Viral Infections in Neonates with Suspected Late-Onset Bacterial Sepsis—A Prospective Cohort Study

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Abstract

Objective   The aim of our study was to evaluate the occurrence of viral infections in infants with suspected late-onset bacterial sepsis in a neonatal intensive care unit.

Methods   In a prospective study, infants with suspected late-onset bacterial sepsis underwent viral testing alongside routine blood culture sampling. Using a multiplex reverse transcription-polymerase chain reaction enzyme-linked immunosorbent assay, nasopharyngeal aspirates were analyzed for adenovirus, respiratory syncytial virus (RSV), influenza virus A and B, H1N1 virus, parainfluenza virus 1 to 4, metapneumovirus, coronavirus, and picornavirus. Stools were examined for adenovirus, rotavirus, norovirus, and enterovirus.

Results   Between August 2010 and March 2014, data of 88 infants with 137 episodes of suspected late-onset bacterial sepsis were analyzed. Six infants were diagnosed with a respiratory viral infection (2 × RSV, 4 × picornavirus). Blood culture–proven bacterial sepsis was detected in 15 infants. Neither viral–bacterial coinfections nor polymerase chain reaction positive stool samples were found.

Conclusion   Respiratory viruses can be detected in a considerable number of neonates with suspected late-onset bacterial sepsis. In contrast, gastrointestinal viral or enterovirus infections appear uncommon in such cases.

Keywords
► virus
► infection
► late-onset bacterial sepsis
► neonatal intensive care unit

Late-onset infections have a significant negative impact on morbidity and mortality of infants hospitalized in a neonatal intensive care unit (NICU).[1,2] These infections are often of bacterial origin, but their diagnosis remains challenging. Positive blood cultures are considered a prerequisite to diagnose bloodstream infections. However, in neonates, blood culture results are positive in only ~25% of the cases.[3]

Numerous cases of clinical deterioration suspicious of infection remain without evidence of a bacterial pathogen.[4]

Based on this, viral infections have also been found to account for a significant burden in neonatal intensive care medicine.[5] A large observational study from the Netherlands showed an overall incidence of viral infections in the NICU of around 1%.[6] Several case reports and observational data

substantiated these findings and focused on respiratory viruses as frequent pathogens.[7–10] Besides, viral outbreaks in NICUs have been reported, indicating significant impact on health outcomes and costs.[11,12]

The emergence of new detection methods such as the multiplex reverse transcription-polymerase chain reaction enzyme-linked immunosorbent assay (multiplex RT-PCR ELISA) has tremendously facilitated the detection of viruses.[13,14] Multiplex RT-PCR ELISA allows screening for a large number of different viruses from a single specimen. There is first evidence that viral infections in term and preterm neonates during birth hospitalization have a significant impact on short- and long-term morbidity.[15–17] In a preceding feasibility study, we demonstrated that this technique can be used to detect respiratory viruses in cases of clinical deterioration in the NICU.[15] The aim of the present study was to further elucidate the occurrence of viral infections in neonates with suspected late-onset sepsis.

Methods

The Unit
We performed a single-center study in the NICU of the University Medical Center of the Johannes Gutenberg University Mainz. This level III unit has 10 intensive care cots, which are allocated to three rooms. Two rooms have five and four cots each; the other is a single room. The unit usually admits patients from the delivery room and the postnatal ward. Both are located nearby in the same building. Previously discharged patients or patients from the special care unit are only readmitted in exceptional cases. The unit is 24/7 open to visitors. Due to limited space, the number of simultaneous visitors is restricted to two per patient. Parents and other visitors are instructed in proper hand hygiene and are repeatedly asked to wear a facemask in case of signs of a minor respiratory tract infection. Siblings of all ages may visit the NICU if clinical examination verifies no signs of infection.

The Study Design
This prospective cohort study was conducted from August 2010 to March 2014. The local ethics committee approved the study. Parents were approached for informed consent on admission. Infants were eligible for the study from 72 hours onward after admission, whether following birth or later in life. Infants in whom intravenous antibiotic treatment was initiated due to suspected late-onset bacterial sepsis were enrolled consecutively. Enrolled infants could be tested repeatedly in the event of independent episodes of suspected sepsis. The decision to start intravenous antibiotics was at the clinician's discretion. Blood culture sampling was mandatory prior to starting antibiotics. One positive blood culture was considered sufficient to diagnose a bacterial bloodstream infection, also in cases of coagulase-negative staphylococci.

Enrolled infants were tested for respiratory and gastrointestinal viruses within the next 72 hours. Nasopharyngeal aspirates (NPAs) were analyzed using an in-house multiplex RT-PCR ELISA as described previously.[15,18] This assay included adenovirus, respiratory syncytial virus (RSV), influenza virus A and B, H1N1 virus, parainfluenza virus 1–4, metapneumovirus, coronavirus, and picornavirus. Stool samples were tested for rotavirus, adenovirus, and norovirus using commercially available antigen tests (Ridascreen, r-Biopharm, Darmstadt, Germany) and for enterovirus via in-house PCR at the local Institute for Virology. In case of a positive antigen test, a confirmation test using PCR was performed from the same sample. Only virus detections via PCR were considered as positive results.

The infants' clinical and demographic characteristics, laboratory markers, and clinical presentation were immediately documented in a written case report form. Following pseudonymization, study personnel transferred the data into a prespecified Microsoft Excel database (Microsoft Office 1997–2013, Versions 8.0–15.0). Before saving, data of all infants with pathogen detection were additionally checked for correctness and completeness by the corresponding author using the original patient files.

The results of the viral tests were not blinded to the clinicians in the unit. In case of a positive test, infants were isolated or hospitalized in cohorts whenever possible. To avoid feelings of guilt especially in affected parents, we resigned the idea of screening close contacts intentionally.

Results

During the study period, 1,013 infants were admitted to our NICU. Parents had given their informed consent in all enrolled cases. In total, 101 infants were eligible for enrollment in the study but 13 infants had to be excluded due to incomplete or nonanalyzable samples. Finally, 88 patients and 137 sepsis episodes were analyzed. This analysis included the sepsis episodes reported in our feasibility study.[15]

Clinical and demographic data of enrolled cases are presented in ►Table 1. Several infants experienced multiple episodes of suspected late-onset sepsis. A total of 60 infants underwent one, 17 infants two, and 11 infants three or more sepsis evaluations. In 83% of all episodes with suspected late-onset bacterial sepsis, neither a bacterial nor a viral pathogen was detected.

A positive respiratory virus test was observed in six infants (6.8%). RSV was detected in two and picornavirus in four infants. One infant had three and another infant two consecutive episodes with a positive PCR result for picornavirus. These episodes were each identified as new clinical deteriorations. The infants had recovered and antibiotics had been stopped between all episodes. Viral testing was not performed during asymptomatic intervals. The two infants with RSV infection had not yet received RSV immunization. The characteristics of the virus-positive infants are summarized in ►Table 2. A virus and a positive blood culture were never detected simultaneously. However, one infant was diagnosed with both, a viral infection (picornavirus, 6 days postnatal) and in the later course with a proven bacterial bloodstream infection (*Klebsiella oxytoca*, 52 days postnatal). Respiratory viral infections did not occur in clusters in the NICU and none of the virus-positive infants had been discharged home previously. Stool examinations showed two

Table 1 Comparative clinical and demographic data of enrolled infants

	All Infants with suspected nosocomial sepsis (n = 88)	Infants with virus detection (n = 6)	Infants with positive blood culture (n = 15)	Infants without pathogen detection (n = 68)
Number of sepsis episodes	137	9	15	113
Gestational age median (IQR)	27 + 4 (25 + 4 to 31 + 4)	25 + 5 (24 + 5 to 36 + 5)	27 + 0 (25 + 5 to 31 + 2)	27 + 5 (25 + 5 to 31 + 5)
Birth weight median (IQR)	852 (714–1,560)	850 (645–2,045)	845 (612–1,365)	858 (720–1,508)
Male/female	50/38	3/3	9/6	38/30
Postmenstrual age at evaluation median (IQR)	31 + 6 (29 + 1 to 35 + 6)	45 + 3 (33 + 6 to 49 + 5)	32 + 2 (28 + 4 to 37 + 1)	31 + 4 (29 + 1 to 35 + 0)
Episodes (%) presenting with respiratory distress[a]	82 (59.9)	9 (100)	11 (73.3)	62 (54.9)
Episodes (%) with positive pressure respiratory support[b]	118 (86.1)	7 (77.8)	12 (80.0)	99 (87.6)
Episodes (%) with central venous line	67 (48.9)	4 (44.4)	10 (66.7)	53 (46.9)
Episodes (%) with open cot nursing	47 (34.3)	7 (77.8)	3 (20.0)	37 (32.7)
Episodes (%) with maximum CrP[c] > 15 mg/L	59 (43.1)	4 (44.4)	9 (60.0)	46 (40.1)
Episodes (%) with ≥ 5 d antibiotic treatment	78 (56.9)	8 (88.9)	15 (100)	55 (48.7)

Abbreviations: CrP, C-reactive protein; IQR, interquartile range.
Note: Gestational age and postmenstrual age is given in weeks + days, and weight is given in grams.
[a]Respiratory distress subsumes tachypnea, dyspnea, and increasing FiO_2.
[b]Positive pressure respiratory support subsumes continuous positive airway pressure and endotracheal ventilation.
[c]Maximum CrP was determined within 5 days.

positive antigen tests for adenovirus and one positive test for norovirus. When retested via PCR, none of the three positive antigen tests could be confirmed. Enterovirus and rotavirus were not detected in any of the studied infants. During the study period, no vaccinations for rotavirus had been performed. Characteristics of the 15 infants with a blood culture–proven sepsis are presented in ►Table 3. During the study period, no fungal bloodstream infection was diagnosed.

Discussion

Virus Infections during Birth Hospitalization
Virus detection in the NICU is an important issue that has been considered to contribute to successful antibiotic stewardship, provided that specific, validated algorithms can be implemented in clinical routine.[13] Although evidence is still lacking that the use of multiplex RT-PCR ELISA to detect respiratory viruses provides a medical or economic benefit to neonatal or pediatric patients,[13] potential benefits from virus detection may arise from early implementation of effective isolation and infection control measures to prevent viral spread. This is of particular importance since modern, family-centered neonatal care that includes kangaroo care and units that are always open to parents and siblings bears an increased risk of virus transmission. In our study, viral infections always presented with respiratory symptoms, albeit their clinical characteristics were highly heterogeneous. When compared with bacterial infections, nosocomial

viral infections occurred rather late during birth hospitalization. It is unclear whether this is due to physiological factors, such as decreasing maternal passive immunity or simply due to long-term hospitalization. Affected infants were either extremely premature infants or term infants with a pulmonary or thoracic disease that presented with respiratory distress. These might be the patients at high risk in whom viral testing should definitely be considered in cases of suspected late-onset sepsis.

Multiplex RT-PCR ELISA and Respiratory Viruses
Multiplex RT-PCR ELISA from nasopharyngeal specimens has been previously used to detect respiratory viruses in NICUs.[15–17,19] Our results are very similar to those recently published by Ronchi et al.[16] Their study had a comparable design and included a similar number of infants. A comparable incidence of 6% of viral respiratory infections in cases of sepsis evaluation in the NICU was observed. Likewise, rhinovirus, which is a member of the picornavirus family, was the dominating pathogen. Bennett et al performed a surveillance study of viral respiratory infections in two NICUs. All infants of a gestational age < 33 weeks were tested twice a week using multiplex RT-PCR ELISA. The authors found that 52% of all tested infants had a positive virus screen at least once during their birth hospitalization. In contrast to our study, they identified infants with a positive virus screen who had no or only minimal clinical symptoms.[17] Another study by Gonzalez-Carrasco et al used a mixed design of weekly

Table 2 Characteristics of virus-positive infants

No.	GA	Birth weight	Age at evaluation	Weight at evaluation	Comorbidities	Clinical presentation at evaluation	Respiratory support at evaluation	Multiplex PCR	Antibiotics ≥ 5 d	CrP[b] > 15 mg/L
1	40 + 2	3,650	95	5,150	Surfactant deficiency due to *ABCA3* STOP mutation	Agitation, tachypnea, dyspnea, increasing FiO_2	Endotracheal tube	Picornavirus	No	No
2	24 + 6	860	6	748	ELBW, RDS, pneumothorax	Fever (38°C), dyspnea, increasing FiO_2	CPAP	Picornavirus	Yes	No
3	26 + 4	840	18	1,048	ELBW, RDS	Tachypnea, apneas, cardiac arrhythmias, vomitus	CPAP	RSV	Yes	No
4[a]	24 + 1	450	155	3,500	ELBW, severe BPD, early NEC	Required intubation due to severe dyspnea and increasing FiO_2	CPAP	Picornavirus	Yes	Yes
			171	3,800		Unwell appearance, muscular hypotonia, dyspnea, rising pCO_2	Tracheostomy	Picornavirus	Yes	Yes
			186	4,200		Tachypnea, dyspnea, and apneas	Tracheostomy	Picornavirus	Yes	Yes
5[a]	24 + 4	580	65	1,750	ELBW, volvulus, ileostomy, BPD	Tachypnea, dyspnea, tachycardia	CPAP	Picornavirus	Yes	No
			90	2,520		Required CPAP due to increasing frequency of apneas and increasing FiO_2	Nasal cannula	Picornavirus	Yes	Yes
6	40 + 0	2,440	66	5,200	Suspected syndromic disorder: small thorax, tracheomalacia, muscular hypotonia	Dyspnea, apneas, increasing FiO_2, feeding intolerance, impaired microcirculation	Nasal cannula	RSV	Yes	No

Abbreviations: BPD, bronchopulmonary dysplasia; CPAP, continuous positive airway pressure; CrP, C-reactive protein; ELBW, extremely low birth weight; FiO_2, fraction of inspired oxygen; GA, gestational age; NEC, necrotizing enterocolitis; PCR, polymerase chain reaction; RDS, respiratory distress syndrome; RSV, respiratory syncytial virus.

Note: Gestational age is given in weeks + days, weight is given in grams, and age in postnatal days.

[a]Patients 4 and 5 had multiple episodes of virus detections.

[b]Maximum CrP was determined within 5 days.

Table 3 Characteristics of blood culture–positive infants

No.	GA	Birth weight	Age at evaluation	Weight at evaluation	Comorbidities	Clinical presentation at evaluation	Respiratory support at evaluation	Blood culture	Antibiotics ≥ 5 d	CrP[a] > 15 mg/L
1	25 + 4	720	12	612	ELBW	Hyperglycemia, respiratory insufficiency, increasing FiO_2	CPAP	CoNS	Yes	No
2	24 + 6	860	52	1,710	ELBW, chorioamnionitis, left-sided IVH III	Dyspnea, impaired microcirculation	CPAP	*Klebsiella oxytoca*	Yes	Yes
3	25 + 6	358	12	394	Placental insufficiency and severe IUGR, symptomatic PDA	Increasing FiO_2, tachypnea	Endotracheal tube	CoNS	Yes	No
4	24 + 2	790	7	690	ELBW	Agitation, increasing FiO_2	Endotracheal tube	CoNS	Yes	No
5	27 + 0	408	59	1,320	ELBW, IUGR, previous NEC	Tachycardia, dyspnea, agitation, feeding intolerance, increasing FiO_2	CPAP	*Enterococcus faecalis*	Yes	Yes
6	31 + 5	1,910	8	1,190	RDS, thrombocytopenia	Apneas, emesis, agitation	None	*Staphylococcus aureus*	Yes	Yes
7	23 + 0	645	12	705	RDS, PDA ligation	Impaired microcirculation, myocloni, rhinorrhea	Endotracheal tube	CoNS	Yes	Yes
8	40 + 5	3,340	12	Not determined	Esophageal atresia	Fever, pleural effusion, periodic breathing	None	CoNS	Yes	Yes
9	26 + 6	845	17	935	ELBW, RDS	Apneas, muscular hypotonia, tachycardia and bradycardia, lethargy, periodic breathing	CPAP	CoNS	Yes	Yes
10	29 + 6	1,150	16	1,465	VLBW, RDS	Apneas, bradycardias, retractions, periodic breathing, emesis	CPAP	CoNS	Yes	Yes
11	27 + 3	580	18	700	ELBW, DORV, RDS	Hyperglycemia, periodic breathing, increasing FiO_2	CPAP	CoNS	Yes	Yes
12	31 + 3	1,560	25	1,920	Chorioamnionitis, bilateral pneumothorax, RDS	Bradycardias, periodic breathing, retractions, tachypnea, increasing FiO_2, impaired microcirculation	CPAP	CoNS	Yes	No
13	37 + 6	3,220	8	3,010	Short rib polydactyly syndrome, pulmonary hypoplasia	Bradycardias, rhinorrhea, unwell appearance	Endotracheal tube	CoNS	Yes	No
14	26 + 6	550	6	565	ELBW, IUGR, placental abruption	Severe agitation	CPAP	*E. faecalis*	Yes	No
15	31 + 0	1,170	57	2,265	Osteogenesis imperfecta	Hypoxemia, retractions, fever, tachycardia, rhinorrhea, agitation, increasing FiO_2	Nasal cannula	CoNS	Yes	Yes

Abbreviations: CoNS, coagulase-negative staphylococci; CPAP, continuous positive airway pressure; DORV, double outlet right ventricle; ELBW, extremely low birth weight; FiO_2, fraction of inspired oxygen; GA, gestational age; IUGR, intrauterine growth restriction; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; VLBW, very low birth weight.
Note: Gestational age is given in weeks + days, weight is given in grams, and age in postnatal days.
[a]Maximum CrP was determined within 5 days.

surveillance and additional tests in the event of respiratory symptoms.[19] In that study, 9.3% of all samples were positive. A total of 13 infants were affected, and 9 of them were symptomatic. Again, rhinovirus was most frequently found (79% of the cases).

Interpretation of Positive Virus Tests

It can be doubted whether the detection of a virus in NPAs really proves an active respiratory viral infection.[20] A birth cohort study from van Benten et al showed that rhinovirus can be detected in 20% of asymptomatic infants.[21] In a matched case–control study, Rhedin et al compared the detection of 16 respiratory viruses in symptomatic versus asymptomatic children aged ≤ 5 years using quantitative real-time PCR. Their results suggest that RSV, human metapneumovirus, and parainfluenza virus are likely to be causative, if detected in symptomatic infants. The detection of other viruses such as rhinovirus, adenovirus, or enterovirus requires prudent evaluation since they were frequently found in asymptomatic infants.[22] This is in contrast to previous studies suggesting that picornaviruses/rhinoviruses are major causative pathogens in infants with lower respiratory tract infection.[23,24] Rhinovirus may be detectable for up to 5 to 6 weeks following infection,[21] but shorter clearance of around 2 weeks has also been described.[24] Since active shedding and clearance occurs, repeated detection of rhinovirus may rather reflect resolving or subclinical upper respiratory tract infection than colonization. It remains unclear how these results should be applied to preterm infants during birth hospitalization.

Directions for Further Research

In large-scale future studies, a screening cohort of asymptomatic infants and serial quantitative PCR analyses might help to evaluate whether clinical deteriorations may be caused by a reactivation of a persisting subclinical viral infection. To allow for discrimination of harmless colonization and true infection, a future study would ideally consist of three cohorts: (1) asymptomatic virus negative, (2) asymptomatic virus positive, and (3) symptomatic virus positive. The additional implementation of host-based RT-PCR gene expression assays into clinical practice might offer a significantly improved tool to diagnose viral infections.[25] Furthermore, the range of viruses, which can be screened for, should be expanded. Quite recently, a study with a comparable design investigated human parechovirus in blood samples of all cases of suspected late-onset sepsis in the NICU and found a high infection rate of 13%.[26] Further research should also focus on modes of virus transmission, patients at risk, and the impact of preventive measures and long-term outcomes of viral infections during birth hospitalization.

Note

The main results of the present study have been orally presented at the 5th International Conference on Clinical Neonatology (Torino, Italy, September 11–13, 2014).[27]

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