med* 2006;**174**:1392–9.
4. **Mosser AG,** Vrtis R, Burchell L, *et al*. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *Am J Respir Crit Care Med* 2005;**171**:645–51.
5. **Mallia P,** Johnston SL. How viral infections cause exacerbation of airway diseases. *Chest* 2006;**130**:1203–10.
6. **Garbino J,** Gerbase MW, Wunderli W, *et al*. Respiratory viruses and severe lower respiratory tract complications in hospitalized patients. *Chest* 2004;**125**:1033–9.
7. **Papi A,** Bellettato CM, Braccioni F, *et al*. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am J Respir Crit Care Med* 2006;**173**:1114–21.
8. **Kaiser L.** Respiratory viruses in immunocompromised hosts. In: Wingard JR, Bowden RA, eds. *Management of infection in oncology patients*. London and New York: Martin Dunitz, 2003:287–320.
9. **Garbino J,** Gerbase MW, Wunderli W, *et al*. Lower respiratory viral illnesses: improved diagnosis by molecular methods and clinical impact. *Am J Respir Crit Care Med* 2004;**170**:1197–203.
10. **Garbino J,** Crespo S, Aubert JD, *et al*. Clinical features associated with coronavirus infections: a prospective and hospital-based study. *Int J Infect Dis* 2006;**10**:S14–5.
11. **Arden KE,** Nissen MD, Sloots TP, *et al*. New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. *J Med Virol* 2005;**75**:455–62.
12. **Regamey N,** Frey U, Deffernez C, *et al*. Isolation of human bocavirus from swiss infants with respiratory infections. *Pediatr Infect Dis J* 2007;**26**:177–9.
13. **Gerna G,** Percivalle E, Sarasini A, *et al*. Human respiratory coronavirus HKU1 versus other coronavirus infections in Italian hospitalised patients. *J Clin Virol* 2007;**38**:244–50.
14. **Deffernez C,** Wunderli W, Thomas Y, *et al*. Amplicon sequencing and improved detection of human rhinovirus in respiratory samples. *J Clin Microbiol* 2004;**42**:3212–8.
15. **Garbino J,** Crespo S, Aubert JD, *et al*. A prospective hospital-based study of the clinical impact of non-severe acute respiratory syndrome (non-SARS)-related human coronavirus infection. *Clin Infect Dis* 2006;**43**:1009–15.
16. **Regamey N,** Kaiser L, Roiha HL, *et al*. Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. *Pediatr Infect Dis J* 2008;**27**:100–5.
17. **Maertzdorf J,** Wang CK, Brown JB, *et al*. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. *J Clin Microbiol* 2004;**42**:981–6.
18. **Wos M,** Sanak M, Soja J, *et al*. The presence of rhinovirus in lower airways of patients with bronchial asthma. *Am J Respir Crit Care Med* 2008;**177**:1082–9.
19. **Falsey AR,** Walsh EE, Hayden FG. Rhinovirus and coronavirus infection-associated hospitalizations among older adults. *J Infect Dis* 2002;**185**:1338–41.
20. **Lee BE,** Robinson JL, Khurana V, *et al*. Enhanced identification of viral and atypical bacterial pathogens in lower respiratory tract samples with nucleic acid amplification tests. *J Med Virol* 2006;**78**:702–10.
21. **Mosser AG,** Brockman-Schneider R, Amineva S, *et al*. Similar frequency of rhinovirus-infectible cells in upper and lower airway epithelium. *J Infect Dis* 2002;**185**:734–43.
22. **Falsey ARW.** Novel coronavirus and severe acute respiratory syndrome. *Lancet* 2003;**361**:1312–3.
23. **Vabret A,** Mourez T, Gouarin S, *et al*. An outbreak of coronavirus OC43 respiratory infection in Normandy, France. *Clin Infect Dis* 2003;**36**:985–9.
24. **Manning A,** Russell V, Eastick K, *et al*. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses (see comment). *J Infect Dis* 2006;**194**:1283–90.
25. **Kesebir D,** Vazquez M, Weibel C, *et al*. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus (see comment). *J Infect Dis* 2006;**194**:1276–82.
26. **Mackay IM.** Human rhinoviruses: the cold wars resume. *J Clin Virol* 2008;**42**:297–320.