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Coronavirus-related nosocomial viral respiratory infections in a neonatal and paediatric intensive care unit: a prospective study

A. Gagneur*, J. Sizun*, S. Vallet†, M. C. Legrand†, B. Picard† and P. J. Talbot‡

**Pediatric Intensive Care Unit, Department of Paediatrics, †Department of Microbiology, University Hospital, Brest, France; and ‡Laboratory of Neuroimmunovirology, INRS-Institut Armand-Frappier, Université du Québec, Laval, Québec, Canada*

Summary: The incidence of nosocomial viral respiratory infections (NVRI) in neonates and children hospitalized in paediatric and neonatal intensive care units (PNICU) is unknown. Human coronaviruses (HCoV) have been implicated in NVRI in hospitalized preterm neonates. The objectives of this study were to determine the incidence of HCoV-related NVRI in neonates and children hospitalized in a PNICU and the prevalence of viral respiratory tract infections in staff. All neonates (age $\leq$ 28 days) and children (age $>$ 28 days) hospitalized between November 1997 and April 1998 were included. Nasal samples were obtained by cytological brush at admission and weekly thereafter. Nasal samples were taken monthly from staff. Virological studies were performed, using indirect immunofluorescence, for HCoV strains 229E and OC43, respiratory syncytial virus (RSV), influenza virus types A and B, paramyxoviruses types 1, 2 and 3 and adenovirus. A total of 120 patients were enrolled (64 neonates and 56 children). Twenty-two samples from 20 patients were positive (incidence 16.7%). In neonates, seven positive samples, all for HCoV, were detected (incidence 11%). Risk factors for NVRI in neonates were: duration of hospitalization, antibiotic treatment and duration of parenteral nutrition ($P < 0.01$). Monthly prevalence of viral infections in staff was between 0% and 10.5%, mainly with HCoV. In children, 15 samples were positive in 13 children at admission (seven RSV, five influenza and three adenovirus) but no NVRI were observed. In spite of a high rate of community-acquired infection in hospitalized children, the incidence of NVRI with common respiratory viruses appears low in neonates, HCoV being the most important pathogen of NRVI in neonates during this study period. Further research is needed to evaluate the long-term impact on pulmonary function.

Keywords: Human coronavirus; virus; nosocomial infection; PNICU; infant; neonate.

Introduction

Nosocomial infections are common in paediatric wards. Their incidence varies between hospitals and within different services of each hospital.[1] A high incidence in paediatric and neonatal intensive care units (PNICUs) has been reported.[2,3]

Bacteria are the most common nosocomial agents identified, but respiratory and intestinal viruses were implicated in 23% of paediatric studies.[1] The

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Author for correspondence: Dr J. Sizun, Unité de Réanimation Pédiatrique, Département de Pédiatrie, CHU, 29609 Brest, France. Fax: +33 2 98 22 39 86; E-mail: Jacques.Sizun@chu-brest.fr

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negative impact of nosocomial viral respiratory infection (NRVI) on high-risk populations (patients who are young, immuno-compromised or afflicted with chronic disease) is well established.[4] Respiratory viruses, including respiratory syncytial virus (RSV), influenza and parainfluenza viruses and rhinovirus are the most common agents. We have previously reported a high incidence of human coronavirus (HCoV)-related infections in preterm neonates.[5]

Effective preventative strategies that target nosocomial infections are based upon an appreciation of their epidemiology and their mechanisms of transmission. The epidemiological profiles of these NVRI are similar to those seen in the community in terms of frequency, season, ages affected and severity of illness.[6] Viruses are introduced into the hospital by patients (symptomatic or asymptomatic), visitors or staff.[7] Many studies have reported NVRI outbreaks in neonatal units but limited information is available on the frequency and mechanisms of NVRI.

This study was undertaken to investigate the epidemiology of HCoV-related NVRI on a combined neonatal and paediatric intensive care unit, and the incidence of community-acquired respiratory infections, and to examine the frequency of nasal viral carriage in medical and nursing staff.

Patients and methods

Patients and staff

This prospective study, approved by the local Ethics Committee, was conducted in a 12 single-room neonatal and paediatric intensive care unit. Every neonate or child admitted into the unit between 24 November 1997 and 2 May 1998 was included. The age, sex, medical history, risk factors, signs and symptoms and duration of hospitalization were recorded for each patient by a single observer. Patients were not studied after discharge.

Specimens and virus detection

In the patient population, nasal specimens were obtained at admission and then weekly thereafter, using a cytological brush.[8] Nasal specimens were taken monthly ('prevalence point') from nursing staff and physicians involved in direct patient care. A short anonymous questionnaire regarding gender, recent medical history and immunization status was given to all staff members.

Nasal specimens were transferred into sterile saline and transport medium (MEM with penicillin-streptomycin 1 mg/100 mL, amphotericin B 10 μg/mL and gentamicin 1 mg/100 mL). Samples were taken to the laboratory and kept at +4°C. Virological analyses were performed within 24 h by indirect immunofluorescence and cell culture.

Indirect immunofluorescence

Samples were washed in phosphate-buffered saline (PBS) and centrifuged at 200× *g* for 10 min. Cells were suspended in PBS and then put into a 10-well slide, dried and fixed in cold acetone for 10 min. Wells were stained with monoclonal antibodies (mAb) against HCoV-229E and OC43 (5-11H.6 and 1-10C.1, INRS-Institut Armand-Frappier, Laval, Canada),[9] RSV, influenza type A and B, parainfluenza types 1, 2 and 3, adenovirus (Biosoft, France), for 30 min at 37°C, washed and air-dried. Fluorescein-labelled anti-mouse immunoglobulin was added to each well for 30 min, washed, fixed with glycerol and kept at −20°C for analysis.

Cell culture

Cultures of MDCK (Madin Darby Canine Kidney), HEp-2 and MRC-5 cells were inoculated at confluence with 200 μL of each sample, incubated at 37°C and observed daily for 10 days for the appearance of cytopathic effects. Immunofluorescence was performed on cell cultures on day 3 for MDCK (influenza), days 5–7 for Hep-2 (RSV and adenovirus) and day 10 for MRC-5 (RSV and adenovirus).

Definitions

Neonates were defined as aged less than or equal to 28 days, and children were defined as older than 28 days. Community-acquired infection was defined as a positive viral detection on admission and nosocomial infection by a negative specimen on admission, with a positive viral detection later. Clinical events associated with a viral infection were defined as events which occurred for a period of three days before and after the positive sample.

Infection control policies

Hand washing with chlorhexidine (Hibiscrub®, Zeneca) or polyvidone–iodine (Betadine®, Asta Medica), individual gowns and masks, and limited

visits (only parents at the time of this study) were routinely used as infection control measures. Gloves were not used for non-invasive procedures.[10]

Statistical analysis

The data was processed using the Epi-Info software (CDC, USA; French version ENSP). Comparisons between groups were performed using the Chi-squared test. Significance was defined as $P < 0.05$.

Results

Demographics

We collected 274 specimens from 120 patients and 110 specimens from staff. Of the patients, 64 were neonates (53.3%) and 56 were children (median age: 1.46 year; range: 4 weeks – 14.5 years). Demographic data are summarized in Table I. Of 123 enrolled patients, data were available for 120 with a gender ratio (M/F) of 1.6.

Immunization against influenza had been administered in 2.5% of children. No RSV prophylaxis was used at the time of the study.

Table I *Demographic data*

	Neonates	Children
N	64	56
Sex ratio (M/F)	40/24	34/22
Mean birthweight (g)	2135.4	
Mean gestational age (weeks)	33.8	
Ventilated (%)	65.8	41.1
Central venous catheter (%)	70.3	28.6
Age < 3 months (%)	100	21.6
Immunodeficiency (%)	3.1	12.5
Mean length of hospitalization (days)	14.3	6.1
Preterm/full term	42/22	
Preterm neonates: mean gestational age (weeks)	31.1	
Preterm neonates: mean birthweight (g)	1573.5	

Table II *Virus detection*

	Neonates	Infant
RSV	0	7
Influenza	0	5
Parainfluenza	0	0
Adenovirus	0	3
HCoV-229E	6	0
HCoV-OC43	2	0
Total	8	15

Viral infections

Twenty-two samples were positive for virus in 120 patients (16.7%) (Table II and Figure 1). For the children, 13 samples were positive for 15 viruses (seven RSV, five Influenza and three Adenovirus), all at admission (defining a community-acquired infection) (incidence of 23.2%). Two samples were positive for both influenza and adenovirus. No NVRI was observed. The only risk factor identified for a positive sample on admission was the existence of a clinical respiratory disease ($P = 0.0004$).

For neonates, seven samples were positive in seven patients (incidence of 11%), all for HCoV: HcoV-229E: $N = 5$, HCoV-OC43: $N = 1$ and one with both HCoV-229E and -OC43. In univariate analysis, risk factors for viral infection in neonates included the length of hospitalization (27.1 vs. 12.8 days for infected and non-infected neonates respectively) ($P < 0.01$), parenteral nutrition (26 vs. 11.5 days) ($P < 0.01$), central venous catheter (CVC) (23.8 vs. 9 days) ($P < 0.01$) and duration of antibiotic treatment (17.9 vs. 5.1 days) ($P < 0.001$) (Table III).

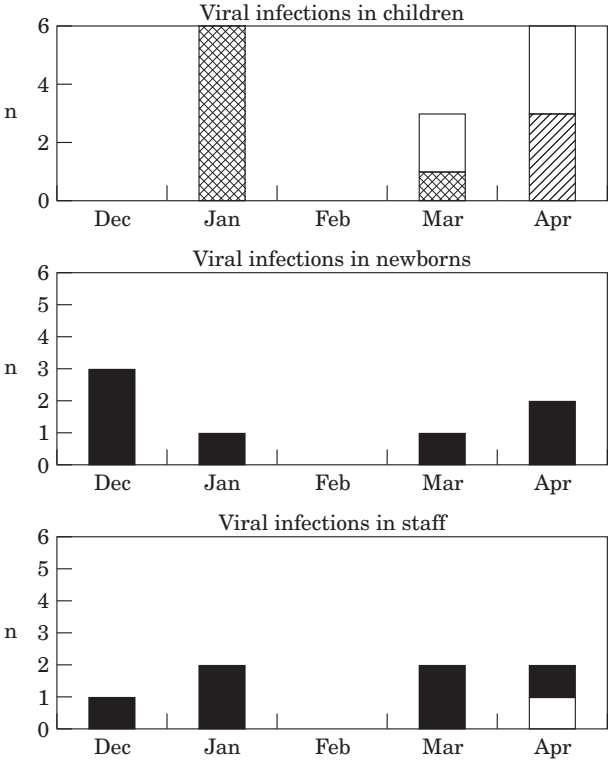

Figure 1 Distribution of viral infections in children, neonates and staff. (■ = HCoV: human coronavirus; □ = Infl.: influenza virus; ▨ = ADV: adenovirus; ⊠ = RSV: respiratory syncytial virus).

Table III *Risk factors for neonatal nosocomial respiratory viral infection*

	Infected $N=7$	Non-infected $N=57$	*P*
Birthweight (g)	1895.7 ± 1320.5	2164.9 ± 920.8	NS
Gestational age (weeks)	32.8 ± 6.1	33.9 ± 4.2	NS
Intubation (*N*)	6	36	NS
Ventilation duration (days)	9.1 ± 7.9	4.9 ± 10.2	0.052
Oxygen (*N*)	6	49	NS
Oxygen duration (days)	14 ± 13.4	7 ± 15.9	NS
CVC*	7	38	NS
CVC* duration (days)	23.8 ± 23.8	9.1 ± 15.7	<0.01
Parenteral feeding (days)	26 ± 24.6	11.5 ± 17.5	<0.01
Antibiotic treatment (*N*)	7	50	NS
Antibiotic treatment duration (days)	17.9 ± 15.7	5.1 ± 6.5	<0.001
Surgery (*N*)	1	8	NS
Hospitalization (days)	27.1 ± 24.4	12.8 ± 17.8	<0.01

NS, not significant. *CVC, central venous catheter.

Clinical events associated with NRVI

NVRI was associated with an increase in the demand for oxygen and the initiation of antibiotic treatment, in 85% of infected neonates. Either intubation or nasal positive pressure support initiation was used in 57% of cases.

Viral carriage in staff members (Figure 1)

A total of 110 samples were collected from 110 staff members. Between 12.5% and 42.1% of staff members had had a respiratory illness the week before the prevalence point. A proportion of 29% were immunized against influenza virus. Six cases of HCoV-related and one influenza virus-related infection were detected in staff members. One sample was positive for both HCoV-0229E and -OC43. No statistically significant association existed between a recent respiratory illness and nasal viral carriage.

Discussion

Our study demonstrates both a high incidence of community-acquired viral infections in children and of NVRI in hospitalized neonates. However, these two types of infection did not show the same epidemiological profile. NVRI were related to HCoV and community-acquired infections were related to common respiratory viruses (RSV, influenza virus and adenovirus). Our results imply a high incidence of HCoV carriage in staff, suggesting staff–patient or patient–staff contamination.

To our knowledge, this is the first prospective study on NVRI in children and neonates in ICU. However, the incidence of NVRI we describe could have been underestimated for three reasons: (i) we have not checked for NVRI occurring after ICU discharge; (ii) rhinoviruses, which are involved in many upper respiratory infections in children,[11] were not searched for with specific diagnostic methods; and (iii) annual variations in the incidence of virus-related diseases are common, which means that our results are only representative of the time period studied.

Only descriptive or retrospective evaluations of NVRI outbreaks in neonatal units have been published which have targeted RSV,[12,13] adenovirus,[14] influenza virus,[15] enterovirus,[16–18] and parainfluenza virus.[19,20]

Only study by Paisley *et al.*, showed that viruses were responsible for 79% of pneumonias in neonates.[21] Abzug *et al.* retrospectively described 40 viral pneumonias in neonates during a five-year period. Causative agents included RSV (55%), rhinovirus (15%), enterovirus (15%), adenovirus (10%) and parainfluenza virus (7.5%). HCoV were not searched for in this study. Viral pneumonia was associated with a high level of morbidity, requiring oxygen and ventilatory support in 90% and 45% of cases, respectively. Prematurity was a contributing factor to severity.[22]

Our study demonstrates a normal distribution of viral agents associated with community-acquired infections. In a prospective regional study on community-acquired infections, Lina *et al.* reported a viral origin in 36.1% of cases. RSV was the most common agent (36%), followed by HCoV (18.4%).[23]

Children hospitalized during the epidemic period often introduce respiratory viruses into the hospital.[7]

Goldwater *et al*. reported a rate of 47% in asymptomatic children hospitalized in a paediatric unit, higher than our 23.2%. However we failed to demonstrate any cross-infections with common respiratory viruses, such as RSV, between infected and non-infected children. This could be an argument against the use of specific RSV chemoprophylaxis in hospitalized high-risk neonates.

Our study confirms previous reports of HCoV-related infections occurring in NICU.[5,24] HCoV are widespread and responsible for one-third of common colds in children and adults.[25] Their role in lower respiratory infection is unclear.[26–28] HCoV have been implicated in NVRI in elderly people attending a daycare unit.[29] HCoV have also been isolated in broncho-alveolar washings in immunocompromised patients,[30] but their role in nosocomial gastrointestinal disease remains unproven.[31] In 1982, an outbreak of necrotizing enterocolitis occurring in near-term babies was reported by Chany *et al*.[32]

In our study, all the HCoV-infected neonates were symptomatic at the time of infection. The need for oxygen and ventilatory support were the main clinical features. In our previous studies, bradycardia and apnoea were the most frequent signs of infection.[5,24]

The lack of knowledge on HCoV epidemiology could be explained by an absence of effective diagnostic methods. Our study was based on indirect immunofluorescence (IIF) results, a standard method for diagnosis of respiratory viruses. We used two monoclonal antibodies specific for each HCoV serogroup, thereby increasing sensitivity.[9] Nucleic acid detection could be of interest in the diagnosis of viral NVRI.[33] PCR has demonstrated a greater sensitivity than IF for HCoV[9] and rhinovirus.[34] Molecular typing might establish mechanisms of transmission and leading to the development of more appropriate infection control measures,[33] noting the limitations of this procedure.[35]

HCoV isolation in both neonates and staff suggests the possibility of patient–staff or staff–patient transmission. HCoV are able to survive in aerosol particles, in suspension and after drying.[36,37] Horizontal transmission is possible therefore, via air or hand contamination, as previously demonstrated for RSV. Hall *et al*. described an outbreak of nosocomial RSV infection in neonates with 34% of staff being contaminated.[38] Moisuk *et al*. reported a 35% rate in staff presenting respiratory symptoms during a parainfluenza virus outbreak occurring in a neonatal unit.[20]

In conclusion, HCoV appear to be involved in NVRI in hospitalized preterm neonates. Further research is needed to evaluate the seasonal variation and the eventual impact on general and pulmonary health.

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