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Research Article

The Role of Serum Endocan as a Prognostic Biomarker in Calves with Enzootic Pneumonia

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Abstract

Respiratory system infections are one of the most critical problems that can cause serious economic losses and mortality in cattle breeding. Our study aimed to investigate the significance of endocan levels, which have been shown to yield successful outcomes in predicting prognosis in various respiratory disorders in human medicine, as a reliable prognostic biomarker in calves affected by enzootic pneumonia (EP). The study also examined the efficacy of lactate levels and a modified respiratory scoring system (MSS) in predicting death, alongside endocan levels. Calves with EP (n=53) and healthy calves (n=27) were included in the study and only single blood sample was collected from each calf in the study. Blood samples were taken from the EP group prior to treatment. Our investigation revealed that endocan levels were considerably lower in calves with EP compared to healthy calves (P<0.001). Our study assessed the diagnostic and prognostic significance of endocan and lactate levels in calves with EP using ROC analysis. The cut-off values for endocan and lactate employed in illness prediction were <70.41 (AUC=0.734, P=0.001) and >2.55 (AUC=0.842, P<0.001), respectively. Cut-off values for mortality prediction were >64.96 for endocan (AUC=0.657, P=0.052), >3.90 for lactate (AUC=0.940, P<0.001), and >7.5 for MSS (AUC=0.848, P<0.001). We concluded that endocan had limited diagnostic utility. However, the modified scoring system showed considerable efficacy in predicting mortality. Furthermore, lactate levels have been shown to exhibit superior accuracy and clinical value for both diagnosis and prognosis.

Keywords: Endocan, Enzootic pneumonia, Lactate, Modified respiratory scoring system, Prognostic biomarker

Introduction

Respiratory tract infections are the second most significant cause of calf mortality [1,2]. Bacterial, viral, and parasitic agents are primarily involved in respiratory diseases of calves [2]. In addition to these pathogens, predisposing factors such as poor nutrition and housing conditions, primary diseases, transportation stress, and adverse weather conditions play a critical role in the onset of disease [2,3]. Affected animals act as important sources of transmission and shed large quantities of viruses and bacteria through nasal discharge. Clinical signs of the disease include fever, nasal discharge, coughing, dyspnea, anorexia, depression, lethargy, and death.

The diversity of causative agents, high morbidity and mortality rates, antimicrobial resistance, and diagnostic limitations in field conditions delay the establishment of effective treatment protocols, resulting in significant calf losses. Furthermore, the disease imposes additional labor and economic burden on farms due to treatment time and medication costs. Therefore, prompt and accurate determination of treatment protocols and prognosis is essential to minimize these costs [2,4]. In this context, the identification and development of novel biomarkers for the diagnosis of respiratory diseases and their transformation into applicable preparations for use in veterinary practice are of great importance for the sustainability of the livestock industry.

Previously known as endothelial cell-specific molecule-1 (ESM-1), endocan is a 50 kDa proteoglycan secreted by various organs, including vascular endothelial cells, cardiomyocytes, lungs, skin, gastrointestinal tract, liver, brain, lymph nodes, and kidneys [5-8]. In humans, it is detectable in both urine and plasma and has been suggested as a useful biomarker for monitoring and predicting the course of various diseases due to its low concentration

and high stability under physiological conditions [7]. In human medicine, endocan has shown promising results in predicting the prognosis of inflammatory conditions such as acute respiratory distress syndrome (ARDS) and hospital-acquired pneumonia [5,6,9]. It has also been reported that endocan modulates several biological processes, including cell proliferation, neovascularization, and cellular adhesion, owing to its ability to interact with bioactive proteins [8]. The prognostic value of endocan has been demonstrated in inflammatory disorders, tumor progression, sepsis, hypertension, diabetes, cardiovascular diseases, and chronic kidney disease. A positive correlation has been reported between endocan levels and cardiovascular risk factors such as hypertension, diabetes mellitus, and chronic renal failure [8,10]. Lassalle et al.[11] found that the secretion of endocan into the bloodstream is upregulated by pro-inflammatory cytokines, particularly interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) [12-14].

This study evaluated the potential of endocan levels as prognostic biomarkers in calves with enzootic pneumonia (EP), based on its proven prognostic value in inflammatory diseases such as ARDS and hospital-acquired pneumonia in human medicine.

Material and Methods

Ethical Statement

This study was conducted with the permission of the Erciyes University Animal Experiments Local Ethics Committee (ERUHADYEK) (Approval date and no: 07.06.2023/128)

Animals

A total of 80 calves, including EP (n=53) and healthy (n=27) calves brought to Erciyes University, Faculty of Veterinary Medicine, Department of Internal Medicine, Ruminant Clinic for examination and general control, were included in our study.

Inclusion and Exclusion Criteria

Animals participating in the study were enrolled after obtaining informed, voluntary consent from their owners and meeting the predefined inclusion and exclusion criteria. The study involved calves aged 2-6 months. Clinically healthy calves with no abnormal findings and a total clinical score of 0 were included as the control group. Calves showing clinical signs consistent with respiratory disease and with a total clinical score ≥1 were included in the EP group.

Calves presenting with symptoms such as cough, nasal discharge, and pathological lung sounds were included in the study, whereas those with congenital anomalies, enteritis, omphalitis, arthritis, other disease symptoms, or a history of prior treatment were excluded.

Clinical Examination and Modified Scoring System

The clinical examinations of the patients presenting at the Veterinary Faculty Education, Research, and Application Hospital were conducted systematically. Vital signs were measured in all calf groups before sample collection. Findings obtained through clinical and laboratory evaluations were recorded and assessed. In addition to clinical examination, a modified clinical respiratory scoring system (MSS) was applied to sick calves for prognostic purposes by modifying the scoring systems used by Hägglung et al. [15] and Love et al. [16]. In this modified score system, clinical symptoms including rectal temperature (°C) [0 point (37.7 - 37.8), 1 point (<37.7 or >37.8)-2 point (<36 or >39.2)], respiratory rate (breath/min) [0 point (36 - 60), 1 point (<36 or >60), 2 point (<24 or >80)], pulse rate (bpm) [0 point (100-120), 1 point (<100 or >120), 2 point (<80 or >140)], nasal discharge [0 point (normal), 1 point (serous), 2 point (seromucous, mucopurulent and purulent)], cough [0 point (no cough), 1 point (rare sporadic cough), 2 point (cough at least once every 10 minutes while the calf is at rest)], lung auscultation sound [0 point (no abnormal sounds), 1 point (pathological sounds, crackles, whistles etc.)] and general condition [0 point (lively, active), 1 point (mildly-depressed), 2 point (moderate to severe depression)] were scored between a minimum of 0 and a maximum of 13.

Similar treatment protocols were applied to all sick calves to ensure uniformity in the study. The owners were contacted 15 days after the calves were discharged, and information about the prognosis of the calves was obtained.

Hemogram and Blood Gases Analysis

Only single blood sample was collected from each calf in the study. Blood samples were taken from the EP group prior to treatment. Blood samples of 4 mL in EDTA K3 tubes, 8 mL in gel tubes, and 1.5 mL in blood gas syringes were taken from the calves' vena jugularis once. Blood samples taken for ELISA analysis were centrifuged at 3000 rpm for 20 min to separate the serum.

Collected samples for hemogram and blood gas analysis were processed within the 15 min following blood-letting. Complete blood count [(Leukocyte (WBC), Lymphocyte (LYM), Monocyte (MON), Granulocyte (GRA), Erythrocyte (RBC), Hemoglobin (HGB), Hemotocrit (HCT) and Platelet (PLT)] was carried out in Exigo Eos device (Haematology analyzer, Boule Medical, Sweden) on K_3 EDTA venous blood samples collected from all calves. Blood gases analysis [pH, pCO_2 (mmHg), pO_2 (mmHg), Potassium, sodium, chloride, calcium, Lactate, bicarbonate, and anion gap (AGP)] was carried out in ABL 80 FLEX (Blood Gas/Electrolyte Analyzer) on the heparinized venous blood samples taken from all calves.

ELISA Analysis

In the collected serum samples, serum endocan levels were measured using the Bovine Endocan ELISA kit (SunRed Biotechnology Company, Cat No: 201-04-4681, Assay range: 5-1500 ng/L, Intra-Assay: CV<10%, Inter-Assay: CV<12%) and the proinflammatory cytokines IL-1β (SunRed Biotechnology Company, Cat No: 201-04-0157, Assay range: 1. 5-400 pg/mL), TNF-α (SunRed Biotechnology Company, Cat No: 201-04-0007, Assay range: 15-4000 ng/L, Intra-Assay: CV<9%, Inter-Assay: CV<11%) and IL-6 (SunRed Biotechnology Company, Cat No: SRB-T-83200, Assay range: 30-6000 ng/L, Intra-Assay: CV<10%, Inter-Assay: CV<12%) Bovine ELISA kits were analyzed in accordance with the ELISA protocol of the kits.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). The normality of the data was evaluated by histograms, Q-Q plots, and the Shapiro-Wilk test. Independent sample t-test (alternative: Mann-Whitney U Test) was used for intergroup comparisons. Pearson correlation analysis was performed on parameters obtained from all animals included in the study (n=80) to determine the direction and strength of the relationship between the variables. ROC (Receiver Operating Characteristic) analysis was performed to evaluate the diagnosis (EP vs healthy calves) and prognosis (died calves with EP vs survived calves with EP) between the groups. Data were expressed as mean±standard deviation and median (min-max). P<0.05 was considered statistically significant.

Results

Of the 80 calves included in the study, 85% (n=68) were Simmental, 6.25% (n=5) were Holstein, 6.25% (n=5) were Montafon, and 2.5% (n=2) were other breeds. 58.8% (n=47) of the calves were male and 41.2% (n=33) were female. 55% (n=52) of the calves were 2 months old and 35% (n=28) were 3-6 months old.

All healthy calves (n=27) were 2 months old and of Simmental breed. Of these calves, 51.9% (n=14) were male and 48.1% (n=13) were female.

Of the calves with EP (n=53), 77.4% (n=41) were Simmental, 9.4% (n=5) were Holstein, 9.4% (n=5) were Montafon, and 3.8% (n=2) were other breeds. Of these calves, 52.8% (n=28) were 3-6 months old and 47.2% (n=25) were 2 months old. Of the calves with EP, 62.3% (n=33) were male and 37.7% (n=20) were female. It was learned that 56.6% (n=30) of the calves with EP were alive and 43.4% (n=23) died.

Statistically significant differences were found in mean rectal temperature and respiratory rate between healthy calves and calves with EP (P<0.001, respectively). Mean rectal temperature (38.1±0.6°C) and respiratory rate (37.6±8.9) in healthy calves were lower than mean rectal temperature (38.9±1.1°C) and respiratory rate (68.6±15.6) in calves with EP. No statistically significant difference was found in mean pulse rate between healthy calves (114.3±9.9 bpm) and calves with EP (107.5±30.3 bpm) (P=0.141).

There was no statistically significant difference between the rectal temperature variables of deceased (39.5±1.5°C) and surviving (38.7±0.8°C) calves with EP (P=0.314). However, there was a statistically significant difference between the respiratory rate and pulse rate variables of deceased and surviving calves (P=0.001, P=0.038, respectively). The respiratory rate (81.5±25.5) and pulse rate (117.3±31.4) of deceased calves with EP were higher than the respiratory rate (58.6±21.2) and pulse rate (99.9±27.7) of surviving calves *(Table 1)*.

In the MSS performed on calves with EP, a statistically significant difference was identified found between the scores of calves that died and survived (P<0.001). The mean score of calves that died with EP (8.74±2.59) was higher than the score of calves that survived (5.13±2.59).

A comparison of the hemogram findings of healthy and EP calves revealed a statistically significant difference in the variables WBC (10^9/L), LYM (10^9/L), GRA (10^9/L), RBC (10^{12}/L), HGB (g/dL), HCT (%) and PLT (10^9/L) (P<0.001, P=0.004, P=0.022, P=0.002, P=0.008, P=0.042, P=0.013, respectively). A statistically significant difference was identified between the WBC (10^9/L), LYM (10^9/L), GRA (10^9/L), RBC (10^{12}/L), HGB (g/dL), HCT (%), and PLT (10^9/L) variables of deceased and surviving calves with EP (P=0.002, P=0.014, P=0.049, P=0.006, P=0.03, P=0.026, P=0.022, respectively) *(Table 2)*.

Table 1. *Vital signs values of calves that died and survived with enzootic pneumonia and healthy calves*

Variable	Healthy Calves (n=27)	Enzootic Pneumonia (n=53)	P
Rectal temperature (°C)	38.1±0.6	38.9±1.1	<0.001
Respiratory rate (min)	37.6±8.9	68.6±15.6	<0.001
Pulse rate (bpm)	114.3±9.9	107.5±30.3	0.141
Variable	**Died Calves (n=23)**	**Survived Calves (n=30)**	**P**
Rectal temperature (°C)	39.1±1.5	38.7±0.8	0.314
Respiratory rate (min)	81.5±25.5	58.6±21.2	0.001
Pulse rate (bpm)	117.3±31.4	99.9±27.7	0.038

Data were expressed as mean±standard deviation. The result is statistically significant at the P<0.05 level

Table 2. *Hematological and blood gases values of calves that died and survived with enzootic pneumonia and healthy calves*

Parameter	**Variable**	**Healthy Calves (n=27)**	**Enzootic Pneumonia (n=53)**	**P**
Hematological Changes	WBC (10^9/L)	7.60 (4.60-13.00)	10.50 (3.40-61.70)	<0.001
	LYM (10^9/L)	2.50 (0.30-5.50)	3.50 (0.60-13.00)	0.004
	MON (10^9/L)	1.00 (0.40-1.70)	0.90 (0.30-10.10)	0.543
	GRA (10^9/L)	4.40 (1.10-8.60)	5.90 (1.40-38.60)	0.022
	RBC (10^{12}/L)	6.22±1.01	7.43±2.14	0.002
	HGB (g/dL)	8.59±1.59	9.94±2.40	0.008
	HCT (%)	24.35±4.11	26.93±7.21	0.042
	PLT (10^9/L)	499.00 (50.00-1308.00)	321.00 (50.00-1527.00)	0.013
	Variable	**Died Calves (n=23)**	**Survived Calves (n=30)**	**P**
	WBC (10^9/L)	12.00 (3.40-23.80)	10.50 (6.20-61.70)	0.002
	LYM (10^9/L)	3.40 (0.90-5.90)	3.50 (0.60-13.00)	0.014
	MON (10^9/L)	0.80 (0.30-1.90)	1.00 (0.30-10.10)	0.555
	GRA (10^9/L)	5.45 (1.40-17.90)	7.40 (1.90-38.60)	0.049
	RBC (10^{12}/L)	7.71±2.22	7.22±2.09	0.006
	HGB (g/dL)	10.16±2.39	9.78±2.44	0.03
	HCT (%)	28.86±7.61	25.45±6.63	0.026
	PLT (10^9/L)	459.00 (50.00-1527.00)	275.00 (50.00-1217.00)	0.022
Blood Gases Changes	**Variable**	**Healthy Calves (n=27)**	**Enzootic Pneumonia (n=53)**	**P**
	pH	7.38±0.05	7.37±0.11	0.551
	pCO_2 (mmHg)	39.49±2.09	43.17±8.79	0.005
	pO_2 (mmHg)	28.93±1.92	32.60±6.03	<0.001
	Na (mmol/L)	141.15±1.75	135.70±4.15	<0.001
	K (mmol/L)	4.54±0.34	4.23±0.74	0.01
	Ca (mmol/L)	1.31±0.02	1.19±0.09	<0.001
	Cl (mmol/L)	95.74±2.16	95.14±4.73	0.443
	Lactate (mmol/L)	2.41±0.49	3.98±1.64	<0.001
	HCO_3 (mmol/L)	32.84±1.77	27.31±4.98	<0.001
	BE (mmol/L)	2.40 (-1.30-9.00)	2.90 (-20.30-10.70)	<0.001
	AGP (mmol/L)	16.66±2.25	20.16±5.29	<0.001
	Variable	**Died Calves (n=23)**	**Survived Calves (n=30)**	**P**
	pH	7.34±0.15	7.39±0.06	0.082
	pCO_2 (mmHg)	43.01±8.74	43.29±8.96	0.910
	pO_2 (mmHg)	30.65±6.35	34.10±5.40	0.038
	Na (mmol/L)	136.00±4.57	135.47±3.87	0.648
	K (mmol/L)	4.19±0.94	4.26±0.56	0.735
	Ca (mmol/L)	1.21±0.08	1.18±0.09	0.340
	Cl (mmol/L)	96.38±4.38	94.27±4.86	0.118
	Lactate (mmol/L)	5.34±1.31	2.93±0.96	<0.001
	HCO_3 (mmol/L)	26.21±5.33	28.15±4.61	0.162
	BE (mmol/L)	3.10 (-20.30-7.30)	2.55 (-7.00-10.70)	<0.001
	AGP (mmol/L)	21.01±6.70	19.57±4.06	0.346

Data were expressed as mean±standard deviation. Data that did not provide normality distribution were expressed as median (min-max). EP: Enzootic pneumonia, WBC: Total leukocytes, LYM: Lymphocyte, MON: Monocyte, GRA: Granulocyte, RBC: Erythrocyte, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelets, pCO_2: Partial pressure of carbondioxide, pO_2: Partial pressure of oxygen, BE: Base excess, AGP: Anion gap

Significant differences were found in blood gas and electrolyte parameters [pCO_2, pO_2, Na^+, K^+, Ca^{2+}, lactate, HCO_3^-, base excess (BE), and anion gap (AGP)] between healthy and EP calves (all P<0.001, except for K^+, P=0.01 and pCO_2, P=0.005). When blood gas findings of deceased and surviving calves with EP were compared, a statistically significant difference was found for pO_2, lactate, and BE variables (P=0.038, P<0.001, P<0.001, P<0.001, respectively) *(Table 2)*.

Serum endocan levels were found to be significantly lower in calves with EP compared to healthy calves (P<0.001). Serum TNF-α levels were significantly higher in calves with EP than in healthy calves (P=0.022). Serum IL-1β levels were significantly higher in healthy calves compared to those with EP (P=0.004). No statistically significant difference was observed in serum IL-6 levels between calves with EP and healthy calves (P>0.05) *(Table 3)*. A

Table 3. *Endocan and other cytokine levels in calves with enzootic pneumonia and healthy calves*

Parameter	**Variable**	**Healthy Calves (n=27)**	**Enzootic Pneumonia (n=53)**	**P**
Comparison of diseased and healthy calves	Endocan (ng/L)	80.88±19.02	63.58±18.61	<0.001
	TNF-α (ng/L)	234.49±69.91	281.72±109.92	0.022
	IL-1β (pg/mL)	37.74±12.36	29.45±11.69	0.004
	IL-6 (ng/L)	313.03±90.17	335.38±110.42	0.367
Parameter	**Variable**	**Died Calves (n=23)**	**Survived Calves (n=30)**	**P**
Comparison of died and survived calves	Endocan (ng/L)	69.60±18.99	58.97±17.23	0.038
	TNF-α (ng/L)	375.82±83.55	209.58±62.95	<0.001
	IL-1β (pg/mL)	34.30±12.65	25.73±9.52	0.007
	IL-6 (ng/L)	414.08±101.94	275.05±72.82	<0.001

Data were expressed as mean±standard deviation. The result is statistically significant at the P<0.05 level, TNF-α: Tumor necrosis factor alfa, IL-1β: Interleukin 1 beta, IL-6: Interleukin 6

Table 4. *Predictive/prognostic value of serum endocan and lactate for enzootic pneumonia*

Predictive	**n**	**Parameters**	**Cut-off**	**AUC (95% CI)**	**Se (%)**	**Sp (%)**	**P**
EP	53	Endocan (ng/L)	<70.41	0.734 (0.621-0.847)	67	64	0.001
		Lactate (mmol/L)	>2.55	0.842 (0.754-0.931)	83	82	<0.001
Mortality	23	Endocan (ng/L)	>64.96	0.657 (0.507-0.807)	61	60	0.052
		Lactate (mmol/L)	>3.90	0.940 (0.879-0.990)	87	83	<0.001
		MSS (point)	>7.5	0.848 (0.735-0.961)	78	80	<0.001

AUC: Area under the curve, CI: Confidence interval, EP: Enzootic pneumonia, MSS: Modified respiratory scoring system, Se: Sensitivity, Sp: Specificity

Table 5. *Pearson correlation values between the modified scoring system, serum endocan, lactate, and proinflammatory cytokines in all calves (n= 80) included in the study*

Parameters		**MSS (point)**	**Endocan (ng/L)**	**Lactate (mmol/L)**	**TNF-α (ng/L)**	**IL-1β (pg/ml)**	**IL-6 (ng/L)**
MSS (point)	Pearson correlation	1	-.278*	.554**	.461**	-.228*	.262
	P		0.12	.000	.000	.042	.019
Endocan (ng/L)	Pearson correlation	-.278*	1	-.029	.106	.369**	.242*
	P	0.12		.799	.350	.001	.031
Lactate (mmol/L)	Pearson correlation	.554**	-.029	1	.546**	-.023	.461**
	P	.000	.799		.000	.843	.000
TNF-α (ng/L)	Pearson correlation	.461**	.106	.546**	1	.181	.446**
	P	.000	.350	.000		.108	.000
IL-1β (pg/mL)	Pearson correlation	-.228*	.369**	-.023	.181	1	.393**
	P	.042	.001	.843	.108		.001
IL-6 (ng/L)	Pearson correlation	.262	.242*	.461**	.446**	.393**	1
	P	.019	.031	.000	.000	.001	

** Correlation significant P<0.05, ** Correlation significant P<0.01. MSS: Modified respiratory scoring system, TNF-α: Tumor necrosis factor alfa, IL-1β: Interleukin 1 beta, IL-6: Interleukin 6*

statistically significant difference was also determined between deceased and surviving calves with EP in terms of serum endocan, TNF-α, IL-1β, and IL-6 levels. Deceased calves with EP exhibited higher serum endocan, TNF-α, IL-1β, and IL-6 levels compared to surviving calves with EP (P=0.038, P<0.001, P=0.007, P<0.001, respectively) *(Table 3)*.

In our study, in the ROC analyses performed to determine the predictive and prognostic value of serum endocan and lactate levels in calves with EP, the cut-off value of endocan for predicting EP was determined as 70.41 and lactate as 2.55. In addition, the cut-off of endocan levels for mortality prediction was determined as 64.96 and lactate as 3.90. The cut-off value of MSS for mortality prediction was 7.5 *(Table 4)*.

Pearson correlation analysis revealed a moderate, positive, and statistically significant correlation between the MSS and lactate levels (r=0.554, P<0.001). Additionally, a moderate, positive, and significant correlation was observed between MSS and TNF-α levels (r=0.461, P<0.001). Serum endocan levels showed a weak but statistically significant positive correlation with IL-1β and IL-6 (r=0.369, P=0.001; r=0.242, P=0.031, respectively). Moderate, positive, and significant correlations were also observed between lactate and TNF-α (r=0.546, P<0.001), lactate and IL-6 (r=0.461, P<0.001), as well as between TNF-α and IL-6 (r=0.446, P<0.001) *(Table 5)*.

Discussion

Respiratory tract infections are one of the most critical problems in cattle breeding that can cause serious economic losses and mortality. In addition to bacterial and viral factors, hygiene conditions, environmental factors, age, and stress factors are also reported to play a role in the occurrence of EP, which has no specific etiology. For the diagnosis of EP in calves aged 2-6 months, cough of variable severity, increased body temperature, shortness of breath, various pathologic lung sounds, and nasal discharge are usually sufficient [17]. In this study, endocan levels, which are reported to give successful results in predicting the possible prognosis in human medicine, were compared in sick calves diagnosed with EP as a result of clinical and laboratory findings and healthy calves.

Clinical scoring systems evaluate data that can be rapidly collected from patients to assess patient health and prognosis and have been used in various human and veterinary medicine applications [15,16]. For the scoring system to be applicable in field conditions: I. the patient's total score should correspond to the risk or probability of disease, II. similar scores should represent similar risks, III. objective methods should be used when scoring clinical data to optimize score performance, IV. clinical signs that are difficult to measure with sufficient precision or require expensive or time-consuming methods to measure should not be included in the scoring [16]. These data were taken into consideration in the creation of the scoring system in our study. Considering the ROC analysis (cut-off = 7.5 point, AUC = 0.848, sensitivity = 78%, specificity = 80%, P<0.001), it was concluded that it is important for veterinarians to evaluate cough (cough at least once every 10 min while the calf is at rest, 2 Point) and general condition (modarate to severe depression, 2 Point) scores in addition to low and high respiratory (<24 or >80, 2 Point) and pulse rate (<80 or >140, 2 Point) scores in determining mortality in EP.

Similar to our study, there is no information in the literature investigating endocan levels in respiratory system infections in calves. However, there are academic studies investigating endocan levels in patients with pneumonia in human medicine. Clinical studies conducted in trauma and septic patients support the hypothesis that endocan has an anti-inflammatory role, as higher endocan concentrations in the blood during admission to intensive care units appear to reduce the risk of developing ARDS. Therefore, it has been suggested that endocan secretion deficiency may be associated with a higher risk of respiratory failure and ARDS [6,18].

Gaudet et al. [19] reported that the possibility of developing ARDS in septic patients would be associated with low blood levels of endocan. Similarly, in our study, serum endocan levels were lower in calves with EP compared to healthy calves. Kechagia et al. [5] interpreted endocan as a predictive biomarker for the development of severe sepsis-induced ARDS. There is also a study indicating that endocan levels, which were significantly increased in patients with severe sepsis and septic shock compared to the control group, were lower in patients who developed acute lung injury and ARDS at 48 and 72 h compared to patients who did not [5]. The researchers explained this situation at the molecular level by decreased endocan release from pulmonary endothelial cells or increased proteolysis by neutrophil serine proteases. Gunaydin et al. [20] found lower serum endocan levels in community-acquired pneumonia patients compared to the control group (P<0.005).

Béchard et al. [12] found that endocan, a proteoglycan, binds to LFA-1 on the cell surface of leukocytes, and this complex reduces leukocyte adhesion via ICAM-1. It has been reported that the reason for lower endocan levels in relatively more severe patients may be neutrophil-derived cathepsin G, which has been shown to increase with neutrophil activation, and that this protein degrades Endocan into a 14 kDa peptide fragment [21]. The hematology device used in the study does not differentiate neutrophil, basophil, and eosinophil granulocytes. Since

neutrophil granulocytes are the most abundant granulocyte type and the most important cellular component of acute inflammation, an increase in granulocyte count was considered as an increase in neutrophil count in this study. In our study, it was determined that calves with EP had higher average GRA (10^9/L) levels than healthy calves, while dead calves had lower GRA (10^9/L) levels than surviving calves. The inverse relationship observed between endocan and GRA in our study was thought to be caused by neutrophil-derived cathepsin G protein, which was reported to increase as a result of neutrophil activation by De Freitas Caires et al. [21] and Kechagia et al. [5].

The apparently contrasting findings of lower endocan levels in calves with EP and higher levels in non-survivors may be explained by differences in the dominant pathophysiological mechanisms and disease severity. Previous studies have shown that endocan levels may decrease in conditions characterized by severe pulmonary involvement, such as ARDS and pneumonia, possibly due to reduced release from injured pulmonary endothelial cells or increased proteolytic degradation by neutrophil-derived serine proteases, particularly cathepsin G [5,19-21]. In contrast, fatal cases of sepsis are characterized by widespread systemic endothelial activation and dysfunction, leading to increased circulating endocan levels. Therefore, lower endocan levels in EP calves may reflect predominant lung-localized endothelial injury and neutrophil-mediated proteolysis, whereas higher endocan levels in non-survivors may indicate severe systemic endothelial activation associated with poor prognosis.

Lactate is produced under hypoxic conditions and poor tissue perfusion and is used as an indirect indicator of tissue hypoxia [22]. There are very few studies evaluating lactate levels in cattle with respiratory system infections [23-25]. In our study, it was determined that EP calves had higher lactate levels than healthy calves and surviving calves from the deceased calves (Cut-off value for mortality <3.90 mmol/L). Consistent with our findings, Ider et al. [25] reported that premature calves with respiratory distress syndrome (RDS) had higher lactate levels than calves in the control group. Additionally, Erdoğan et al. [26] reported in their study that calves with pneumonia and L-lactate levels above 4 mmol/L did not survive. Coghe et al. [23] and Šoltésová et al. [24] reported that plasma lactate levels increase with the severity of the disease process. This is thought to be due to an imbalance between anaerobic and aerobic metabolism and, possibly, an increasing imbalance between lactate production and lactate clearance.

TNF-α and IL-1β are powerful triggers that increase endocan synthesis in endothelial cells [11,14]. Clinically, high levels of TNF-α, IL-1β, and IL-6 are associated with severe inflammation, tissue damage, and mortality in sepsis. In neonatal sepsis studies, these cytokines are used for early diagnosis, follow-up, and prognosis determination [27]. In our study, TNF-α, IL-1β, and IL-6 levels were found to be higher in calves that died compared to those that survived. This difference may reflect variations in the stage and severity of the inflammatory response between calves with EP and those with fatal outcomes. Additionally, our study identified weak but positive and significant correlations between serum endocan levels and IL-1β and IL-6 levels. However, endocan exhibits a dual role in inflammatory conditions. While endocan expression can be increased by pro-inflammatory cytokines [11,12], circulating endocan levels have been reported to be decreased in conditions characterized by severe pulmonary endothelial damage such as pneumonia, acute lung injury, and ARDS [5,19-21]; this is likely due to impaired endothelial release or increased proteolytic degradation. Furthermore, in the present study, serum TNF-α and IL-6 levels were significantly lower in healthy calves compared with those in the EP group, whereas IL-1β levels were significantly higher in the healthy group than in calves with EP. IL-1β has been reported to play a physiological role in innate immune responses against infections, while its excessive release during pathological inflammatory processes is associated with tissue damage [28]. In addition, it has been reported that during the later stages of systemic inflammatory conditions such as sepsis, suppression of certain proinflammatory cytokines may occur [29]. Because cytokine dysregulation, activation of negative feedback mechanisms, and the predominance of anti-inflammatory responses in septic conditions may lead to attenuation of the proinflammatory cytokine response, these mechanisms may have contributed to the relative decrease in IL-1β levels observed in the present study [30]. This finding was considered to account for the lower IL-1β levels observed in calves in the EP group compared with control group.

In a study conducted by Gaudet et al. [19], it was reported that serum endocan levels showed high diagnostic accuracy in predicting ARDS, with an AUC value of 0.93 (95% CI= 0.87-1; P<0.001). In our study, based on the ROC analysis, considering the threshold value determined for serum endocan levels, it is suggested that endocan may only provide limited clinical benefit as a biomarker in the diagnosis of EP. However, when blood lactate levels were evaluated, it was found that blood lactate levels are a biomarker with superior diagnostic accuracy and clinical utility compared to endocan in predicting PE.

Tang et al. [9] reported that ARDS patients who survived had lower endocan levels compared to those who did not survive. These data are consistent with our study findings. In our study, serum endocan levels in calves with EP that survived were found to be statistically significantly lower than those in calves that died. Additionally, Behnoush et al.

[31] confirmed the high plasma endocan levels in deceased patients, which is consistent with our study findings.

Tang et al. [9] determined the AUC value to be 0.715 (95% CI = 0.555-0.875, P<0.017) when a cut-off of 4.96 ng/ml was used for plasma Endocan in predicting mortality. In the current study, however, ROC analysis revealed that serum endocan levels did not demonstrate similar success in predicting mortality. This finding indicates that endocan has limited clinical utility in the diagnosis of EP and also lacks sufficient diagnostic performance in predicting mortality. On the other hand, the prognostic value of lactate levels (>4 mmol/L) reported by Coghe et al. [23] was also supported in this study, and it was determined that mortality could be predicted with high accuracy using lactate levels in the ROC analysis. Furthermore, the ROC analysis revealed that MSS has significant discriminatory power in predicting mortality. All these findings indicate that serum endocan levels have limited clinical utility in mortality prediction, whereas blood lactate levels, consistent with the literature, continue to be a strong and reliable prognostic biomarker.

Our study has methodological limitations. The most important of these is that repeated measurements could not be performed on the calves included in the study. This limited the ability to track long-term changes in serum endocan and related proinflammatory cytokines. Another limitation of this study is that the etiological agents of EP could not be identified and bronchoalveolar lavage (BAL) sampling was not performed, limiting pathogen-specific assessment of inflammatory responses and disease severity. In addition, necropsy examinations were not conducted in deceased calves, restricting pathological–clinical correlation. Also, differences in age, sex, and breed among groups may also have influenced biomarker levels.

High endocan levels are known to have prognostic value in various inflammatory diseases. In our study, consistent with the literature, serum endocan levels were found to be lower in calves with EP compared to the control group. However, although serum endocan levels may be partially useful as a biomarker in calves with EP, it was found that they are not a successful biomarker for predicting mortality.

In conclusion, the modified scoring system was found to have significant discriminatory power in predicting mortality, while lactate levels demonstrated high diagnostic accuracy in both EP and mortality prediction. Furthermore, although endocan has been shown to have prognostic potential, this finding should be confirmed in further standardized studies involving homogeneous patient populations.

Declarations

Availability of Data and Materials: The data used in this article will be provided by the corresponding author (ET) upon request.

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