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ORIGINAL ARTICLE

Detection of Bacterial Infection Based on Age-Specific Percentile-Based Reference Curve for Serum Procalcitonin Level in Preterm Infants

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SUMMARY

Background: **Considering the physiological changes in serum procalcitonin (PCT) levels in newborns due to age, we recently established an age-specific percentile-based reference curve for serum PCT level. The present study aimed to determine the best cutoff percentile line using this reference curve for the differentiation between infected and colonized preterm infants.**
Methods: **A total of 52 preterm infants with positive bacterial culture (9 with bacterial infection, 43 with colonization) were enrolled within the study period. The 97.5th, 95.0th, 92.5th, 90.0th, 80.0th, 70.0th, 60.0th, and 50.0th percentile lines were drawn in the reference curve. PCT levels in infected or colonized infants were used, and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. The best cutoff percentile line was determined in the receiver operating characteristic curve analysis.**
Results: **Of the 52 preterm infants, 9 were infected (5 and 4 infants with an onset of < 7 days and ≥ 7 days after birth, respectively), whereas 43 were colonized (6 and 37 infants with an onset of < 7 days and ≥ 7 days after birth, respectively). The best cutoff percentile lines were the 90.0th percentile (sensitivity, 0.800; specificity, 0.833; PPV, 0.800; NPV, 0.833) and 97.5th percentile (sensitivity, 1.00; specificity, 0.973; PPV, 0.800; NPV, 1.00) in infants with an onset of < 7 days and ≥ 7 days after birth, respectively.**
Conclusions: **The age-specific percentile-based reference curve for serum PCT level is clinically applicable as a new tool for diagnosing infections in preterm infants with positive culture results, particularly at ≥ 7 days after birth.**
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colonization, infection, neonatal intensive care unit, physiological change, reference curve, procalcitonin

INTRODUCTION

Neonatal bacterial infections including sepsis and pneumonia are a major cause of neonatal mortality globally [1] and can be categorized into two subgroups: early-onset infection (< 72 hours or 7 days after birth) and late-onset infection (≥ 72 hours or 7 days after birth) [2,

3]. Early-onset infection usually occurs *in utero* or during vaginal delivery and is mainly due to group B streptococcus and *Escherichia coli* [4]. In contrast, late-onset infection is often transmitted through the contaminated hands of healthcare personnel, particularly in preterm infants during neonatal intensive care unit (NICU) stay, and are primarily caused by varied pathogens [3,5]. Therefore, the longer the length of NICU stay, the higher the infection rate.

Coagulase-negative staphylococci, *Staphylococcus aureus*, and other gram-negative rods are often detected in infected or colonized preterm infants with or without symptoms during NICU stay [3,6]. In the early stage of infection, symptoms are absent in preterm infants or are difficult for physicians or nursing staff to identify. In addition, although white blood cell (WBC) count and serum C-reactive protein (CRP) levels can be used to distinguish both conditions, their precision is insufficient in adult and pediatric patients with bacterial diseases [7-9].

Serum procalcitonin (PCT) level is a more sensitive marker for bacterial infection or sepsis than WBC count and serum CRP level [7-9]. PCT-guided decision-making has recently been reported to reduce the duration of antibiotic therapy in newborns with suspected early-onset infection [10]. Reference values and cutoff point for serum PCT levels in adult and newborn patients with bacterial infection and other diseases have been proposed [7,8,11-18]. Considering the physiologically elevated serum PCT level in newborns after birth, PCT concentration should be carefully interpreted when diagnosing an infection [14-18]. Therefore, we established a postnatal age-specific reference curve for serum PCT level in preterm infants during NICU stay [18]. In our previous study, the serum PCT level at onset in three preterm infants with sepsis or bacterial infection was clearly over the 95th percentile reference value for PCT level [18]. Nevertheless, the effectiveness of postnatal age-specific reference curve in preterm infants, particularly in the differential diagnosis of colonization and infection, has not been fully investigated. Furthermore, the best cutoff percentile line in the age-specific reference curve for PCT level has not yet been determined. The present study aimed to determine the best cutoff percentile line between the 50th and 97.5th percentiles in our reference curve for the differentiation between infected and colonized preterm infants.

MATERIALS AND METHODS

Study design, subjects, and definitions

This retrospective study enrolled preterm infants admitted to the NICU of Kobe University Hospital (Kobe, Japan) between June 2014 and March 2016. Residual serum samples following completion of routine laboratory tests were used for PCT measurement. Data collection and the use of human materials for this study were approved by the ethics committee of Kobe University Graduate School of Medicine (approval no. 1688), and written informed consent was obtained from parents of all enrolled newborns.

The preterm infants included in this study were identified using surveillance cultures or cultures of the collected samples when patients exhibited any clinical signs and symptoms. A total of 52 preterm infants with positive bacterial culture during NICU stay were enrolled within the study period. Infection was defined as positive bacterial culture, abnormal peripheral WBC counts or serum CRP levels, and treatment with antibiotics, as previously described [19]. Colonization was defined as positive bacterial culture, but without clinical signs and symptoms and without the use of antibiotics. Based on the definition of early-onset and late-onset infections in the current textbook of pediatrics, subjects were divided into two subgroups according to the days when specimens were collected from infants with positive bacterial culture: < 7 days and ≥ 7 days after birth [2]. In infants with positive bacterial culture, serum PCT level was measured on the same day when the culture was positive (median [min to max]: 0 [-1 to 1] day for infected infants or 0 [-2 to 6] day for colonized infants).

Serum PCT measurement

Briefly, serum PCT level was measured in 30 μL of serum by electrochemical luminescence immunoassay using the COBAS 8000e analyzer (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions [18]. Data are shown as ng/mL. This measurement was the same method used for establishing the previously reported postnatal age-specific percentile-based reference curve [18].

Culture specimens

Clinical culture specimens were collected based on clinical symptoms or surveillance. With respect to surveillance, weekly surveillance cultures of nasal swab specimens for methicillin-resistant *Staphylococcus aureus* (MRSA) were performed while the infants were staying at the NICU. Bronchoalveolar lavage fluid (BALF) was obtained from intubated infants by once-weekly screening. A culture sample was obtained at the time of tracheal tube or catheter removal in infants treated with tracheal intubation or catheterization (e.g., umbilical or peripherally inserted central catheter).

Culture method

Bacterial culture was performed using conventional methods. Briefly, for swab samples acquired from body surfaces (including the nasal cavity), BALF, tracheal tube, or catheter, Gram staining and culture were performed using sheep blood and chocolate agars to isolate organisms.

For blood samples, 1 mL of blood was obtained by venipuncture or through arterial lines using sterile techniques. The acquired blood was injected into a BacT/ALERT PF bottle (bioMérieux Inc., Durham, NC,

USA). Subsequently, the bottle was incubated in the BacT/ALERT 3D microbial detection system (bioMérieux Inc., Durham, NC, USA). Aliquots removed from bottles flagged as positive by the instrument were Gram-stained and subcultured in sheep blood and chocolate agars. Isolated organisms were identified by matrix-assisted laser desorption-ionization (MALDI) time-of-flight mass spectrometry using the MALDI Biotyper and Biotyper 2.0 database (Bruker Japan Inc., Kanagawa, Japan).

Weekly surveillance cultures of nasal swab specimens for MRSA were screened using ST tubes (P series; Corona Giken Industry Co., Ltd., Matsudo, Japan). In the event of a positive result, cultures were performed using trypticase soy agar with 5% sheep blood (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and MRSA selective agar (MDRS-K agar™; Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan). In order to identify MRSA, antimicrobial susceptibility test for the isolated strain was subsequently performed using the WalkAway 96 plus™ system with PMIC3.3J panel (Beckman Coulter Inc., Brea, CA, USA).

Definitions of abnormal WBC counts and serum CRP levels

Abnormal peripheral WBC counts and serum CRP levels were defined as $\geq 20 \times 10^3/\mu L$ or $< 5 \times 10^3/\mu L$ and ≥ 0.5 mg/dL, respectively [3,19].

Study methods and statistical analyses

Serum PCT level was evaluated using our previously reported postnatal age-specific percentile-based reference curve: from birth to 6 days after birth and from 1 week to 9 weeks after birth (Figure 1) [18]. The 97.5th, 95.0th, 92.5th, 90.0th, 80.0th, 70.0th, 60.0th, and 50.0th percentile lines were drawn in the reference curve (Figure 1). For the determination of the best percentile line that could distinguish infection from colonization, PCT levels in infected or colonized infants were used, and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. The best cutoff percentile line was determined using the maximum Youden index in the receiver operating characteristic curve analysis. The Youden index is the farthest point from the boundary delineating the area under the curve (AUC) and represents the value of [sensitivity + specificity - 1] [20].

RESULTS

Characteristics of enrolled preterm infants

The characteristics of infected and colonized preterm infants are summarized in Table 1. Of the 52 preterm infants with positive bacterial culture, 9 were infected (5 infants with an onset of < 7 days after birth and 4 infants with an onset of ≥ 7 days after birth), whereas the remaining 43 were colonized (6 infants with an onset of < 7 days after birth and 37 infants with an onset of ≥ 7 days after birth). The median gestational age and median birth weight were significantly lower in infected infants than in colonized infants (median gestational age: 24 vs. 28 weeks, $p < 0.01$; median birth weight: 544 g vs. 1,030 g, $p < 0.01$ by the Mann-Whitney *U* test). Bacteria were detected in the blood samples from 56% of infected infants and in the BALF and tracheal tube or nasal cavity samples from 47% and 23% of colonized infants, respectively.

Characteristics of infected infants

Table 2 presents the characteristics of infected infants. Of the 9 infected infants, 6 (67%), 2 (22%), and 1 (11%) were diagnosed by attending neonatologists with sepsis or clinical sepsis, ventilator-associated pneumonia, and dermatitis, respectively. Some infants with positive bacterial culture did not exhibit abnormal peripheral WBC counts or serum CRP levels. With respect to pathogenic bacteria, *Bacillus cereus* was detected in 3 infants who developed sepsis at < 7 days of life, whereas *Enterobacter cloacae* was identified in 2 infants who had sepsis at ≥ 7 days after birth.

Cutoff lines for serum PCT level to distinguish infection from colonization

Serum PCT levels in infected or colonized preterm infants were plotted in the postnatal age-specific percentile-based reference curve (Figure 1). The sensitivity, specificity, PPV, and NPV for the differentiation between infection and colonization using cutoff percentile lines from 50.0th to 97.5th percentiles are summarized in Table 3 and Supplementary Data 1 - 3. Overall, sensitivity decreased from 1.00 to 0.667 in enrolled infants; conversely, as expected, specificity increased from 0.372 to 0.953 as percentile lines increased. In all lines, the sensitivity, specificity, and NPV in infants with an onset of ≥ 7 days were higher than or equal to those in infants with an onset of < 7 days. In all lines except for the 97.5th percentile, the PPV in infants with an onset of ≥ 7 days was lower than or equal to that in infants with an onset of < 7 days.

The best cutoff percentile line based on the Youden index was the 90.0th percentile (sensitivity, 0.889; specificity, 0.860; PPV, 0.571; NPV, 0.974). Furthermore, the best cutoff percentile lines for serum PCT level were the 90.0th percentile in infants with an onset of < 7 days after birth (sensitivity, 0.800; specificity, 0.833; PPV, 0.800; NPV, 0.833) and the 97.5th percentile in infants with an onset of ≥ 7 days after birth (sensitivity, 1.00; specificity, 0.973; PPV, 0.800; NPV, 1.00).

DISCUSSION

We attempted to distinguish infection from colonization in preterm infants with positive bacterial culture using the age-specific percentile-based reference curve for serum PCT level that we developed [18]. In our previous study [18], the PCT level gradually decreased after peak

Table 1. Characteristics of enrolled preterm infants.

	Infection			Colonization (n = 43)
	Overall (n = 9)	Onset of < 7 days (n = 5)	Onset of ≥ 7 days (n = 4)	
GA, weeks	24 (22 - 27)	24 (22 - 25)	24 (22 - 27)	28 (22 - 33)
Birth weight, g	544 (364 - 1,014)	530 (364 - 822)	600 (482 - 1,014)	1,030 (356 - 2,688)
Gender (male/female)	4/5	2/3	2/2	20/23
Age at positive culture, day	5 (1 - 42)	3 (1 - 5)	30.5 (13 - 42)	12 (4 - 50)
Culture sample				
Blood	5 (56)	3 (60)	2 (50)	0 (00)
BALF/tracheal tube	2 (22)	0 (0)	2 (50)	20 (47)
Catheter	0 (0)	0 (0)	0 (0)	8 (19)
PICC	0 (0)	0 (0)	0 (0)	4 (9)
Umbilical	0 (0)	0 (0)	0 (0)	4 (9)
Scraping swabs	2 (23)	2 (40)	0 (0)	5 (11)
Nasal cavity	0 (0)	0 (0)	0 (0)	10 (23)
Skin	1 (11)	1 (20)	0 (0)	3 (7)
Umbilicus	1 (11)	1 (20)	0 (0)	0 (0)
Eye discharge	0 (0)	0 (0)	0 (0)	2 (5)

Data are presented as median (range) or number (%). GA - gestational age, BALF - bronchoalveolar lavage fluid, PICC - peripherally inserted central catheter.

Table 2. Characteristics of infected infants.

	Case	Diagnosis	Pathogenic bacteria	Culture sample	Age at positive culture (weeks/ days)	PCT (ng/mL)	CRP (mg/dL)	WBC (/μL)	Antibiotics
Onset of < 7 days after birth	A	Dermatitis	MRS	Skin	0/5	0.49	< 0.03	4,200	ABPC + FCZ
	B	Sepsis	*B. cereus*	Arterial blood	0/1	30.6	0.74	2,000	ABPC
	C	Sepsis	*B. cereus*	Arterial blood	0/4	2.46	1.23	1,200	ABPC
	D	Sepsis	*B. cereus*	Arterial blood	0/3	38.64	3.33	8,600	ABPC + AMK
	E	Clinical sepsis	*C. freundii*	Umbilicus	0/1	> 100	0.66	16,700	ABPC + AMK
Onset of ≥ 7 days after birth	F	Sepsis	*E. cloacae*	Venous blood	2/20	> 100	0.21	4,500	TAZ/PIP
	G	VAP	*P. aeruginosa*	Tracheal tube	1/13	3.18	1.75	32,500	CMZ
	H	VAP	*K. pneumoniae*	BALF	5/41	1.49	0.62	7,400	CMZ
	I	Sepsis	*E. cloacae*	Venous blood	6/42	43.01	13.64	16,900	CTX

ABPC - ampicillin, AMK - amikacin, BALF - bronchoalveolar lavage fluid, CMZ - cefmetazole, CRP - C-reactive protein, CTX - cefotaxime, MRS - methicillin-resistant staphylococcus, PCT - procalcitonin, TAZ/PIP - tazobactam/piperacillin, VAP - ventilator-associated pneumonia, WBC - white blood cell.

Table 3. Precision of percentile lines for serum PCT level to distinguish infection from colonization.

	Cutoff line, percentile	Sensitivity	Specificity	PPV	NPV	Youden index
Overall (n = 52)	50.0	1.000	0.372	0.250	1.000	0.372
	60.0	1.000	0.488	0.290	1.000	0.488
	70.0	1.000	0.628	0.360	1.000	0.628
	80.0	0.889	0.721	0.400	0.969	0.610
	90.0	0.889	0.860	0.571	0.974	0.749
	92.5	0.667	0.930	0.667	0.930	0.597
	95.0	0.667	0.930	0.667	0.930	0.597
	97.5	0.667	0.953	0.750	0.932	0.620
Onset of < 7 days (n = 11)	50.0	1.000	0.167	0.500	1.000	0.167
	60.0	1.000	0.333	0.556	1.000	0.333
	70.0	1.000	0.500	0.625	1.000	0.500
	80.0	0.800	0.667	0.667	0.800	0.467
	90.0	0.800	0.833	0.800	0.833	0.633
	92.5	0.400	0.833	0.667	0.625	0.233
	95.0	0.400	0.833	0.667	0.625	0.233
	97.5	0.400	0.833	0.667	0.625	0.233
Onset of ≥ 7 days (n = 41)	50.0	1.000	0.405	0.154	1.000	0.405
	60.0	1.000	0.514	0.182	1.000	0.514
	70.0	1.000	0.649	0.235	1.000	0.649
	80.0	1.000	0.730	0.286	1.000	0.730
	90.0	1.000	0.865	0.444	1.000	0.865
	92.5	1.000	0.946	0.667	1.000	0.946
	95.0	1.000	0.946	0.667	1.000	0.946
	97.5	1.000	0.973	0.800	1.000	0.973

The maximum Youden index is indicated in underlined line. PCT - procalcitonin, PPV - positive predictive value, NPV - negative predictive value.

concentrations were achieved at 1 day after birth; in preterm infants, the PCT level takes 9 weeks to decrease to 0.1 ng/mL, which is within the normal range for adults [11]. In order to utilize serum PCT levels in diagnosing bacterial infections in preterm infants, taking such physiological changes into account is necessary. Even with positive results of culture, evaluating whether the patient is infected or colonized is required. We conducted the present study, as we thought that a reference curve for PCT level is useful for the accurate diagnosis of infection.

Low birth weight infants tend to be prone to infections [3]. In the present study in which subjects were limited to preterm infants, gestational age at birth and birth weight were lower in infected infants than in colonized infants (Table 1). In addition, numerous cases of bloodstream infections were identified in more preterm and low birth weight infants. As sepsis and bloodstream infections are directly associated with prognosis [3], an appropriate marker and criteria are required, which can be helpful for early diagnosis.

PCT level is superior to WBC count and serum CRP level as a marker of sepsis in adults and children [7-9]. However, as indicated by the results of the present study, the PCT level in several colonized infants was > 0.5 ng/mL (Figure 1), which was the standard value for diagnosing a bacterial infection [7,15,21]. Criteria that take physiological changes into consideration, such as the reference curve for PCT level, are believed to be required for preterm infants.

Although standard values for CRP level (> 0.5 mg/dL) and WBC count (< 5,000 or > 20,000/μL) were set, there were some cases of infection in which these standard values could not be fulfilled (Table 2). Not only preterm infants with elevated CRP level and WBC count (e.g., Case I) but also preterm infants in whom the CRP level did not increase (e.g., Case F) were identified. Hence, the PCT level should be appropriately used

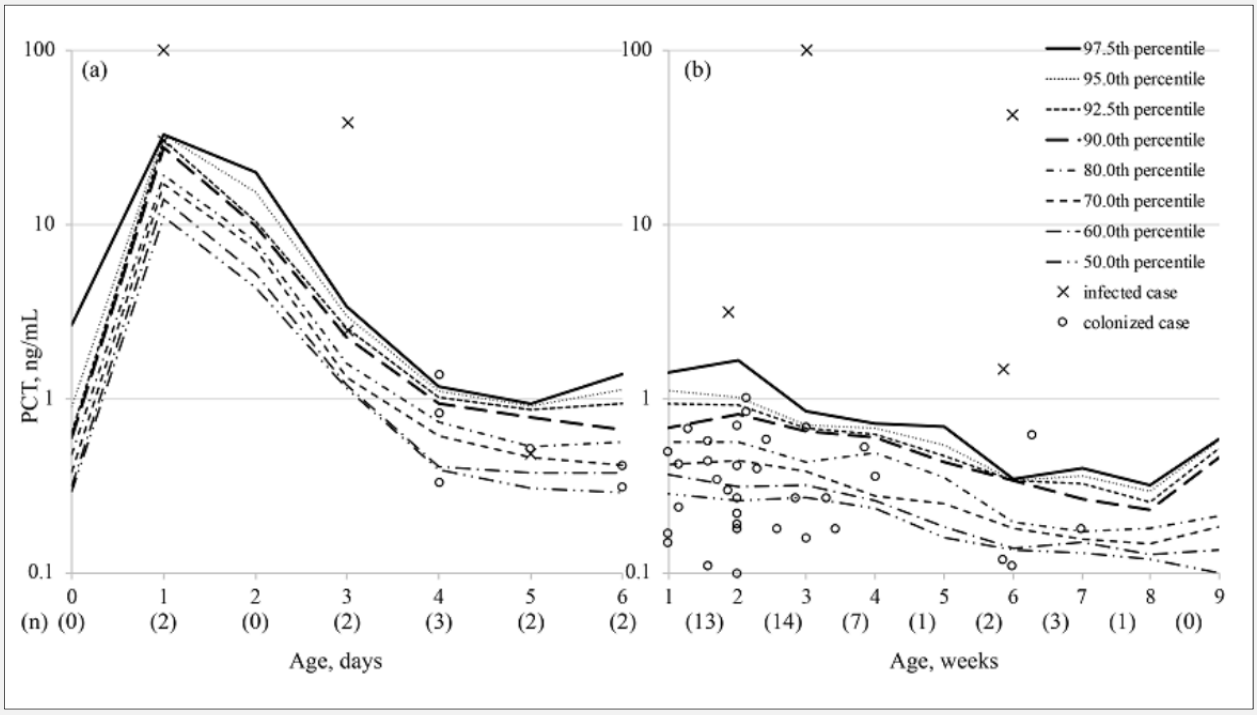

Figure 1. Postnatal age-specific percentile-based reference curves for serum PCT levels: (a) from birth to 6 days after birth and (b) from 1 week to 9 weeks after birth. The 97.5th, 95.0th, 92.5th, 90.0th, 80.0th, 70.0th, 60.0th, and 50.0th percentile lines were drawn in the reference curve.

Serum PCT levels in infected or colonized infants were plotted. The number of infected or colonized infants is presented as (n). PCT - procalcitonin.

when evaluating infections in preterm infants. Hahn et al. [22] reported that serum PCT level and PCT/CRP ratio were more useful than serum CRP level alone in distinguishing confirmed sepsis from suspected sepsis in preterm infants aged 7 - 16 days. Eschborn and Weitkamp reviewed 39 clinical studies directly comparing PCT with CRP levels for diagnosis of early-onset or late-onset sepsis, and reported the sensitivity and sensitivity in PCT were higher than those in CRP [23]. Furthermore, a recent meta-analysis has shown that the combination of PCT and CRP improves the accuracy of diagnosis of neonatal sepsis [24].

Assuming that the 95th percentile reference value for PCT level was set as the cutoff [18], sensitivity and specificity were 0.400 and 0.833, respectively, in preterm infants with an onset of < 7 days after birth and 1.000 and 0.946, respectively, in preterm infants with an onset of ≥ 7 days after birth. Based on the results of the present study, the most suitable cutoff percentile lines were the 90.0th and 97.5th percentiles in preterm infants with an onset of < 7 days and ≥ 7 days, respectively. Thus, there was a difference in suitable cutoff percentile lines between preterm infants with an onset of < 7 days and ≥ 7 days, which is thought to be due to the PCT levels that greatly varied depending on physiological changes and individual differences during a certain postnatal period. Thus, sensitivity and specificity decreased in preterm infants with an onset of < 7 days, because the unevenness of physiological changes overlapped the PCT level elevation due to infection. Nevertheless, the differentiation between infection and colonization became clear after 7 days of life, which is the period in which physiological changes settle down. The results of a meta-analysis even indicated better diagnostic accuracy in infants with late-onset sepsis (≥ 72 hours of life) than in those with early-onset sepsis (< 72 hours of life) (AUC, 0.78 vs. 0.95) [25]. In Japan, the high risk of infection and colonization due to prolonged NICU stay is currently a clinical practice issue concerning preterm infants with an onset of ≥ 7 days. Based on the results of the present study, distinguishing infection from colonization may be possible using the 97.5th percentile line for PCT level.

As for the precision in identifying infection in preterm infants with an onset of < 7 days, Turner et al. compared sepsis and non-sepsis in preterm infants and reported

sensitivity, specificity, PPV, and NPV of 0.74, 0,54, 0.53, and 0.78, respectively, just after diagnosis, assuming that the cutoff value was 0.5 ng/mL [15]. In our study, assuming a 90.0th percentile cutoff value (e.g., 0.6 ng/mL at birth, 27.7 ng/mL at 1 day after birth, 0.7 ng/mL at 6 days after birth) in preterm infants with an onset of < 7 days after birth, the sensitivity, specificity, PPV, and NPV were 0.80, 0.83, 0.80, and 0.83, respectively (Table 3). Considering the substantial physiological changes and individual differences in preterm infants with an onset of < 7 days after birth, there may be limitations in setting and determining the cutoff value based on uniform PCT level.

With respect to the precision in detecting infection in preterm infants with an onset of ≥ 7 days after birth, the sensitivity, specificity, PPV, and NPV had been reported to be 0.97, 0.57, 0.76, and 0.92, respectively, assuming a cutoff value of 0.5 ng/mL in preterm infants with very low birth weight aged 7 days or more [21]. In our study, assuming a 97.5th percentile cutoff value (e.g., 1.65 ng/mL at 2 weeks after birth, 0.6 ng/mL at 9 weeks after birth) in preterm infants with an onset of ≥ 7 days after birth, the precision for the detection of infection improved, with the sensitivity, specificity, PPV, and NPV being 1.00, 0.97, 0.80, and 1.00, respectively (Table 3). The PCT reference percentile line based on the postnatal age may be more suitable for infection evaluation than the cutoff value based on the number.

The present study has some limitations. The number of infection cases was small. The incidence of sepsis in Japanese NICU has been reported to be extremely low (0.13% for early-onset sepsis and 0.61% for late-onset sepsis during 2006 and 2008) [3]. They were around one tenth in comparison with those in English NICU during the same period (0.9% or 6.1%, respectively) [26]. Thus, amassing many cases of bacterial infection or sepsis in a single-center observational study was difficult. As a limitation in the diagnosis of infection by serum PCT level, the detection of localized infection such as dermatitis proved to be difficult (Table 2). Furthermore, even with the use of the reference curve for PCT level, there was a limitation in the precision for detection in preterm infants with an onset of < 7 days after birth who had substantial physiological changes.

CONCLUSION

The age-specific percentile-based reference curve for serum PCT level is clinically applicable as a new tool for diagnosing bacterial infections in preterm infants, particularly at ≥ 7 days after birth. To establish the utility of this PCT reference curve in the diagnosis of bacterial infection, further multicenter studies enrolling a large number of preterm infants are required to confirm our results.

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Declaration of Interest:
All authors declare no conflicts of interest related to this work.

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