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Viral Respiratory Tract Infections in the Neonatal Intensive Care Unit: The VIRIoN-I Study

Andrea Ronchi, MD[1,2], Ian C. Michelow, MD, DTM&H[3,4], Kimberle C. Chapin, MD, D(ABMM), FCAP[4,5], Joseph M. Bliss, MD, PhD[4,6], Lorenza Pugni, MD[2], Fabio Mosca, MD[2], and Pablo J. Sánchez, MD[1,7]

Objective To determine the frequency of respiratory viral infections among infants who were evaluated for late-onset sepsis in the neonatal intensive care units (NICUs) of Parkland Memorial Hospital, Dallas, Texas; and Women & Infants Hospital, Providence, Rhode Island.

Study design Prospective cohort study conducted from January 15, 2012 to January 31, 2013. Infants in the NICU were enrolled if they were inborn, had never been discharged home, and were evaluated for sepsis (at >72 hours of age) and antibiotic therapy was initiated. Infants had a nasopharyngeal specimen collected for detection of respiratory viruses by multiplex polymerase chain reaction within 72 hours of the initiation of antibiotic therapy. Their medical records were reviewed for demographic, clinical, radiographic, and laboratory data until NICU discharge.

Results During the 13-month study, 8 of 100 infants, or 8 (6%) of the 135 sepsis evaluations, had a respiratory virus detected by polymerase chain reaction (2, enterovirus/rhinovirus; 2, rhinovirus; 2, coronaviruses; and 2, parainfluenza-3 virus). By bivariate analysis, the infants with viral detection were older (41 vs 11 days; $P = .007$), exposed to individuals with respiratory tract viral symptoms (37% vs 2%; $P = .003$), tested for respiratory viruses by provider (75% vs 11%; $P < .001$), and had lower total neutrophil counts ($P = .02$). In multivariate regression analysis, the best predictor of viral infection was the caregivers' clinical suspicion of viral infection ($P = .006$).

Conclusions A total of 8% of infants, or 6% of all NICU sepsis evaluations, had a respiratory virus detected when evaluated for bacterial sepsis. These findings argue for more respiratory viral testing of infants with suspected sepsis using optimal molecular assays to establish accurate diagnoses, prevent transmission, and inform antibiotic stewardship efforts. *(J Pediatr 2014;165:690-6)*.

Respiratory viral infections among infants in the neonatal intensive care unit (NICU) can result in substantial morbidity and mortality. Limited data exist on their occurrence, however, because testing for viral pathogens is not performed routinely in many NICUs. In addition, most reports on the prevalence of respiratory viral infections in NICUs have centered on outbreaks or prospective surveillance of clinically stable infants,[1-15] and thus significant knowledge gaps remain.

The contribution of respiratory viruses to clinical signs of infection among infants in the NICU is largely unknown. These infants are evaluated for possible sepsis, yet their bacterial cultures often are sterile. Because of diminished confidence in culture results, infants may receive prolonged antibiotic therapy.[16] Because preterm infants in the NICU may not have classic "cold" symptoms that are observed in older infants and children,[1,15,17,18] the possibility that a viral respiratory pathogen is the causative agent may not be considered.

The advent of new molecular technologies has facilitated the detection of respiratory viruses in children and adults, yet this technology has not been applied routinely to high risk infants in the NICU.[19-22] The objective of this study was to determine the frequency and role of respiratory viral infections, as detected by polymerase chain reaction (PCR) testing, among infants who are evaluated for possible late-onset sepsis in 2 Level 3 NICUs: Parkland Memorial Hospital (PMH), Dallas, Texas, and Women & Infants Hospital (WIH), Providence, Rhode Island.

Methods

This was a prospective cohort study of all infants who were hospitalized in the NICUs at PMH and WIH from January 15, 2012, to January 31, 2013. The PMH NICU is a 90-bed, Level 3C, predominantly inborn unit with

NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
PMH	Parkland Memorial Hospital
RSV	Respiratory syncytial virus
WIH	Women & Infants Hospital

From the [1]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX; [2]Neonatal Intensive Care Unit, Department of Clinical Sciences and Community Health, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; [3]Department of Pediatrics, Rhode Island Hospital; [4]Alpert Medical School of Brown University; [5]Department of Pathology and Medicine, Rhode Island Hospital; [6]Department of Pediatrics, Women & Infants Hospital, Providence, RI; and [7]Department of Pediatrics, Center for Perinatal Research, Nationwide Children's Hospital - The Ohio State University, Columbus, OH

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approximately 1200 admissions annually. Gowning is not required for entrance into any areas of the NICU, and neither is the use of gloves for all patient contacts. Parents have unlimited access except in the high-acuity area, where visiting is discouraged from 9 a.m. to noon, when daily patient rounds are held. Visitors are limited to 2 per visit; siblings ≥12 years of age may visit anytime with a parent. Siblings <12 years of age may visit with a parent twice a week under the supervision of a Child Life specialist; they must have received all the recommended childhood vaccinations, including influenza vaccine.

The WIH NICU is an 80-bed, Level 3-4 regional facility comprising predominantly single-family rooms that has on average 1200 admissions annually. Gowning is not required for entrance into any area of the NICU, and neither is routine use of gloves for patient contacts. Parents have unlimited access and they may have up to 2 visitors per visit. Siblings of any age may visit provided they have received all age-appropriate vaccinations, including influenza vaccine, and after undergoing screening to confirm lack of fever, respiratory, or gastrointestinal symptoms or recent exposure to individuals with such symptoms. Parents and visitors are requested to perform a 1-minute fingertip-to-elbow disinfectant scrub upon first entering the infant's room and use hand sanitizer after touching any surfaces and before handling the infant.

Infants were eligible if they were inborn, had never been discharged to home, and were evaluated for possible late-onset sepsis and antibiotic therapy was initiated at >72 hours of age. Eligible infants were identified by daily review of all antibiotics provided by the NICU pharmacists. Infants who received antibiotics for only superficial skin or surgical-site infection were excluded.

After obtaining informed consent, enrolled infants had a nasopharyngeal specimen collected for detection of respiratory viruses by PCR within 72 hours of initiation of antibiotic therapy. Their medical records were reviewed for pertinent maternal and infant demographic, clinical, radiographic, and laboratory data until discharge from the NICU. In addition, at the time of consent, the mother or legal guardian of the infant was asked whether any one at home had symptoms of suspected respiratory viral infection. The study was approved by the institutional review boards of the University of Texas Southwestern Medical Center, WIH, and Rhode Island Hospital.

Nasopharyngeal Specimens

Respiratory specimens were obtained using sterile flexible flocked nylon swabs (Copan Diagnostics Inc, Murrieta, California; Becton, Dickinson and Co, Sparks, Maryland), which were inserted in each nostril and the posterior nasopharynx and subsequently placed in 1 mL of Universal Transport Medium (Copan Diagnostic Inc; Becton, Dickinson and Co). After sample collection, specimens were provided a number code and refrigerated at 4°C for up to 24 hours, after which samples were stored at −70°C before they were shipped on dry ice to the Microbiology Laboratory at Rhode Island Hospital-Brown University, where respiratory viral PCR testing was performed by technicians blinded to patient identity and site.

Respiratory Viral PCR Testing

Nasopharyngeal specimens were tested in batches by 2 multiplex reverse-transcriptase-PCR assays: (1) xTag Respiratory Viral Panel (Luminex Inc, Austin, Texas) for 14 respiratory viruses (influenza A H1, H3, and nonspecific; influenza B; respiratory syncytial virus (RSV) A and B; parainfluenza virus 1, 2, 3, 4; coronavirus group [229E, NL63, HKU-1, and OC43]; rhinovirus/enterovirus; adenovirus; and human metapneumovirus); parainfluenza 4 and the coronavirus group are not part of the Food and Drug Adminsitration–approved assay but were validated separately by one of the authors (K.C.); and (2) eSensor XT-8 Respiratory Viral Panel (GenMark Diagnostics, Inc, Carlsbad, California) for 19 respiratory viruses (influenza A H1, H3, 2009 H1N1; influenza B; RSV A and B; parainfluenza virus 1, 2, 3, 4; human rhinovirus; adenovirus groups B, C, and E; human metapneumovirus; and coronavirus types 229E, HKU1, OC43, and NL63). Viral detection on either test was considered positive.

Definitions

Neonates were infants aged 28 days or less. Hypothermia was defined as axillary temperature ≤36°C,[23] and fever was temperature ≥38°C.[24] Respiratory signs suggestive of infection included rhinorrhea, nasal congestion, cough, tachypnea (>60 breaths per minute), retractions, or hypoxia (oxygen saturation <90%). The diagnosis of bacterial pneumonia was based on clinical findings, and included fever, tachypnea, abnormal chest radiograph, and/or prolonged antibiotic therapy for ≥7 days.[17] Tachycardia was ≥180 beats per minute, and hypotension was blood pressure below the fifth percentile for the age of the infant at the time of the evaluation and for which vasopressor therapy was provided.[25] Bronchopulmonary dysplasia was determined by the guideline proposed by Jobe and Bancalari.[26] Central line-associated bloodstream infection was defined as a positive blood culture for a clinically relevant bacterial pathogen in an infant who had a central venous catheter at the time of or in the previous 24 hours before the onset of the event, without any other source of infection.[27] Diagnosis of urinary tract infection was based on the neonatologist's assessment in the medical record, bacterial growth on urine obtained by either suprapubic bladder aspiration (any growth gram-negative bacilli; >50 000 colonies/mL gram-positive cocci) or catheterization (>50 000 colonies/mL), and/or receipt of ≥7 days of appropriate antibiotic therapy.

Statistical Analyses

For descriptive statistics, normality of continuous covariates first was assessed using the Kolmogorov-Smirnov test. For normally distributed data, means with SD were derived for descriptive statistics (eg, patient demographics and characteristics), and median values with IQR were calculated for non-normally distributed data. Where appropriate, 95%

CIs were used to compare differences in mean and median values. Proportions were calculated for categorical data. Bivariate analyses were performed to determine the association between the dependent variable (presence of virus) and independent covariates using *t* tests for normally distributed data and the Mann-Whitney *U* test for nonparametric data. Chi-square or Fisher exact tests were used for categorical variables as appropriate. Covariates that had a $P < .1$ from the bivariate analyses were entered in a mixed stepwise logistic regression model to determine which variables independently predicted viral infection. The maximum and minimum threshold significance levels for an effect to be entered and retained in the model were 0.25 and 0.1, respectively. A 2-tailed $P < .05$ was considered to be statistically significant.

Results

During the 13-month study period, 100 (70%) of 143 eligible infants were enrolled; 86 infants were in the PMH NICU (93% of 92 eligible infants) and 14 were at WIH (27% of 51 eligible infants). The mothers/legal guardians of 42 (5, PMH; 37, WIH) infants declined enrollment in the study, and 1 infant was missed at PMH. An additional infant died before consent could be obtained.

Most mothers were Hispanic and delivered their infants via cesarean delivery (Table I); 4 (4%) mothers at PMH were infected with HIV, but the infants were uninfected. None had intrapartum fever or symptoms suggestive of a viral infection at delivery. The infants were mostly male, preterm (81% <37 weeks' gestation; 71% <34 weeks' gestation), and of low birth weight (66% <2000 g birth weight; 56% <1500 g; 29% <1000 g; Table I). The onset of clinical signs of infection, or the chronologic age when evaluated for late-onset sepsis, occurred at a median age of 11.5 days, and the median duration of hospitalization was 66 days. The 100 infants received 135 evaluations with initiation of antimicrobial therapy for possible sepsis; 21 of them had 2 sepsis evaluations, 10 had 3, and 4 had 4 performed.

Table I. Characteristics of the 100 mothers and enrolled infants

	PMH, n = 86	WIH, n = 14	Total, n = 100
Mothers			
Age, y, mean ± SD (range)	29 ± 8 (14-43)	29 ± 6 (18-40)	29 ± 7 (14-43)
Ethnicity, n (%)			
Hispanic	69 (80)	3 (21)	72
Non-Hispanic white	2 (2)	5 (36)	7
Non-Hispanic black	14 (16)	2 (14)	16
Unknown	1 (1)	4 (29)	5
Type of delivery			
Vaginal	27 (31)	8 (57)	35
Cesarean	59 (69)	6 (43)	65
Infants			
GA, wk, median (IQR)	30 (27-36)	31 (26-35)	30 (27-36)
Weight, g, median (IQR)	1360 (900-2236)	1570 (825-2461)	1380 (900-2262)
Sex, M	51	10	61
Age at sepsis evaluation (days, median, IQR)	11 (7-22)	22 (6-52)	12 (7-27)
Duration of hospitalization (days; median, IQR)	65 (36-95)	67 (30-120)	66 (35-98)

GA, gestational age; *M*, male.

A nasopharyngeal swab was collected at all of the 135 sepsis evaluations. Eight (8%) of the 100 infants, or 8 (6%) of the 135 sepsis evaluations, had a respiratory virus detected from the nasopharyngeal swab that included 6 (7%) of the 86 infants at PMH and 2 (14%) of the 14 infants at WIH (Table II). All of the respiratory viruses were detected at the time of the first sepsis evaluation. None of the 8 patients whose nasopharyngeal swab detected a respiratory virus at the first evaluation received another sepsis evaluation and, therefore, none was retested. The respiratory viruses detected were enterovirus/rhinovirus (n = 2), rhinovirus (n = 2), coronaviruses (1, HKU-1; 1, OC43), and parainfluenza-3 virus (n = 2). The Luminex xTag Respiratory Viral Panel identified 5 of the 8 positive infants and the GenMark eSensor Respiratory Viral Panel detected 6 of the 8, but only 3 infants (1, rhinovirus/enterovirus; 2, parainfluenza-3 virus) were detected by both panels (Table II). The 8 infants who had a respiratory virus detected at the time of sepsis evaluation had hypothermia (n = 2), tachypnea (n = 6), and apnea (n = 6). Only one had fever and one had cough, with the latter infant as well as another one diagnosed with pneumonia due to parainfluenza-3 virus. Hematochezia occurred in 2 infants with rhinovirus, but neither was diagnosed with necrotizing enterocolitis. Five (5%) infants had sick contacts who had presumed respiratory viral infection. Three of the 5 infants had a respiratory virus detected and a parent, grandparent, or nurse had rhinorrhea, and 2 infants who did not have a respiratory virus detected had a mother or siblings with cough, sore throat, and rhinorrhea. None of the contacts was tested for viral infection.

Overall, the main reasons for the sepsis evaluations were respiratory signs (86%, 86/100), mainly tachypnea (77%, 77/100) and chest retractions (57%, 57/100); and gastrointestinal signs (45%, 45/100), principally feeding intolerance (33%, 33/100) and abdominal distension (29%, 29/100) (Table III). Compared with infants whose respiratory virus PCR test was negative, infants who had a respiratory virus detected during the sepsis evaluation were more likely to be older (41 vs 11 days, $P = .007$; 95% CI, 4.4-55.6), exposed to individuals with respiratory viral symptoms (37% vs 2%; $P = .003$; 95% CI, 0.1-0.7), and had rhinorrhea (25% vs 1%; $P = .02$; 95% CI, 0.05-0.6) and congestion (25% vs 1%; $P = .02$; 95% CI, 0.05-0.6) with a lower total neutrophil count (1790 cells/mL vs 4530 cells/mL; $P = .02$; 95% CI, 906-4573; Table III). They also were more likely

Table II. Characteristics of the 8 infants in whom a respiratory virus was detected by multiplex PCR testing

Sites	GA, wk	Weight, g	Sex	Age (d) and weight (g) at sepsis evaluation	Clinical manifestations	Maximum respiratory support	Date of detection	Virus (test)	Antibiotic use, d	Diagnosis (infectious)
PMH	27	900	M	130/4115	Fever, tachypnea, retractions, feeding intolerance	Nasal cannula	2/16/2012	Coronavirus HKU1*	Gentamicin (7)/Ampicillin (2)	None
PMH	26	900	F	40/1510	Apnea, tachypnea, retractions, rhinorrhea, hematochezia‡	Nasal cannula	3/02/2012	Rhinovirus*,†	Gentamicin (4)/Ampicillin (3)/Oxacillin (4)	None
PMH	29	1150	F	52/2410	Hypothermia, apnea, bradycardia, tachypnea, retractions, feeding intolerance, emesis‡	Mechanical ventilation	7/10/2012	Parainfluenza 3*,†	Gentamicin (8)/Oxacillin (8)	Pneumonia
PMH	25	760	M	62/1885	Hypothermia, apnea, bradycardia, tachypnea, retractions, rhinorrhea, congestion‡	Mechanical ventilation	9/23/2012	Coronavirus OC43*	Gentamicin (6)/Oxacillin (2)	Urinary tract infection
PMH	31	1250	F	15/1500	Apnea, retractions, tachycardia, congestion‡	Nasal cannula	10/03/2012	Enterovirus/rhinovirus†	Gentamicin (3)/Oxacillin (3)	None
PMH	29	1210	M	42/2615	Apnea, tachypnea, retractions, tachycardia, cough‡,§	Nasal cannula	1/31/2013	Parainfluenza 3*,†	Gentamicin (2)/Oxacillin (2)	Pneumonia
WIH	34	2705	M	6/2620	Tachypnea, hematochezia	None	7/20/2012	Rhinovirus*	Gentamicin (2)/Ampicillin (2)/Acyclovir (2)	None
WIH	26	420	F	36/1030	Apnea‡,§	Mechanical ventilation	11/23/2012	Enterovirus/rhinovirus†	Gentamicin (1)/Oxacillin (4)/Fluconazole (6)	None

F, female.
*GenMark eSensor.
†Luminex xTag.
‡Tested for respiratory virus by provider (PMH, direct fluorescent antibody test; WIH, Luminex xTag).
§Respiratory virus detected by provider testing at birth hospital.

to have been tested for a respiratory virus by their provider (6/8, 75% vs 10/92, 10%; $P < .001$; 95% CI, 0.3-0.8). However, only 2 of the 8 infants in whom a respiratory virus was detected had a virus identified by provider testing at the birth hospital (Table II). The other 10 infants tested by the provider also had negative respiratory viral testing results at the birth hospital (Table III).

None of the infants in whom a respiratory virus was detected had a bacterial agent isolated from blood or cerebrospinal fluid. One infant who had coronavirus OC43 detected was diagnosed with a urinary tract infection; urine obtained by suprapubic bladder aspiration yielded 30 000 colony-forming units/mL of coagulase-negative staphylococci, but 2 blood cultures were sterile. On the other hand, 13% (9/71) and 16% (15/92) of infants whose nasopharyngeal swab did not detect any respiratory virus had bacterial infection of the urinary tract or bacterial bloodstream infection, respectively (Table III). Nine of the 15 bloodstream infections were central line–associated bloodstream infections. In addition, infants who had a positive respiratory viral PCR test had a median duration of antibiotic use of 6.5 days (IQR 2.2-7.7) vs a median of 3 days (IQR 2-7) in those whose PCR was negative ($P = .3$; 95% CI, 0.07-7.07; Table III). There also was no significant difference in incidence of bronchopulmonary dysplasia between the 2 groups.

The covariates with $P < .1$ on bivariate analyses (Table III) were entered in a logistic regression model to determine which factors most accurately predicted viral infection after adjusting for other confounders; these included gestational age, birth weight, age at sepsis evaluation, absolute total neutrophil count, exposure to individuals with symptoms of upper respiratory infection, and clinical suspicion of viral infection by attending physicians. After we adjusted for confounding, the model's only predictor of viral infection was the caregivers' clinical suspicion of viral infection as indicated by their ordering a viral study independent of the study protocol ($P = .006$). Exposure to individuals with symptoms of upper respiratory infection was not a significant predictor ($P = .07$) in this small study.

Discussion

Using 2 commercially available PCR-based respiratory viral panels, we found that 8% of high-risk infants who were evaluated for late-onset sepsis and had antibiotic therapy initiated in the NICU had a respiratory virus detected. Overall, a respiratory virus was detected in 6% of sepsis evaluations. The best predictor of detecting a respiratory virus in these infants was the NICU provider ordering respiratory viral testing, which was performed in 6 (75%) of the 8 PCR-positive infants and was positive in only 2 infants (Tables II and III). However, at PMH, a direct fluorescent antibody test was performed, which lacks optimal sensitivity and would not detect enterovirus/rhinovirus and coronaviruses. In addition, these 6 infants who were tested mostly had tachypnea and apnea, and only 2 had

Table III. Characteristics of the 100 infants who had a sepsis evaluation, antibiotic therapy initiated, and a respiratory viral PCR test performed in the NICU

	Respiratory virus detection		
	Yes	No	*P* value
No. of infants	8 (8%)	92 (92%)	-
GA, wk, median (IQR)	28 (26-33)	30 (27-36)	.15
Birth weight, g median (IQR)	1025 (795-1240)	1424 (918-2388)	.06
Age at sepsis evaluation, d median (IQR)	41 (26-57)	11 (7-22)	**.007**
Weight at sepsis evaluation, g median (IQR)	2143 (1503-2619)	1671 (1055-2729)	.4
Exposure to individuals with respiratory viral symptoms	3 (37%)*	2 (2%)†	**.003**
T instability			
Hypothermia (T ≤36°C)	2 (25%)	14 (15%)	.6
Fever (T ≥38°C)	1 (12%)	6 (6%)	.4
Apnea	6 (75%)	42 (46%)	.14
Respiratory signs			
Any	7 (87%)	79 (86%)	1
Tachypnea	6 (75%)	71 (72%)	1
Retractions	6 (75%)	51 (55%)	.5
Rhinorrhea	2 (25%)	1 (1%)	**.02**
Congestion	2 (25%)	1 (1%)	**.02**
Cough	1 (12%)	2 (2%)	.2
Increased or need for oxygen	7 (87%)	57 (62%)	.2
Maximum respiratory support	7 (87%)	60 (64%)	.2
Nasal cannula	4 (57%)	15 (25%)	.1
CPAP	0	22 (37%)	.08
Mechanical ventilation	3 (43%)	23 (37%)	1
Duration, d, mean (±SD)	3.7 (±3)	6.9 (±6.5)	.4
Gastrointestinal signs			
Any	4 (50%)	41 (43%)	1
Feeding intolerance	2 (25%)	31 (37%)	1
Emesis	1 (12%)	21 (23%)	.7
Diarrhea	0	1 (1%)	1
Abdominal distension	0	29 (31%)	.1
Hematochezia	2 (25%)	8 (9%)	.2
Hypotension	0	10 (11%)	1
Fluid bolus (saline; intravenous)	0	10 (11%)	1
Inotropic agents	0	5 (5%)	1
Neurologic signs			
Any	0	18 (20%)	.2
Lethargy	0	10 (11%)	1
Hypotonia	0	9 (10%)	1
Irritability	0	4 (4%)	1
Hematologic values			
White cell blood count, cells/mL, median (IQR)	9785 (5600-12 250)	11 535 (8740-16 288)	.19
Absolute total neutrophils, cells/mL, median (IQR)‡	1790 (1232-4418)	4530 (2850-7871)	**.02**
Lymphocytes, cells/mL, median (IQR)‡	5124 (3304-5412)	4120 (2665-5715)	.5
Platelets, no./mL, median (IQR)	265 000 (117 250-389 250)	265 000 (145 000-363 000)§	.9
Hemoglobin, g/dL, median (IQR)	10.3 (9.4-12.8)	11.8 (10.1-13.7)	.3
Hematocrit, %, median (IQR)	30 (28-37)	35 (30-41)	.3
Bacterial coinfection			
Urine culture positive	1/7 (14%)¶	9/71 (13%)‖	1
Blood culture positive	0	15 (16%)**	.6
Duration of hospitalization, d, mean (±SD)	84 (±48)	68 (±43)	.3
Bronchopulmonary dysplasia			
Any	3 (37%)	25 (27%)	.7
Mild	1 (33%)	9 (36%)	1
Moderate	0	2 (8%)	1
Severe	2 (67%)	14 (56%)	1
No. of infants who had respiratory viral testing performed by provider	6 (75%)	10 (11%)††	**.001**
Antibiotic duration, d median (IQR)	6.5 (2.2-7.7)	3 (2-7)	.3
Death	0	4 (4%)	1

CPAP, continuous positive airway pressure; *T*, temperature.
Significant *P* values (< .05) are represented in bold.
*Contacts were parents, grandparents, and nurse.
†Contacts were mother and siblings.
‡Performed on 7 and 91 infants with and without viral detection, respectively.
§Performed on 91 infants without viral detection.
¶1, coagulase-negative staphylococcus (3×10^4 colonies/mL) by suprapubic aspiration.
‖3, *Enterococcus faecalis* (>2×10^4 colonies/mL by catheterization); 1, *Enterobacter cloacae* (>10^4 colonies/mL) by catheterization; 1, *Klebsiella pneumoniae* (10^4 colonies/mL) by catheterization; 1, coagulase-negative staphylococcus (3×10^4 colonies/mL) by suprapubic aspiration; 1, *Escherichia coli* (10^5 colonies/mL) by suprapubic aspiration; 1, *K pneumoniae* (8×10^3 colonies/mL) and *E faecalis* (6×10^3 colonies/mL) by suprapubic aspiration; 1, *Enterococcus sp.* and *Klebsiella oxytoca* (collection method and colony counts not known).
**2, *K oxytoca*; 3, *K pneumoniae*; 6, *coagulase-negative staphylococci*; 1, *Enterobacter aerogenes*; 1, *E coli*; 1, *E faecalis*; 1, group B *Streptococcus*.
††All 10 infants had negative respiratory viral testing results at birth hospital.

rhinorrhea (Table III). These results argue for not only more frequent testing of infants but also the use of optimal molecular diagnostic methodologies.[18-21,28]

Although both multiplex assays that were used in this study, Luminex xTag and GenMark eSensor panels, offer a larger menu of potential viral pathogens compared with traditional virologic methods, not all of the viruses identified in the patients were detected by both test kits. Only 3 infants (1, rhinovirus/enterovirus; 2, parainfluenza-3 virus) were detected by both multiplex PCR panels. This result likely is attributable to the differences in both the composition of their multiplex primer targets for different viruses as well as the lower limit of detection of viral particles by each assay. Our findings support the better sensitivity of GenMark for detection of coronaviruses and rhinovirus.[22] The 2 infants who were positive for enterovirus/rhinovirus by Luminex xTag testing but negative by the GenMark eSensor panel could have been infected with enterovirus rather than rhinovirus because enterovirus is not detected by the GenMark eSensor kit. Unfortunately, viral culture or specific enteroviral PCR testing was not performed in either infant.

In a similarly designed study of 60 infants evaluated for late-onset sepsis in a German NICU, 6 (10%) infants had a respiratory virus detected by multiplex PCR testing. RSV was detected in 1 infant and picornaviruses in 5 infants. The detection rate was similar to our study, and of note, there also was no bacterial bloodstream coinfection detected, which occurred in 5% (3/60) of enrolled infants in the NICU.[29] The authors concluded that there was no specific presentation or laboratory marker that differentiated infants with viral detection and those with positive blood cultures. Our data are supportive of the nonspecific clinical presentation because few had rhinorrhea, congestion, or cough.

In a prospective surveillance study using multiplex PCR testing for detection of respiratory viruses among infants in 2 NICUs during a 1-year period, Bennet et al[1] found that 52% (26/50) of infants <33 weeks' gestational age tested positive for a respiratory virus at least once during their birth hospitalization. Viruses detected in the 26 infants were parainfluenza virus (20 patients), human metapneumovirus (9 patients), RSV (15 patients), entero/rhinovirus (7 patients), and influenza B (4 patients). Unlike in our study, 28% of the positive swabs included more than one virus, and 14 infants had sequentially positive specimens for the same virus, with clusters observed. None of our patients who had a respiratory virus detected was sampled subsequently because they did not undergo another sepsis evaluation, but there was no clustering of positive infants in either NICU. Also different from our study was that a viral infection was not clinically recognized by the NICU providers, whereas 6 of our 8 infants who had a respiratory virus detected had been tested for clinical reasons. Importantly, however, Bennet et al[1] found that infants who tested positive for a respiratory virus had longer length of hospitalization, greater use and duration of supplemental oxygen, prolonged ventilator support, twice the rate of bronchopulmonary dysplasia, and greater number of clinical deterioration events.[1] In our study, the number of infants who tested positive for respiratory viruses was too small to be able to find significant associations with such variables.

The small sample size in our study made it impossible to evaluate the differences between the NICU environments at PMH which has shared bays, and WIH that has individual patient rooms. A multicenter study would have the ability to look at differences in viral infection rates between different NICU designs, and could help identify safer hospital practices that may reduce the risk of viral infection. Similarly, the small sample size and the lack of real time knowledge of the results of viral testing did not allow for detection of potential differences in antibiotic use among infants with and without viral infection. Future studies that enroll a larger cohort of infants with rapid feedback of results to providers are needed to determine how respiratory viral testing can inform antibiotic stewardship in the NICU.

Limitations of this study include the small sample size, the low rate of enrollment in WIH, and small number of infants in which a respiratory virus was detected despite the fact that both RSV and influenza circulated in each community during the year-long study.[30,31] On the one hand, this argues for good infection prevention practices in both NICUs because horizontal transmission did not seem to occur based on lack of temporal or spatial clustering of cases. On the other hand, all 8 cases represented health care–associated infections that if appropriately diagnosed, could lead to optimal strategies to prevent their occurrence. Another limitation is that detection of nucleic acid from the respiratory tracts of infants in the NICU could represent false-positive results, prolonged shedding from a preceding infection, or even colonization rather than acute infection.[32]

A strength of this study was its prospective nature that identified infants with clinical signs of possible sepsis and studied the potential impact of a respiratory viral infection. Nonetheless, the number of respiratory viral infections may have been underestimated if the clinical signs of a viral respiratory infection were confused with other events like frequent desaturation episodes or increasing oxygen requirement and not a bacterial infection that would have made the infant eligible for the study. The question also remains whether the virus was causing the clinical signs or whether it was a previously unidentified infection in which shedding was ongoing. The latter issue may require the inclusion of healthy infants in future studies of respiratory viral testing in the NICU. In addition, future studies involving microarray analysis for studying the gene expression profiles of infected infants may help to resolve this issue by differentiating infection from possible colonization.[33] ■

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Reprint requests: Pablo J. Sánchez, MD, Nationwide Children's Hospital - The Ohio State University, Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, 700 Children's Drive, RB3, WB5215, Columbus, OH 43205-2664. E-mail: Pablo.Sanchez@nationwidechildrens.org

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