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Measurement of serum procalcitonin concentrations in calves with bovine respiratory disease

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Abstract

Bovine respiratory disease (BRD) is an important and complex disease caused by various pathogens and stressors. Although BRD is often caused by viruses, secondary bacterial infections can worsen symptoms and prognosis. Due to this viral-bacterial synergy, the evaluation of bacterial infections in BRD is important for the diagnosis and prognosis of BRD. In this study, we analyzed the serum concentrations of procalcitonin (PCT), a biomarker of bacterial infection, in 41 calves with BRD. We demonstrate that when calves are categorized into PCT-positive and PCT-negative groups based on serum PCT concentrations, the PCT-positive group shows more persistently high BRD scores. These findings suggest that the measurement of serum PCT is potentially useful for the diagnosis and prognosis of calves with BRD.

Key Words: bovine respiratory disease, procalcitonin, respiratory score

Bovine respiratory disease (BRD) is a complex disease caused by various pathogens, including viruses and bacteria. BRD is one of the most important diseases affecting beef and dairy calves because it causes retarded growth and affects industrial productivity, resulting in significant economic losses[19,25)]. Therefore, effective tools are required for early diagnosis and prognosis.

Various biomarkers have been evaluated for the diagnosis of human community-acquired pneumonia, including procalcitonin (PCT), C-reactive protein (CRP), tumor necrosis factor-related apoptosis ligand, interferon-γ-induced protein 10, erythrocyte sedimentation rate, white blood cell (WBC) count, and neutrophil percentage. Among these, PCT has shown

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Table 1. Clinical signs and blood chemistry results on day 1 in relation to PCT status

	PCT+ group	PCT− group	Normal value[a)]
Age (days)	218.5 ± 25.7 [b)]	172.0 ± 18.6	
Temperature (℃)	40.2 ± 0.3	40.4 ± 0.1	38-39
Heart rate (/min)	109.5 ± 6.2	119.3 ± 5.4	40-80
Respiration (/min)	72.0 ± 12.7	63.1 ± 3.6	12-36
BRD score	9.1 ± 1.4	9.4 ± 0.6	
WBC (/μl)	8870 ± 621	10028 ± 590	4000-12000
Neu (/μl)	2105 ± 427	2021 ± 319	600-4000
Band (/μl)	163 ± 55	121 ± 56	0-120
Lym (/μl)	6388 ± 406*	7839 ± 453	2500-7500
Mon (/μl)	250 ± 81	162 ± 39	25-840
Eos (/μl)	155 ± 63	81 ± 24	0-2400
RBC ($\times 10^4$/l)	803 ± 10	801 ± 24	500-1000
Ht (%)	32.8 ± 0.8	33.9 ± 1.0	29-39
Hb (g/dl)	10.7 ± 0.1	11.3 ± 0.7	8-15
AST (IU/l)	67.9 ± 4.6*	52.7 ± 2.2	43-127
TP (g/dl)	6.5 ± 0.3	6.4 ± 0.1	6.7-7.5
Alb (g/dl)	2.4 ± 0.1	2.8 ± 0.2	3-3.6
Tchol (mg/dl)	88.8 ± 9.4	100.0 ± 7.7	80-120
Glu (mg/dl)	77.3 ± 5.7	78.9 ± 3.9	45-75
BUN (mg/dl)	11.7 ± 1.0	10.8 ± 1.0	20-30
NEFA (μEq/l)	242.1 ± 49.7	224.0 ± 25.2	144.6 ± 4.3

a) Normal value cited from the references [5, 14, 15, 22]
b) Data represent mean ± SE (*: $P < 0.05$ vs PCT− group; Welch's t test)

Table 2. Clinical symptoms of the 41 calves diagnosed with BRD on initial examination

		n	(%)
Ausculation of abnormal lung sounds			
Discontinuous sounds	Coarse crackles	2	(4.9)
	Fine crackles	0	(0)
Continuous sounds	Wheezes	8	(19.5)
	Rhonchi	3	(7.3)
Pleural friction rub		1	(2.4)
Bronchial sounds in the lung fields		11	(26.8)
Clinical signs			
Adbominal respiration		7	(17.1)
Oral respiration		1	(2.4)
Cough		41	(100)
Purulent nasal discharge		2	(4.9)
Dyspnea		22	(53.7)
Polypnea [a)]		36	(87.8)
Low auricular reflex		19	(46.3)
Cyanosis		0	(0)

a) Nomal respiratory rates of adult and young (< 30 days of age) are 12-36 and 30-60, respectively.

excellent diagnostic and prognostic performance for severe bacterial infections[6,16,20,23)]. PCT is a precursor of the calcium-regulating hormone calcitonin (CT). CT is produced intracellularly by proteolytic splicing of PCT and is normally secreted by parafollicular cells of the thyroid gland in response to hypercalcemia. In contrast, PCT does not enter the bloodstream of healthy animals. However, in response to bacterial infection, PCT is induced directly by lipopolysaccharide and other bacterial toxins and indirectly by proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6, which are secreted in response to infection[18)]. Prominent increases in serum PCT concentrations were observed in systemic bacterial infections, but not in noninfectious inflammatory responses or viral infections[7,11)]. During viral infection, PCT expression is suppressed by interferon-γ, which plays a central role in antiviral immunity[12)]. Bacterial infection can worsen the severity of BRD even if the primary cause of the disease is a viral infection. This interaction is called viral-bacterial synergy[10)]. Therefore, the severity of BRD may be related to biomarkers specific to bacterial infections such as PCT.

In addition to humans, elevated serum PCT has been detected in mice[3,26)], hamsters[17)], pigs[24)], horses[2)], and calves[4,9)] in response to bacterial infection. Similarly, blood PCT concentrations increase in calves with sepsis[4,9)]. A previous study has shown that serum concentrations of PCT, along with other biological markers, are increased in BRD-suspected calves with any of the BRD symptoms when compared with healthy calves[8)]. However, whether PCT concentrations of BRD are associated with prognosis remains unclear. Therefore, in this study, we investigated the relationship between serum PCT positivity on the first medical day (day 1) and the subsequent severity of BRD using a scoring system that quantifies the severity of BRD.

This study included 41 calves (30 Japanese Black, 1 Holstein, and 10 first filial (F1) generations between Japanese Black and Holstein) fed commercial feedlots in Ehime prefecture, Japan, which were diagnosed with BRD. These calves were examined for California BRD scores,

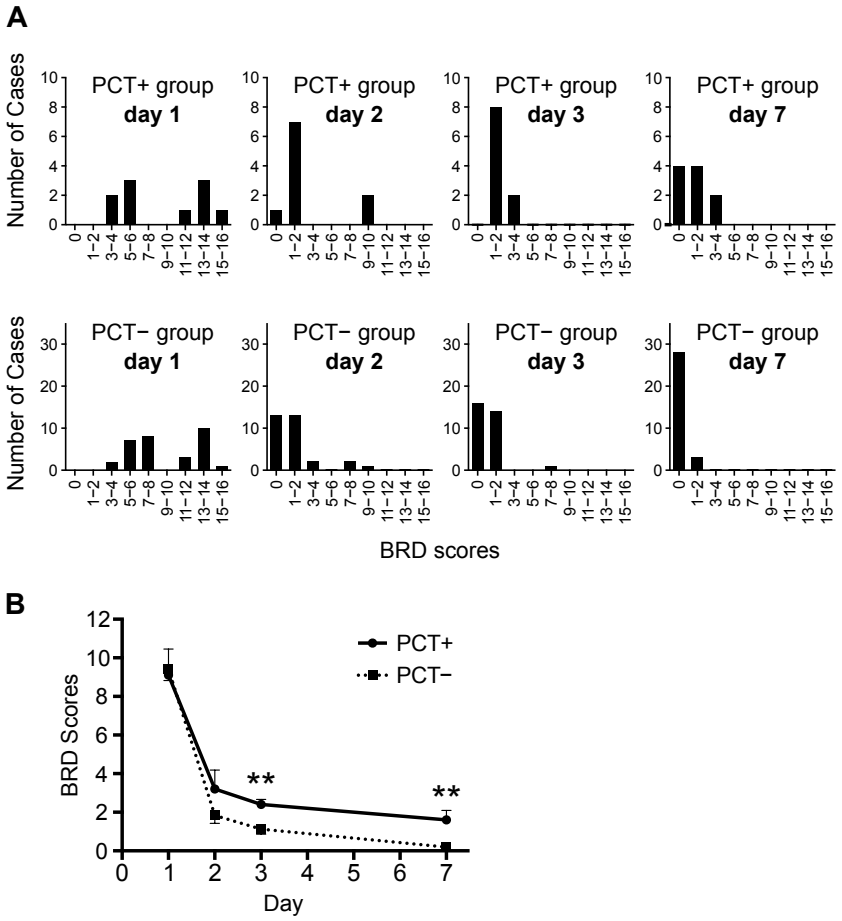

Fig. 1. BRD scores in relation to PCT status
(A) Distribution of BRD scores in the BRD calves of the PCT+ (upper panels, n = 10) and PCT− (lower panels, n = 31) groups. (B) Time course of BRD scores in the BRD calves of the PCT+ and PCT− groups. Data represent mean ± SE. The BRD scores of the PCT+ group are significantly higher than those of the PCT− group on days 3 and 7 (**: $P < 0.01$; Mann−Whitney's U test)

which were determined by the presence/absence of an eye discharge (score of 0 or 2), nasal discharge (score of 0 or 4), ear droop/head tilt (score of 0 or 5), cough (score of 0 or 2), forced breathing (score of 0 or 2), and rectal temperature (score of 0 or 2; ≥39.2 °C)[1]. BRD scores of ≥5 are proposed to be "BRD positive"[13]. Most (37 of 41) calves exhibited BRD scores of ≥5 on day 1. The BRD score was also evaluated on days 2, 3, and 7. The ages of the calves ranged from 38 to 399 days. None of the patients died during the study. More detailed clinical findings, including blood cell counts, serum biochemistry, and clinical symptoms, are summarized in Tables 1 and 2. BRD cases showed low serum albumin, increased band neutrophils, slightly increased lymphocytes, decreased blood urea nitrogen (BUN), and increased levels of non-esterified fatty acids (Table 1). Auscultation revealed coarse crackles in two cases (4.9%), wheezing in eight cases (19.5%), rhonchi in three cases (7.3%), pleural friction rub in one case (2.4%) and bronchial sounds in the lung fields in 11 cases (26.8%) (Table 2). In addition to BRD calves, five healthy F1 calves were used as controls.

Whole blood and serum samples were collected from the jugular vein of the calves on days 1, 2, 3, and 7 using collection tubes with and without an anticoagulant (heparin). Whole blood samples were immediately analyzed using a multi-parameter automated blood cell counter (KX-21NV; Sysmex, Kobe, Japan) and tested for differential WBC counts. Blood samples were centrifuged at 2500×g

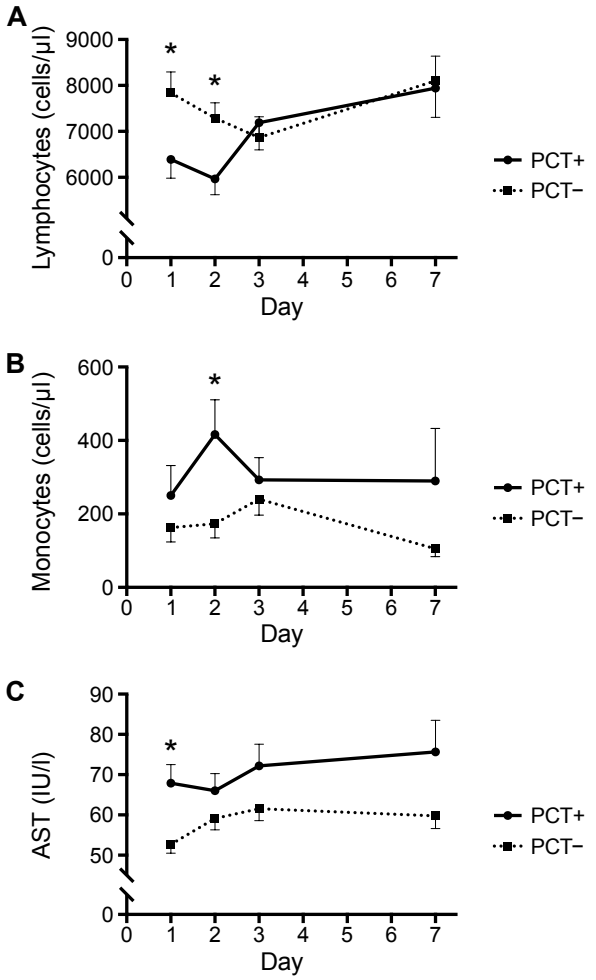

Fig. 2. Time course of lymphocyte counts, monocyte counts, and AST concentrations in relation to PCT status

(A) Time course of lymphocyte counts in BRD calves of the PCT+ and PCT− groups.

(B) Time course of monocyte counts in the BRD calves of the PCT+ and PCT− groups.

(C) Time course of AST concentrations in the BRD calves of the PCT+ and PCT− groups.

Data represent mean ± SE. The asterisks indicate significant differences between the groups (*: $P < 0.05$; Welch's t test).

for 20 min at 4°C and stored at -20°C until analysis of blood biochemistry using a chemistry analyzer (AU400; Beckman Coulter, Brea, CA, USA). Serum PCT concentration was measured using a bovine procalcitonin ELISA kit (Cusabio Biotech, Houston, TX, USA). The detection range of the ELISA kit is 40–2500 pg/mL. In all control calves, serum PCT concentrations were below the detection limit. In contrast, elevated serum PCT concentrations were detected in 10 of the 41 calves with BRD on day 1 or day 2. The calves were categorized into the PCT+ group. The PCT+ calves continuously showed high PCT concentrations during the test period (mean ± SE values of 411 ± 160, 358 ± 156, 569 ± 231, and 530 ± 254 pg/mL on days 1, 2, 3, and 7, respectively). The remaining 31 BRD calves, in which serum PCT concentrations were lower than the detection limit (40 pg/mL) on day 1, were categorized into the PCT− group.

The relationship between BRD scores (days 1, 2, 3, and 7) and PCT positive/negative status (day 1) is shown in Fig. 1. Although the distribution of the BRD scores was not significantly different on day 1 (Fig. 1A), the BRD scores of the PCT+ group were significantly higher than those of the PCT− group on days 3 and 7 (Fig. 1B; $P = 0.003$ and 0.048, respectively; Mann–Whitney's U test).

The clinical signs and serum biochemistry were then analyzed in relation to the positive/negative status of PCT on day 1. Table 1 lists the measurements on day 1. Among the measured parameters, the lymphocyte count and aspartate aminotransferase (AST) concentration showed significant differences on day 1. Lymphocyte counts in the PCT+ group were significantly lower than those of the PCT− group, and AST concentrations in the PCT+ group were significantly higher than those in the PCT− group (Table 1). The other parameters did not show statistical significance between the PCT+ and PCT− groups on day 1. The time course of lymphocyte counts showed that lymphocyte numbers were higher in the PCT− group on days 1 and 2 (Fig. 2A). In contrast, we observed that monocyte counts on day 2 were significantly higher in the PCT+ group than in the PCT− group (Fig. 2B). Monocytes are increased by acute stress and in the healing phase of acute and chronic infections[21], suggesting that elevated PCT concentrations might reflect these activities. AST concentrations were also higher in the PCT+ group than in the PCT− group on day 1 (Fig. 2C). Elevated AST concentrations may have resulted from more severe tissue damage in the PCT+ group. Although lymphocytes, monocytes, and AST showed significant differences between the

PCT+ and PCT− groups, these values were within the normal range in both groups, and thus, using them alone as biomarkers will be difficult. In contrast, serum PCT is not usually detected in uninfected healthy calves, making it particularly specific for infection by bacteria and useful as a biomarker.

Bacteriological tests for two major bacterial species that cause BRD, *Pasteurella multocida* and *Mannheimia haemolytica*[8], were performed on 11 cases (4 PCT+ and 7 PCT−). On Day 1, samples were taken from the right and left nasal cavities of affected calves using sterile swabs, applied to 5% sheep blood agar plates (Nissui Pharmaceutical, Tokyo, Japan), and incubated aerobically at 37°C for 24 h. *Pasteurella multocida* and *Mannheimia haemolytica* were attempted to be identified by microscopic observation after Gram staining and using ID Test HN-20 Rapid (Nissui Pharmaceutical) according to the analysis profile of the kit. *Pasteurella multocida* was isolated in all cases except for one PCT− case. In contrast, *Mannheimia haemolytica* was not detected in any case. Thus, at least for *Pasteurella multocida* and *Mannheimia haemolytica*, no clear association was noted between bacterial infection and PCT positive/negative status.

BRD is often diagnosed based on clinical signs (e.g., coughing, breathing difficulty, and fever), physical examination, auscultation, and laboratory tests. The BRD scoring system used in this study provides a simple and convenient method for quantifying multiple clinical signs. While it is useful, the distribution of the BRD scores was not apparently different between the PCT+ and PCT− groups (Fig. 1). However, our data indicated that PCT status on day 1 correlated with BRD scores on days 3 and 7 (Fig. 1B). These findings suggest that measurement of serum PCT concentrations can provide an additional layer of information for the diagnosis and prognosis of BRD; the PCT-positive status on day 1 is associated with subsequent severity of BRD. The specificity of PCT for bacterial infection is particularly important for the evaluation of the state of viral-bacterial synergy that exacerbates symptoms. In summary, this study showed the potential diagnostic and prognostic utility of PCT measurement in BRD. Further investigation of the relationship between changes in serum PCT concentration and the various bacterial species underlying the pathogenesis of BRD, as well as changes in PCT in animals with more chronic or severe BRD, will provide a more accurate and extensive understanding of the PCT response to BRD.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the content of this manuscript.

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