Neonatal Infections
—Clinical Manifestation and Diagnosis—

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The clinical characteristics of infections in neonates are different from those in children and adults. In particular, some neonates suffer from opportunistic infections and critical infections because neonates are physiologically immunocompromised. It is important that neonatologists make an early diagnosis and begin early treatment. However, early diagnosis is sometimes difficult because early-stage clinical symptoms are non-specific. Neonatologists should suspect neonatal infections when neonates have subtle symptoms. The diagnosis should be confirmed by laboratory data. Serial evaluation of C-reactive protein is useful for diagnosis of neonatal infections.

Introduction

Neonatal infection is one of most critical diseases that affect the prognosis of a child. Unlike infection in children and adults, the condition of the mother's body is also involved. Vertical infection such as congenital cytomegalovirus, and ascending infection and birth canal infection caused by intra-vaginal bacteria, can occur only during the perinatal period. In addition, the neonate is in an aseptic condition immediately after birth, and in many cases bacteria existing in the environment of the Neonatal Intensive Care Unit (NICU) become established and cause opportunistic infections, especially in premature infants whose immunocompetence is immature and who are physiologically susceptible to infection.

A basic principle of avoiding death and serious complications due to infection is early diagnosis and early treatment. Slight changes in clinical symptoms must not be overlooked, and confirmation of an infection diagnosis by blood testing or other means is important. This article describes the manifestation of neonatal infection and methods of diagnosis.

Manifestation of Neonatal Infection

As shown in Table 1, all organs of the body are a target of neonatal infection. Unlike children and adults, the types of infection include congenital infection through the placenta, infection through the birth canal during delivery, and infection through breast-fed milk. Like children and adults, infection related to medical treatment is an important route of infection.

Table 1 Neonatal infection

Central nerve infection
Meningitis, Subdural abscess
Respiratory tract infection
Pneumonia, Lung abscess
Blood infection (blood flow) infection
Septicemia, Catheter infection
Digestive tract infection
Bacterial enteritis, Candida enteritis
Skin infection
Impetigo, Staphylococcal scalded skin syndrome
Urinary tract infection
Osteomyelitis
Eye infection
Surgical wound infection

Neonatal infection is divided into early-onset infection and late-onset infection, depending on the time of onset. Although there are no strict definitions, onset within 72 hours after birth is commonly regarded as early-onset infection, and onset after 72 hours is regarded as late-onset infection. Early-onset infection is related to pregnancy and delivery complications such as water breaking before the onset of labor pains, fever in the mother during delivery, harboring of Group B

Table 2 Symptoms of neonatal infection

General condition	Cardiovascular symptoms
Not doing well Poor skin color Fever Low body temperature Peripheral cold sensation Lack of vigor Oliguria	Bradycardia Tachycardia Low blood pressure
	Digestive symptoms
	Poor suckling Vomiting Increase of stomach residue Abdominal distention Diarrhea
Respiratory symptoms	**Neurological symptoms**
Apnea Polypnea Respiratory distress Increase of respiratory tract secretion	Convulsions Somnolence

Streptococcus (GBS) bacteria in the mother, and chorioamnionitis. Late-onset infection has little relation to delivery complications.

Clinical Symptoms

As shown in Table 2, infections in neonates present a variety of clinical symptoms. Care is needed because in many cases, early-stage symptoms are non-specific. In neonatal medicine, a vaguely poor condition is expressed as "Not doing well". "Not doing well" is a comprehensive term that includes poor feeding, poor skin color and other conditions, and when an infant is "not doing well", close observation and detailed diagnosis are necessary. If a change is observed in body temperature, respiratory rate, pulse rate, or other vital signs, it is critical that infection be checked for.

Various Tests for Neonatal Infection

Blood culture

When neonatal infection is suspected, various cultures are performed before an antibiotic is administered. A blood culture is indispensible for the diagnosis of septicemia and bacteremia, and thus the sample is drawn by means of an antiseptic procedure that prevents bacteria occurring naturally on the skin from contaminating the sample. To avoid iatrogenic anemia, there is often a tendency to use a small blood sample volume, however, the work of Connell et al. indicates that if a sufficient volume is not collected, the cultivation positive rate drops by about half (5.1% vs. 2.6%).[1] and this creates the risk of a false negative diagnosis for septicemia and bacteremia. It is possible to reduce the sample volume by using a blood cultivation bottle that enables cultivation of both aerobic and anaerobic bacteria.

Blood testing

A variety of test parameters are used as infection markers for the diagnosis of infection, and in particular, a blood count and C-reactive protein (CRP) is widely used as an infection marker. To interpret the results, the sensitivity and specificity for diagnosis must be understood.

Blood count

Neonatal WBC (White blood cell) alone has little value for diagnosis due to its susceptibility to the effects of the gestation period, pregnancy-induced hypertension, and other complications in the mother's body. Differential WBC is useful for the diagnosis of infection. When the ratio of immature neutrophils to the total NEU (I/T ratio) is 0.2 or higher, infection is suspected. Based on this criterion, sensitivity is 90% to 100% and specificity is 50% to 78%.[2] However, WBC and I/T change dramatically immediately after birth, and thus caution is required when interpreting the results of a test performed immediately after birth.[3] Severe infection is accompanied by a drop in platelets, however, it is reported that this has low sensitivity and specificity for the diagnosis of infection.[4, 5]

C-reactive protein (CRP)

CRP is an acute phase reactant that increases when an inflammation reaction or tissue destruction occurs, and CRP has this name because it combines with C-polysaccharide of pneumococcus. CRP accompanies infection and inflammation, and increases over a reaction time of 6 to 8 hours, and thus is regarded as having low sensitivity for early diagnosis. Benitz et al. studied infection diagnosis and CRP value at the onset of infection symptoms, CRP value 8 to 24 hours after the onset of symptoms, maximum CRP value 24 to 48 hours after the onset of symptoms, and maximum CRP value within 48 hours after the onset of symptoms (6). When the CRP cutoff value was 1.0 mg/dL, the sensitivity was low at 35% for CRP at the onset of symptoms of early-onset infection; however, at the maximum value of CRP within 48 hours after the onset of symptoms, the sensitivity was 88.9 %. (Table 3) In late-onset infection, the CRP sensitivity at the onset of symptoms was 61.5%, higher than early-onset infection. However, it must be kept in mind that CRP at the time of onset is normal in about 40% of neonates with late-onset infection. Like early-onset infection, the diagnosis sensitivity of late-onset infection improves with serial CRP evaluation. (Table 3) The relation between maximum CRP value 8 to 48 hours after the onset of infection and the positive predictive value for infection diagnosis in late-onset infection is a positive predictive value of 20% or higher at a CRP value of 2.0 mg/dL. In early-onset infection, the

Table 3 Usefulness of CRP in bacterial infection and mycotic infection

Early-onset infection

	CRP #1	CRP #2	CRP #2 & #3	CRP×3
Sensitivity (Confidence interval 95%)	35.0% (30.2-40.6)	78.9% (72.0-86.4)	88.9% (80.8-94.3)	88.9% (80.8-94.3)
Specificity (Confidence interval 95%)	90.0% (88.1-91.9)	78.4% (75.8-81.0)	73.8% (71.1-76.6)	70.5% (67.7-73.4)
Positive predictive value (Confidence interval 95%)	6.7% (1.9-11.4)	6.7% (3.4-10.0)	6.0% (3.1-8.8)	5.2% (2.2-7.7)
Negative predictive value (Confidence interval 95%)	98.6% (97.8-99.3)	99.5% (99.3-99.6)	99.7% (99.5-99.9)	99.7% (99.5-99.8)

Late-onset infection

	CRP #1	CRP #2	CRP #2 & #3	CRP×3
Sensitivity (Confidence interval 95%)	61.5% (48.3-74.8)	84.4% (80.5-87.3)	96.4% (90.9-99.1)	97.5% (93.6-99.4)
Specificity (Confidence interval 95%)	68.9% (61.0-76.8)	74.6% (66.7-82.7)	71.8% (63.6-79.9)	60.5% (52.0-68.9)
Positive predictive value (Confidence interval 95%)	43.8% (32.5-55.2)	47.4% (34.4-60.5)	45.0% (32.4-57.6)	43.3% (33.1-53.6)
Negative predictive value (Confidence interval 95%)	82.0% (74.8-89.1)	94.6% (93.3-95.5)	98.8% (97.0-99.7)	98.7% (96.7-99.7)

CRP #1: CRP value when infection symptoms first appear (initial evaluation), CRP #2: CRP value 8 to 24 hours after infection symptoms appear (2nd evaluation), CRP #2 & #3: Higher CRP value 8 to 48 hours after infection symptoms appear (2nd, 3rd evaluation), CRP×3: Highest CRP value within 48 hours after infection symptoms appear (1st to 3rd evaluation) (revision of citation from reference 6)

CRP value must be over 6.0 mg/dL in order for the positive predictive value to rise to 20% or higher, and thus infection is often present even when the CRP value is low.

For this reason, a low CRP value when symptoms of infection are observed is not sufficient to validate refraining from antibiotic treatment. In addition, because the negative predictive value of CRP concentration is low at the initial onset of infection, it is dangerous to conclude that there is no infection when CRP is negative. Serial evaluation of CRP is critical to the diagnostic evaluation of neonatal infection.

Cerebrospinal fluid testing

It is said that the majority of cases of bacterial meningitis in small children and neonates is hematogenous infection, however, a NICU retrospective study in the United States indicated that 38% of neonatal bacterial meningitis is blood culture-negative, and thus inferring the presence or absence of bacterial meningitis from a blood culture result is dangerous.[7] Cerebrospinal fluid testing is indispensible to the diagnosis of bacterial meningitis, and it is necessary to perform a lumbar puncture on an infant that shows symptoms of infection. However, a lumbar puncture is invasive, and when the general condition of the neonatal is poor, it is also acceptable to perform this procedure after the general condition of the neonatal stabilizes sufficiently. In mature infants that have a risk of early-onset infection because the mother has a fever or otherwise, but there are no symptoms of infection, the frequency of occurrence of bacterial meningitis is very low and thus there is no need to perform a lumbar puncture.[8]

Because there is no fixed viewpoint regarding the diagnosis of neonatal bacterial meningitis, a comprehensive judgment must be made based on cerebrospinal cell count, glucose, and protein content. It is reported that with a cell count of 21/mm^3 as the cutoff value, sensitivity is 79% and specificity is 81%. With a cell count of 100/mm^3 as the cutoff value, sensitivity is 66% and specificity is 94%.[7] Regarding protein in cerebrospinal fluid, it is thought that a value under 100 mg/dL is normal in neonates. However, in premature infants, this value is high and caution is required when interpreting the results.

Current State of Blood Testing for Neonatal Infection

The weight of neonates is about 2500 to 3000 g, even for full-term infants, and thus circulating plasma volume is low compared to adults. For example, in infants with an extremely low birth weight of 750 g, 1 mL of collected blood is equivalent to 100 mL in an adult male weighing 75 kg. If CBC (complete blood count) and CRP are repeatedly tested because infection is suspected, iatrogenic anemia will be evoked. The ability to perform high-accuracy testing with micro-volumes is therefore desirable.

We examined whether sufficient testing accuracy could be obtained with micro-volume samples using a hematology analyzer, Microsemi CRP (LC-667CRP manufactured by HORIBA, Ltd.)[9] Using 10 healthy adults, we collected approximately 60 µL using a lancet from the left fourth finger (micro-volume sample group), and 2 mL from an elbow vein using a vacuum blood collection tube (regular volume sample group). Using these two sample types, we measured WBC, RBC, Hgb, Hct, PCT and other parameters. Examining the correlations between the two sample types, we found a very high correlation for WBC, RBC, Hgb, and Hct, which indicated that a high accuracy result is obtained even with micro-volume samples. (Figure 1) Next, we used 41 samples from 19 neonates (birth weight 378 g to 3430 g, median 1948 g) in our NICU, and compared analysis results using the Microsemi CRP (LC-667CRP) to analysis results using the analyzers in our central laboratory (blood count: XE-2100, Sysmex Corporation; CRP: JCA-BM225, JEOL Ltd.). A high correlation was observed for WBC, RBC, PLT and CRP (Figure 2), suggesting that micro-volumes can be analyzed with high

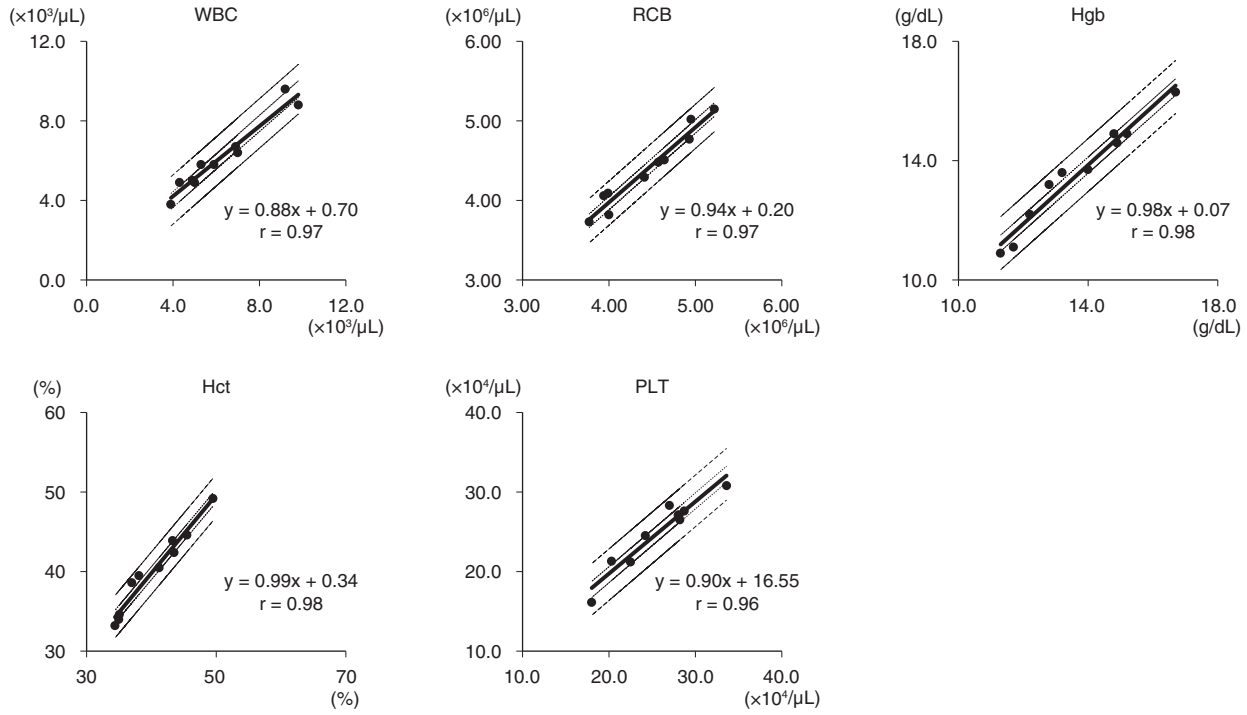

Figure 1 Comparison of blood counts of micro-volume samples and regular volume samples
Horizontal axis of each graph: Micro-volume sample group (analysis results using 60 μL samples (approx.) collected from the left 4th finger using a lancet)
Vertical axis of each graph: Regular volume sample group (analysis results using 2 mL samples collected from elbow vein using a vacuum blood collection tube)

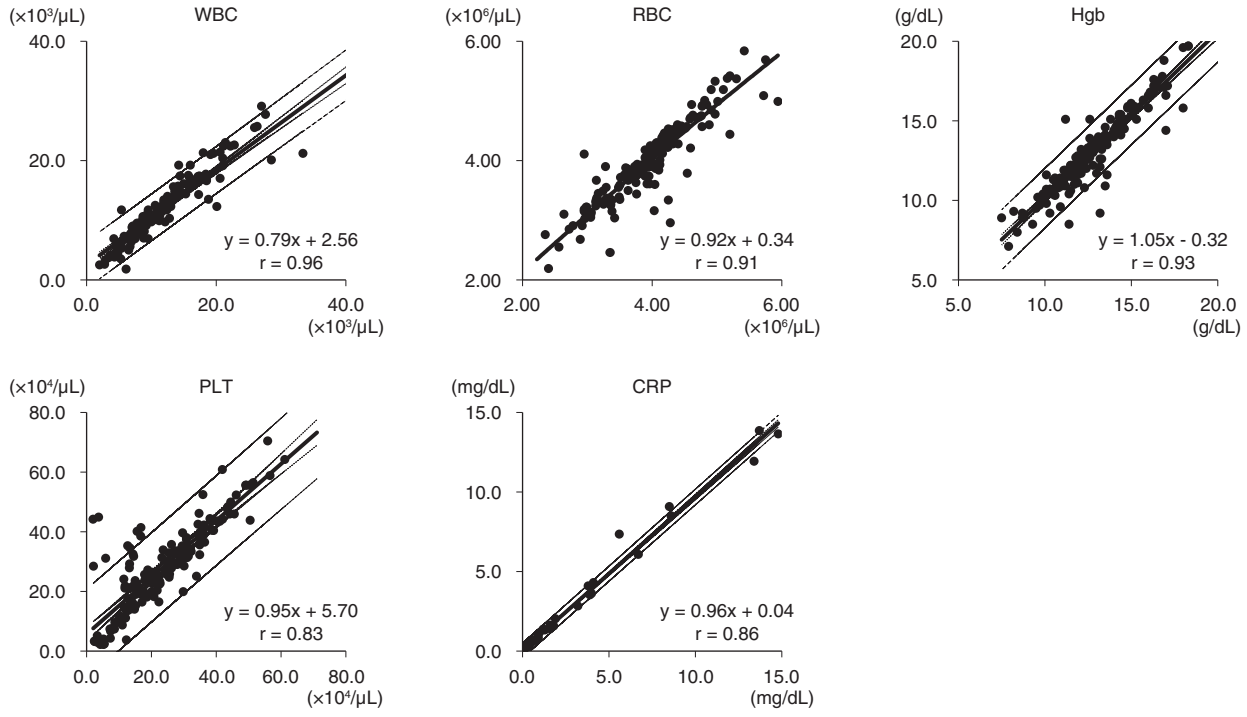

Figure 2 Comparison of blood count and CRP results using the Microsemi CRP (LC-667CRP) and our central laboratory analyzers
Horizontal axis of each graph: Analysis results using the Microsemi CRP(LC-667CRP) Vertical axis of each graph: Analysis results using analyzers in our central laboratory (blood count: XE-2100, Sysmex Corporation; CRP: JCA-BM225, JEOL Ltd.)

accuracy on the Microsemi CRP(LC-667CRP).[10] This indicated that the Microsemi CRP (LC-667CRP) is useful for management of neonatal infection that requires serial evaluation of CRP.

Conclusions

Neonatal infection has a significant effect on the prognosis of an infant, however, symptoms are non-specific in the incipient stage, and thus it is very difficult

to diagnose infection with certainty from clinical symptoms alone. In addition, CRP is often negative in the incipient stage, and thus definitive diagnosis is difficult in early diagnosis. Observation of changes in clinical symptoms and serial evaluation of CRP are critical, however, frequent collection of blood creates a risk of evoking iatrogenic anemia. It is desirable to manage neonatal infection using the Microsemi CRP analyzer for blood analysis and CRP measurement (LC-667CRP), which allows testing with micro-volume blood samples.

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