REGULAR ARTICLE

Children with multiple viral respiratory infections are older than those with single viruses

Maria Rotzén-Östlund (maria.rotzen-ostlund@karolinska.se) [1,2], Margareta Eriksson [3], Annika Tiveljung Lindell [1,2], Tobias Allander [1,2], Benita Zweygberg Wirgart [1,2], Lena Grillner [1,2]

1.Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden
2.Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden
3.Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden

ABSTRACT

Aim: To study the clinical impact of multiple viral respiratory infections compared to single infections.

Methods: Demographic data from 37 multiple infection periods in children <5 years of age were compared to data from 193 episodes with single infections. Clinical data derived from patient records of the multiple infection episodes were further compared to data from 93 matched control episodes with single infections.

Results: The mean age of patients with multiple viral findings was 12.7 months, compared to 5.7 months for those with single findings (p < 0.01). Wheezing was the most common diagnosis in both groups, except among children who were only infected with the coronavirus. No differences were found regarding duration of hospitalisation, oxygen treatment or admittance to the intensive care unit.

Conclusion: Children with multiple viral findings in their respiratory secretions were older than those with a single detected virus. Otherwise, no major differences in comorbidity, presentation or clinical outcome were observed between the two groups.

INTRODUCTION

Acute respiratory infections are the most common cause of morbidity and hospitalisation in children. The diagnostic methods have greatly improved by new molecular techniques.

The most common respiratory viruses found in hospitalised children under two years of age are respiratory syncytial virus (RSV) and influenza virus A and B (1,2). Parainfluenzavirus 1-3 (PIV), rhinovirus and adenovirus are also important pathogens among children with respiratory infections (3,4).

During recent years, at least five new pathogens causing respiratory disease have been described: human metapneumovirus (hMPV), three new coronaviruses (HCoV-NL63, HCoV-HKU1 and HCoV-SARS) and human bocavirus (HBoV). Detection of these viruses has been made possible by molecular diagnostics (5–7).

The reported frequency of multiple viral findings in a respiratory specimen varies with the diagnostic methods used and between different studies, with reported frequencies between 2% and 27% (8–15). Detection of multiple viruses in the respiratory tract may be explained by simultaneous or overlapping sequential infections.

It has been discussed whether quantification of the viral load in respiratory specimens could be used to distinguish between simultaneous and overlapping infection. The amount of virus could be measured semi-quantitatively,

expressed as the cycle threshold value. In our previous study (16), we compared the cycle threshold values for different viruses in specimens containing multiple and single findings. For bocavirus, the viral load was higher in single than in double infections. No difference was seen for the other compared viruses (influenza A-virus, picornavirus, coronavirus, adenovirus or RSV). Discrimination between single and coinfections was not possible using cycle threshold values.

This study is a retrospective investigation of multiple respiratory viral findings in children under 5 years of age. The object of the study was to examine the clinical impact of dual infections by comparing demographic and clinical

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Key notes

- Demographic and clinical data from patients with multiple viral findings were compared retrospectively with data from patients with single infections.
- Children with multiple viral findings (37 episodes) were older than those with a single detected virus (193 episodes), with mean ages of 12.7 and 5.7 months, respectively.
- No other major differences in the presentation or clinical outcome were observed between the two groups.

data from patients with multiple viral findings with those from patients with single findings.

MATERIALS AND METHODS

Study population

As part of an evaluation of a new diagnostic platform for respiratory viruses (16), 585 consecutive nasopharyngeal aspirates were collected from 517 hospitalised children at the Astrid Lindgren Children's Hospital, Karolinska University Hospital, from July 2004 to June 2005. Demographic data (age and gender of the patients) were available for 582 of the samples. Multiple viruses were found in 41 specimens from 39 patients younger than 5 years of age with 39 episodes of respiratory infection (16). Single virus findings were found in 279 samples (Table 1).

Sampling

Hospitalised children with respiratory symptoms and with a predicted stay of more than 2 days were routinely sampled in order to facilitate cohort care. Nasopharyngeal aspirates were also collected from children with suspected virus infection such as febrile convulsion and encephalitis.

Cases

The 39 episodes with multiple findings were divided into three groups of coinfections: those including RSV, picornavirus and coronavirus. All but two of the 39 episodes involved picornavirus, coronavirus or RSV and these 37 episodes were selected for further studies. Based on these criteria, 12 patients were included in two of the groups; five patients with picornavirus and coronavirus, three patients with picornavirus and RSV and four patients with RSV and coronavirus (Table 1). Only age and gender were available for one patient and, as a result, they were

only included in the demographic comparison. Hence, data from 36 patients (three with triple findings and 33 with dual findings) were retrieved retrospectively from patient records.

Controls

For comparison of the demographic data, all patients with episodes of single infection with either RSV, corona or picornavirus were included (n = 193). As controls for the patient record study, all episodes with a single rhinovirus (n = 34) or coronavirus finding were included (n = 19). It was not possible to review all records for the patients with a single infection with RSV and therefore two age-matched controls with a single finding of RSV were chosen for each coinfection with RSV, resulting in a total of 40 RSV-positive controls. We selected those that were as close as possible to the corresponding case child, in terms of age and time of hospitalisation.

In total, clinical data were obtained from the patient record from 93 episodes of single viral findings in 91 patients and demographic data from 193 episodes.

Diagnostic methods

The specimens had initially been analysed with antigen detection and virus isolation and then stored at −70°C (17). They were then evaluated using an in-house real-time polymerase chain reaction (PCR) diagnostic panel including 11 respiratory viruses: adenovirus, HBoV, coronaviruses (HCoV: HKU-1, NL63, 229E and OC43), influenzaviruses A and B, hMPV, picornavirus and RSV as previously described (16). Parainfluenza viruses were not investigated by PCR during the time of the study, but only diagnosed by IF and virus isolation. The different coronaviruses, HKU-1, NL63, 229E and OC43, were diagnosed by separate PCR assays, but in the present study they were considered to be a single group due to the small numbers of cases. The most common coronavirus was HCoV-OC43 (seven patients), followed by HCoV-NL63 (six patients). HCoV-HKU1 was only found in one patient and there were no findings of HCoV-229E. The picornavirus PCR assay was optimised for rhinoviruses, but could also detect most enterovirus species.

Clinical data

Data on comorbidity including atopic characteristics, duration of hospitalisation, diagnosis at discharge, C-reactive protein (CRP), oxygen treatment, chest x-ray results, and admission to the paediatric intensive care unit (PICU) were collected from the patient records. The investigator was blinded to whether the patients had single or multiple viral findings when reviewing the records.

Ethical approval

The study was approved by the Stockholm Regional Research Ethics Committee.

Statistical analysis

Age was counted in whole months. Categorical data were examined using the χ^2 test. Wilcoxon and Mann–Whitney

Table 1 Number of episodes of single findings and of combinations of viruses in children younger than 5 years

	Coronavirus	Picornavirus	RSV	Single finding
Adenovirus	2	2	6	11
Bocavirus	2	2	4	8
Coronavirus		5*	3**	19
Influenzavirus A	0	0	4	28
Influenzavirus B	0	0	0	7
Metapneumovirus	1	0	0	8
Parainfluenzavirus	0	1	0	24
Picornavirus	5*		3***	35
RSV	3**	3***		139
Adeno- and coronavirus		0	1**	
Adeno- and bocavirus	0	1	0	
Adenovirus and RSV	1**	0		
Total	14	14	21	279

RSV = Respiratory syncytial virus.
*Included in both picornavirus and coronavirus groups.
**Included in both RSV and coronavirus groups.
***Included in both RSV and picornavirus groups.

tests were used to compare continuous data in two groups, and were performed with the SPSS and Statistica software.

RESULTS

Demographic data

The mean ages of patients with multiple and single coronavirus findings were 14.6 months and 7.2 months, respectively, and for picornavirus findings, 11.8 months and 9.8 months, respectively. The mean age of all the children with mixed infections, including RSV, was 12.7 months, compared to 5.7 months for single infections (p < 0.01). Males dominated the series and accounted for 60% of patients with single findings and 62% of those with multiple findings.

Clinical data

Data from patient records were obtained from 129 episodes in 127 patients (Table 2). There was no difference in clinical data such as CRP, oxygen treatment, duration of hospitalisation and admission to PICU between patients with single and double infections.

A chest x-ray was performed in 60% of the children. There was a greater tendency to perform a chest x-ray if the patient had multiple detectable viruses than if there was a single finding, (67% versus 58%,), but the difference was not statistically significant (p = 0.33).

Diagnosis at discharge

The main diagnoses at discharge were croup, lower respiratory infection (LRI), upper respiratory infection (URI) and wheezing. Wheezing was the most common diagnosis in all groups (36–88%) except among children only infected with the coronavirus. No differences regarding diagnoses were found between children with multiple findings and those with a single finding.

DISCUSSION

This retrospective case-control study aimed to develop a better understanding of the relevance of multiple virus detection in nasopharyngeal specimens by comparing demographic and clinical data from patients with multiple respiratory virus findings with data from patients with single virus findings.

The frequencies of multiple findings in published studies differ depending on the study populations, the disease entities included, the microbiological diagnostic methods, the number of pathogens investigated and the study period. In reports from recent years, the frequency of multiple findings varied between 14% and 27% (10–15,18). The lower frequency of multiple findings (7.1%) in the present evaluation study from Stockholm might be explained by the rather heterogeneous study population, resulting from sampling on different clinical criteria.

The frequency of multiple findings also depends on the year of the study. RSV, influenza, and hMPV occur in yearly epidemics of variable magnitudes. Multiple findings would be more frequent during periods of simultaneous epidemics of more than one pathogen. For example, hMPV was an uncommon finding in Stockholm during the 2004–2005 winter season and the seasonal influenza epidemic was mild.

The main finding of the present study is that children with multiple viruses were older than those with a single detected virus. A possible explanation may be that older children are more frequently exposed to virus infections than infants, due to more frequent contact with other children in various settings, including the parent's social activities and day care. The majority of children over 12 months of age attend day care in Sweden. The age for admission to day care and the percentages of children attending day care could be factors that vary between different countries and contribute to different findings in studies of single and multiple infections. If older children are indeed exposed to more viruses, this may lead to more

Table 2 Diagnosis at discharge and chest x-ray findings for the 129 disease episodes with available clinical data for the children. Twelve children are included in two groups.

	All infections		Coronavirus infections		Picornavirus infections		RSV infections	
	Multiple No. (%)	Single No (%)	Multiple No. (%)	Single No. (%)	Multiple No. (%)	Single No. (%)	Multiple No. (%)	Single No. (%)
Total	36	93	14	19	13	34	21	40
Diagnosis								
Croup	1 (3)	2 (2)	0 (0)	2 (11)	1 (8)	0 (0)	0 (0)	0 (0)
LRI	6 (17)	9 (10)	4 (29)	4 (21)	1 (8)	4 (12)	3 (14)	1 (3)
URI	9 (25)	27 (29)	5 (36)	12 (63)	5 (38)	11 (32)	2 (10)	4 (10)
Wheezing	20 (56)	51 (55)	5 (36)	1 (5)	6 (46)	15 (44)	16 (76)	35 (88)
Other*	0 (0)	4 (4)	0 (0)	0 (0)	0 (0)	4 (12)	0 (0)	0 (0)
Chest x-ray								
Pneumonia	9 (25)	17 (18)	5 (36)	3 (16)	2 (15)	7 (21)	5 (24)	7 (18)
Perihiliar infiltrate with or without hyperinflation	9 (25)	25 (27)	3 (21)	2 (11)	4 (31)	10 (29)	5 (24)	13 (32)
Discrete	2 (6)	5 (5)	0 (0)	0 (0)	1 (8)	2 (6)	1 (5)	3 (8)
Normal	4 (11)	7 (8)	2 (14)	2 (11)	2 (15)	5 (15)	2 (10)	0 (0)
Not done	12 (33)	39 (42)	4 (28)	12 (63)	4 (31)	10 (29)	8 (38)	17 (42)

*One adenitis and three asymptomatic children, two of whom receiving prophylactic palivizumab treatment and one twin sibling of an admitted child.

frequent sequential infections, and the presence of virus from one infection may be more likely to overlap the next viral infection.

Previous reports on the clinical significance of multiple virus findings, as compared to single findings, are contradictory (10–12,18–21). The diverging results support the view that any differences in disease severity, if present, are minor.

Respiratory infections are frequent among children and prolonged shedding after a symptomatic infection may explain the finding of multiple agents. There is incomplete knowledge about the duration of shedding of respiratory viruses as determined by molecular techniques. Kaiser et al. (22) demonstrated that HCoV-NL63 was still detectable 3 weeks after the initial infection, and there are indications that HBoV is frequently shed for prolonged periods with ensuing diagnostic problems (23,24). Asymptomatic carriage of rhinovirus is well known, and shedding may occur for weeks (25). The significance of a rhinovirus finding in a child with acute respiratory infection is therefore often unclear in the individual case. However, for most respiratory viruses such as RSV, asymptomatic carriage has not been considered common, and hence is not a likely explanation for multiple pathogens in respiratory specimens. RSV, influenza and hMPV appear in epidemics that often overlap. Thus, sequential infections may be the main mechanism behind multiple virus detection.

It is reasonable to assume that the amount of virus will decline during an infection (rising cycle threshold values). In a previous study, we compared cycle threshold values in specimens with single and multiple findings and did not find any differences except for the case of bocavirus (16). In order to shed more light on this issue, sequential sampling and quantification every week or every second week during more than one season would be required. Such studies are complicated to undertake, both from an economical and ethical point of view. Understanding the significance of multiple virus findings is important for the correct interpretation of assay results regarding respiratory infections.

CONFLICT OF INTEREST

No conflict of interest.

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