

Neonatal nosocomial respiratory infection with coronavirus: a prospective study in a neonatal intensive care unit

J Sizun¹, D Soupre¹, MC Legrand², JD Giroux¹, S Rubio¹, JM Cauvin³, C Chastel², D Alix¹ and L de Parscau¹

Department of Pediatric and Neonatal Intensive Care Unit¹, Department of Microbiology² and Medical Information Department³, University Hospital, Brest, France

Sizun J, Soupre D, Legrand MC, Giroux JD, Rubio S, Cauvin JM, Chastel C, Alix D, de Parscau L. Neonatal nosocomial respiratory infection with coronavirus: a prospective study in a neonatal intensive care unit. *Acta Paediatr* 1995;84:617–20. Stockholm. ISSN 0803–5253

The aim of this prospective study was to evaluate the incidence of viral respiratory infection in hospitalized premature newborn infants and to assess the role of coronaviruses. All hospitalized premature infants with a gestational age less than or equal to 32 weeks were included. Tracheal or nasopharyngeal specimens were studied by immunofluorescence for coronaviruses, respiratory syncytial virus, adenoviruses, influenza and parainfluenza viruses. Forty premature infants were included; 13 samples were positive in 10 newborns (coronaviruses $n = 10$; influenza 1 $n = 2$; adenovirus $n = 1$). None was positive at admission. All premature infants infected with coronaviruses had symptoms of bradycardia, apnea, hypoxemia, fever or abdominal distension. Chest X-ray revealed diffuse infiltrates in two cases. However, no significant difference was observed between infected and non-infected premature infants for gestational age, birth weight, duration of ventilation, age at discharge, incidence of apnea or bradycardia. Nosocomial respiratory tract infection with coronaviruses appears to be frequent. The clinical consequences should be evaluated in a larger population. □ *Coronavirus infections, prematurity, viral infections.*

J Sizun, Department of Pediatrics and Neonatal Intensive Care Unit, University Hospital, 29609 Brest Cedex, France

Viral pneumonia which develops in neonatal intensive care units is potentially life-threatening. Indeed, young age (less than 1 month) and prematurity are two major risk factors (1). Nosocomial respiratory infections can occur sporadically or as an epidemic. The precise incidence has not been established. The variability or even absence of clinical symptomatology, especially in very immature newborn infants (2), makes all retrospective evaluations difficult. Numerous viral agents have been implicated. The most frequently reported viruses are respiratory syncytial virus (RSV) (1, 3), adenoviruses (4), influenza viruses A and B, and parainfluenza viruses I, II and III (5). The coronavirus (CV) has also been implicated. We have previously reported four cases of upper respiratory tract infection in premature newborns presenting with attacks of apnea of sudden onset (6). The pathogenic role of CVs during the neonatal period is, however, poorly established.

The aim of this prospective study undertaken from November 1, 1991 to March 1, 1993, was to evaluate, in the pediatric intensive care unit, the incidence of upper respiratory tract viral infection in hospitalized premature newborn infants, to assess the role of CV and to analyze the associated symptomatology.

Patients and methods

The study was approved by the Hospital Center Ethics Committee. All newborn infants less than 3 days of age, with a gestational age less than or equal to 32 weeks and hospitalized in a resuscitation unit throughout the duration of the study, were entered into the trial. Infants who died before day 7 of life were excluded a posteriori.

Samples

Samples were obtained on admission and then weekly. The methodology was as follows: administration of 0.5 ml of physiological saline via the intranasal route in non-intubated newborns or via the tracheal route in intubated newborns, followed by aspiration using a disposable Vygon-type catheter. Samples were transported at room temperature to the microbiology department and stored at +4°C until analysis.

Viral antigenic study using immunofluorescence (IF)

Samples were treated with a mucolytic solution (Mucomyst, Eurobio, France). Cells were then washed twice in phosphate buffered saline (PBS). After the last

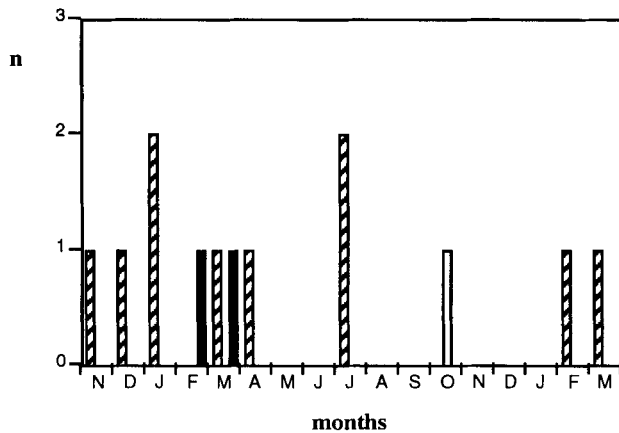


Fig. 1. Positive nasotracheal samples during the study. ▨ = Corona-virus, □ = adenovirus, ■ = influenza virus.

centrifugation, the precipitate was resuspended in PBS and distributed into slide wells. After drying and fixation in cold acetone for 10 min, a standard indirect IF assay was carried out using a panel of monoclonal antibodies directed against respiratory viruses: influenza viruses A and B, parainfluenza viruses I, II and III, adenovirus, RSV and CV. All were available through Clonatec (France) as was the second antibody, fluorescein-labeled antimouse IgG + IgM.

According to the manufacturer, monoclonal antibody against CV is a 110/120 kDa peplomer group specific and recognizes bovine, porcine and human CV strains.

Clinical data and data obtained from the infant's family were recorded at the time of entry into the study and weekly by the same observer.

Gestational age was established from obstetric data. Apnea was defined as absence of respiratory movements over a 20-s period and bradycardia as heart rate less than 90 beats/min, detected by a Hewlett-Packard cardiorespiratory monitor. Days on which more than six episodes of bradycardia of less than 60 beats/min, or more than eight episodes greater than 60 beats/min, or more than five apneic events occurred, were noted.

Table 1. Clinical characteristics of the infants. No significant differences between groups

	Neonates infected with coronavirus (n = 8)	Neonates not infected (n = 30)
Birth weight (g)	1285 ± 258 (1070–1860)	1374 ± 312 (760–2000)
Gestational age (weeks)	30.3 ± 1.7 (27–32)	30 ± 1.5 (26–32)
Male (n)	5	11
Hyaline membrane disease (%)	50	53

Chest X-rays were interpreted according to four parameters: atelectasis, effusion, unilateral infiltrate or a bilateral infiltrate.

Statistical analysis

Statistical analysis was carried out using the chi-square test with Yates' correction for qualitative variables and by parametric or non-parametric tests for quantitative variables. A p value < 0.05 was considered statistically significant.

Results

Forty infants were included in the study. Initial samples were negative in all infants. Thirteen subsequent samples were found to be positive in 10 newborns: 10 for CV, 2 for myxovirus influenzae type 1 and 1 for adenovirus. Two children were CV positive on two successive samples. Another was positive for CV and then positive for myxovirus 4 weeks later. No sample was doubly positive. The seasonal distribution is shown in Fig. 1.

Previous perinatal histories are summarized in Table 1. The group infected with CV was comparable to the non-infected group in terms of gestational age, birth weight, sex ratio and past history of hyaline membrane disease. All infants infected with CV were symptomatic at the time of infection (Table 2). Symptomatology was predominantly bradycardia and apnea. Digestive signs were present in five newborns but virology was not performed on the stools. Mean age at the time of infection was 17 ± 7 days. Chest X-ray revealed bilateral interstitial infiltrate in two infants, 3 and 6 days after the date of the first positive CV sample. No statistically significant differences with respect to frequency of bradycardia or apnea were observed between the CV-infected and non-infected infants (Table 3). Laboratory signs of an inflammatory syndrome were not observed in any CV-infected newborns (normal C-reactive protein concentration). Blood cultures were negative.

The development of a neonatal viral infection did not significantly alter the therapeutic management of these infants, since the duration of intubation, administration of atropine, need to interrupt feeding and conceptional mean age at the time of discharge were comparable in both groups. Two newborn infants in the non-infected group died, the first on day 13 with multiple organ failure and the second on day 30 due to cardiorespiratory arrest during surgical closure of a persistent ductus arteriosus; no infant in the infected group died.

Discussion

The incidence of viral infection in this study was high,

Table 2. Clinical features in patients with coronavirus infection.

Patient No.	Age (days)	Apnea	Hypoxemia	Bradycardia	Rhinitis	Fever	Abdominal distention
1	17	-	+	-	-	-	-
2	16	-	-	+	+	-	+
3	11	+	-	+	-	-	-
4	8	-	-	+	-	-	-
5	16	-	-	+	-	+	+
6	15	+	+	+	-	-	+
7	32	+	+	+	-	-	+
8	27	-	+	-	-	-	+

affecting 25% of the newborn infants under investigation. No comparable evaluation exists in the literature. The negative initial sampling performed before the third day of life tends to support the diagnosis of nosocomial rather than materno-fetal contamination.

RSV, the primary cause of bronchiolitis in infancy, has also been implicated as the cause of epidemics of chest infections in neonatal intensive care units (2, 3) and can worsen pre-existing respiratory disease. In this study, no sample was positive for RSV. On the other hand, we have seen a high rate of occurrence of infection with CV. CVs are RNA viruses with a spherical shape covered with projections similar to a "crown". Two main strains of human CV have been identified: 229 E and OC (7). These viruses are commonly implicated in benign upper respiratory tract infections in adults (7), reproduced experimentally in healthy volunteers (8). They are also implicated in acute decompensation in chronic respiratory insufficiency (9).

Epidemiological data in young infants are rare and vary depending on the study. In infants, CVs can be the cause of respiratory infections such as pneumonia or bronchiolitis, without clinical or radiological specificity compared with other respiratory viruses. In a serological study carried out in infants aged less than 18 months and hospitalized for respiratory tract infection, this cause was identified in 8.2% of cases (10). On the

other hand, none of the 106 children less than 12 years of age hospitalized for respiratory infection and investigated by McIntosh et al. using IF was found to have a positive nasopharyngeal secretion for these viruses (11). Similarly, in a prospective study by Ray et al., based on serological analysis and cell culture from acute lower respiratory tract infections in children less than 3 years of age, human CV viruses, as initial infecting agents, were not found to be responsible (12). The difficulty in culturing CVs and the absence of serological specificity probably explain these findings (13).

The pathogenic role of CVs in the newborn is poorly understood. An association with enterocolitis in full-term newborn infants has been reported (14). No data are available in the literature regarding the possibility of nosocomial respiratory tract infections in the newborn. We reported four apneic episodes associated with bradycardia of sudden onset in the absence of an explicable etiology and with positive nasotracheal samples for CV on IF (6). CV has also been isolated using IF in infants with chronic respiratory insufficiency requiring prolonged mechanical ventilation (15).

All the infected neonates in our prospective study had symptoms at the time of infection, which supports the recent onset of an acute process. However, we were unable to prove any significant difference between infected or non-infected infants with respect to symptomatology and therapeutic measures necessary. Several hypotheses can be proposed: these symptoms (apnea, bradycardia and digestive disorders) are not very specific in the premature infant and can be found in many pathological conditions; moreover, duration of infection is short compared with duration of hospitalization.

The diagnosis of a CV infection is based mainly on IF (15). The special culture conditions required make this procedure expensive and difficult to achieve. Serological methods are not very reliable (14). The polymerase chain reaction has been used in the diagnosis of murine CV by Homberger et al. (16). This technique appears to be faster and as sensitive as the usual techniques of inoculation of suckling mice or sero-conversion after mouse inoculation, but it is not yet applicable to human CV, even though the genetic M

Table 3. Symptomatology and treatment.

	Neonates infected with coronavirus (n = 8)	Neonates not infected (n = 30)
Days with apnea	3.6 ± 4.6	2.6 ± 3.4
Days with bradycardia > 60/min	2.2 ± 1.3	3.2 ± 3.6
Days with bradycardia < 60/min	1.7 ± 1.1	1.2 ± 1.6
X-ray infiltrate(n)	2	8
Atropine treatment (n)	5	22
Feeding interruption (n)	4	10
Duration of ventilation (days)	13.3 ± 15.6	11.4 ± 10.9
Duration of oxygenation (days)	15.8 ± 23.1	8.9 ± 12.4
Postconceptional age at discharge (weeks)	38.6	37.4
Death(n)	0	2

sequence of OC43 is the counterpart of that of the murin virus.

This study suggests a significant occurrence of upper respiratory tract viral infection in hospitalized premature newborns, mainly with CVs. The pathogenic role of CVs, as suggested by simultaneous viral infection and development of symptoms, requires a larger series of patients to be confirmed.

References

1. Abzug MJ, Beam AC, Gyorkos EA, et al. Viral pneumonia in the first month of life. *Pediatr Infect Dis J* 1990;9:881-5
2. Gouyon JB, Fantino M, Couillault G, et al. Infections à virus respiratoire syncytial du nouveau-né. *Arch Fr Pediatr* 1986;43:93-7
3. Hall CB, Kopelman AE, Douglas G, et al. Neonatal respiratory syncytial virus infection. *N Engl J Med* 1979;300:393-6
4. Abzug MJ, Levin MJ. Neonatal adenovirus infection. *Pediatrics* 1991;87:890-6
5. Jenista JA, Powell KR, Menegus MA. Epidemiology of neonatal enterovirus infection. *J Pediatr* 1984;104:685-90
6. Sizon J, Soupre D, Giroux JD, et al. Nasal colonization with coronavirus and apnea of the premature newborn. *Acta Paediatr* 1993;82:238
7. Bradburne AF, Tyrell DAJ. Coronaviruses of man. *Prog Med Virol* 1971;13:373-403
8. Bradburne AF, Bynoe ML, Tyrrell DAJ. Effects of a "new" human respiratory virus in volunteers. *BMJ* 1967;23 September:767-9
9. Riski H, Hovi T. Coronavirus infections of man associated with diseases other than the common cold. *J Med Virol* 1980;6:259-65
10. McIntosh K, Chao RK, Krause HE, et al. Coronavirus infection in acute lower respiratory tract disease of infants. *J Infect Dis* 1974;130:502-7
11. McIntosh K, McQuillin J, Reed SE, Gardner PS. Diagnosis of human coronavirus infection by immunofluorescence: method and application to respiratory disease in hospitalized children. *J Med Virol* 1978;2:341-6
12. Ray CG, Holberg CJ, Minnich LL, et al. Acute lower respiratory illnesses during the first three years of life: potential roles for various etiologic agents. *Pediatr Infect Dis J* 1993;12:10-4
13. Monto AS. Coronaviruses. *Viral infections of humans—epidemiology and control*, 3rd ed. New York: AS Evans, 1991
14. Chany C, Moscovici O, Lebon P, et al. Association of coronavirus infection with neonatal necrotizing enterocolitis. *Pediatrics* 1982;69:209-14
15. Sizon J, Soupre D, Giroux JD, et al. Rôle pathogène des coronavirus en réanimation pédiatrique: analyse rétrospective de 19 prélèvements positifs en immunofluorescence indirecte. *Arch Fr Pediatr* 1994;1:477-80
16. Homberger FR, Smith AL, Barthold SW. Detection of rodent coronaviruses in tissues and cell cultures by using polymerase chain reaction. *J Clin Microbiol* 1991;29:2789-93

Received May 19, 1994. Accepted Jan. 20, 1995

Errata

Acta Pædiatrica 83: Suppl 399, edited by B Lippe, M Ritzén and R Gunnarsson

In the article entitled "Bone mineralization after treatment of growth hormone deficiency in survivors of childhood malignancy", by Nussey et al. on pp. 9-14, the name of one of the authors (M Pazianas) was inadvertently left out.

We apologize to Dr Pazianas for this omission.

Acta Pædiatrica 84: Suppl 407, edited by F Güttler and R Zetterström

Reference no. 3 (p. 10) in "The discovery of phenylketonuria" by I. Følling is given incorrectly in the above supplement. It should read:

3. Følling A, Mohr OL, Ruud L. Oligophrenia phenylpyrouvica. A recessive syndrome in man. The Norwegian Academy of Science and Letters 1944; Skrifter, I, M.-N. 13. 48 pp.

We apologize for this inconvenience.