Unrecognized Viral Respiratory Tract Infections in Premature Infants during their Birth Hospitalization: A Prospective Surveillance Study in Two Neonatal Intensive Care Units

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Objective To determine the frequency and effects of nosocomial respiratory viral infections (RVIs) in premature neonates, including those who may be asymptomatic.

Study design We performed a year-long surveillance for RVIs in infants <33 weeks gestational age admitted to 2 Syracuse neonatal intensive care units. Infants were enrolled within 3 days of neonatal intensive care unit admission and were sampled for RVIs until discharge using a multiplex polymerase chain reaction assay capable of detecting 17 different respiratory viruses or subtypes.

Results Twenty-six of 50 prematurely born infants (52%) tested positive for a respiratory virus at least once during their birth hospitalization. Testing positive for a respiratory virus was significantly associated with longer length of stay (70 days vs 35 days, $P = .002$) and prolonged ventilatory support (51 vs 13 days, $P = .002$). Infants who tested positive for a respiratory virus during their birth hospitalization had more than twice the rate of developing bronchopulmonary dysplasia ($P < .05$).

Conclusion Nosocomial RVIs were frequent in our study population, despite the absence of clinical indicators of illness. Length of hospital stay was significantly longer and a diagnosis of bronchopulmonary dysplasia was more common in infants who had respiratory viruses detected. *(J Pediatr 2012;161:814-8)*.

Recent work has challenged the widely-held assumption that newborns in neonatal intensive care units (NICUs) are protected from infections present in the community. Outbreaks of common respiratory viruses among hospitalized infants have been described, including influenza virus, respiratory syncytial virus (RSV), coronavirus, parainfluenzavirus (PIV), adenovirus, enterovirus, and rhinovirus.[1-8] In most of these published studies, virus detection was ascertained only in symptomatic infants. In others, surveillance for respiratory virus pathogens was initiated only after an outbreak was established. Of note, respiratory viral infections (RVIs) were detected in minimally symptomatic and even asymptomatic infants when widespread screening was instituted during outbreaks.[3] Moreover, premature infants often have atypical symptoms of infection, such as feeding difficulty, periodic breathing, or apnea.[8-10] Reports of NICU outbreaks generally have focused on a single infectious agent, and studies extending to multiple viruses are hampered by the technical limitations associated with routine assays such as antigen detection and virus culture. Recent developments in multiplex polymerase chain reaction (PCR) testing permit rapid, sensitive testing for multiple viruses from a single specimen, including viruses for which culture is unavailable and/or unreliable or for which rapid antigen assays do not exist. In this study, we report the results of a year-long surveillance study in 2 NICUs in Syracuse, New York, in which infants' nasopharyngeal specimens were tested for 17 different viruses in longitudinal fashion from birth to discharge.

Methods

We performed a prospective, observational study of premature infants in 2 NICUs in Syracuse, NY, during 1 calendar year (2009). Infants were eligible if they

BPD	Bronchopulmonary dysplasia
GA	Gestational age
hMPV	Human metapneumovirus
MRSA	Methicillin-resistant *Staphylococcus aureus*
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
PIV	Parainfluenzavirus
RSV	Respiratory syncytial virus
RVI	Respiratory viral infection

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Funded by the Children's Miracle Network of New York (to J.D. and N.B.) and the National Institute of Allergy and Infectious Diseases, Division of Intramural Research (Z01-AI000943 to H.R.). The authors declare no conflicts of interest.

 10.1016/j.jpeds.2012.05.001

were born at less than 33 weeks, 0 days gestational age (GA), and were available for enrollment within 3 days of arrival in the NICU, which included transfer from another facility or following hospital birth. Neonates with medical and surgical diagnoses were included. GA was determined by menstrual dates and confirmed by ultrasound. Infants were excluded if they had a known or suspected immune deficiency, or were born to a human immunodeficiency virus-positive mother. After obtaining informed consent, nasopharyngeal specimens were collected using flocked swabs (Copan Diagnostics, Inc, Corona, California) from each infant by study personnel trained in the proper procedure for obtaining a nasopharyngeal specimen (including intubated infants to ensure sample consistency) within 3 days of birth and on a regular twice weekly (Monday/Thursday) schedule thereafter until discharge. Sampling was deferred on a particular day if the procedure was deemed to place the infant at risk. Specimens were placed in 1.5 mL universal transport media (Copan) and frozen at −70°C prior to testing with the xTAG respiratory viral panel (RVP; Luminex Molecular Diagnostics, Inc, Toronto, Ontario, Canada). RVP is a multiplex PCR assay that detects influenza A H1, H3, and nonspecific; influenza B; RSV A and B; PIV 1, 2, 3, and 4; coronavirus 229E, NL63, HKU-1, and OC43; rhinovirus/enterovirus; adenovirus; and human metapneumovirus (hMPV). Clinical and demographic data were collected from the medical records. At each sampling timepoint, the use of mechanical ventilation and percent of inspired oxygen was recorded, along with the results of any microbiologic testing performed for appropriate clinical purposes. Clinical events (episodes of oxygen desaturation, bradycardia, or apnea) were recorded daily according to the existing criteria being used routinely by the nursing staff in both NICUs. To qualify as an event, the aforementioned change had to persist for longer than 20 seconds. Oxygen saturation below 88% and a heart rate below 100 beats per minute were the cutoff points used for desaturation and bradycardia events, respectively. Clinical deteriorations were defined as a sustained (lasting 3 days) increase in daily oxygen desaturation events from the prior baseline rate, an increase in oxygen requirement (as judged by the current inhaled oxygen concentration required for stable oxygen saturations, excluding transient desaturations associated with feeding), or an increased level of respiratory support (moving from nasal cannula to continuous positive airways pressure, or to intubation or mechanical ventilation). A diagnosis of bronchopulmonary dysplasia (BPD) was made if an infant required supplemental oxygen at 36 weeks post-conceptional age.

The 2 NICUs in the study were a level 3 unit with a daily average census of approximately 12 infants, and a level 4 regional center with a daily average census of approximately 55 infants. Throughout the study period, each NICU operated under its usual infection control procedures: all staff employed an extended hand and arm scrub on arrival to the unit, and standard precautions were in place at all times. All direct patient contact by medical staff required gloves. Contact precautions also were in place (gown and glove) for patients known to be colonized or infected with methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA screening by PCR was performed on all patients at the regional center on a weekly basis. All children (age 17 years and younger) were excluded from the units and adult visitors were excluded if ill. Parents who had respiratory symptoms were discouraged from visiting but allowed to visit if they wore gowns, gloves, and a surgical mask. In the regional center NICU, twin and triplet births were separated into different nursing areas. No healthcare personnel were aware of the results of the respiratory virus detection tests during the study.

Statistical comparisons between groups were performed using Fisher exact test or Student *t* test as appropriate. Time to infection was calculated as the interval between birth date and infection date for the first infection and the interval between previous and current infection dates for repeated infections. The marginal effects of several variables, including NICU units, sex, GA, and birth weight on time to infection were presented by Kaplan-Meier infection-free probability curves and tested by Log-rank test. A Cox proportional hazards regression model was further fitted to examine the effect of each covariate, adjusting the effect of other covariates. A random-effect term was included to adjust the dependence among the multiple observations from the same infant. The proportional hazards assumption and possible outliers were visually examined through Schoenfeld and deviance residual plots. A path model, focusing on the direct and indirect effects of infection on the hospital stay, was proposed and confirmed by using SAS v. 9.2 PROC CALIS (SAS Institute, Cary, North Carolina) under which the algorithm was optimized via a Quasi-Newton method. Statistical significance was defined at a level of $P = .05$.

The research was approved by the Institutional Review Boards of St. Joseph's Hospital Health Center and Crouse Irving Memorial Hospital, both of Syracuse, NY (Institutional Review Board for the Protection of Human Subjects #2008.147 and #2008.4657, respectively).

Results

Approximately one-half of the parents approached for the study consented to enrollment. The most common reason given for refusal was a concern for an additional procedure on a very small baby, which may have skewed our population towards enrolling neonates of later GA. Fifty infants were enrolled into the study; 27 (54%) were male. The average GA was 28 weeks (range 24 to 32). Eighteen infants (36%) had GA <28 weeks. The average duration of birth hospitalization was 54.6 days. Twenty-five infants (50%) were intubated at some time during hospitalization, for a mean of 12.5 days. Twenty-eight infants (56%) required supplemental oxygen at some time during hospitalization, and 17 (34%) met criteria for diagnosis of BPD. Eleven infants were enrolled from the level 3 NICU, and 39 were from the level 4 regional NICU. One infant died.

A total of 708 specimens were obtained and tested from the 50 patients over the 52-week period, for an average of 13.6 specimens per week. No weeks were missed but the level of