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

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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

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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%