

Serum Neopterin, Procalcitonin, Endothelin-1 and Hematological Parameters in Cows with Traumatic Reticuloperitonitis and Traumatic Pericarditis

Key words

endothelin-1;
cow;
neopterin;
traumatic pericarditis;
procalcitonin;
traumatic
reticuloperitonitis

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Abstract: Traumatic reticuloperitonitis (TRP) and traumatic pericarditis (TP) are disorders that occur because of cows swallowing metal foreign bodies due to malnutrition or different etiological reasons. Foreign bodies cause serious complications by piercing the peritoneum or sinking into the pericardium. This study analyzed the levels of neopterin, procalcitonin, and endothelin-1 and various hematological parameters in blood samples of cows with TRP and TP. This study included 2–7-year-old Simmental cows with TRP (n=35) or TP (n=35) and healthy controls (n=20). Glutaraldehyde tests were used to assess inflammation levels, and radiography confirmed the diagnoses. Total leukocyte count was found to be significantly higher in the TP and TRP groups compared to the control. In addition, while lymphocyte, monocyte and granulocyte counts were found to be higher in the TP group compared to other groups, erythrocyte and hematocrit levels were significantly lower. TP cows had higher neopterin levels than those of TRP and control cows. TRP cows had higher procalcitonin and endothelin-1 levels than those of TP and control cows. These increased levels were diagnostically important. Procalcitonin had the highest sensitivity and specificity for distinguishing between TRP and TP cases. As a result, neopterin, procalcitonin and endothelin-1 levels in cows with TRP and TP were found to be important in confirming the diagnosis and determining the severity of the disease.

Received: 10 March 2023

Accepted: 28 February 2024

Introduction

The consumption of foreign objects that cannot be digested by cows and buffaloes is an extensive problem globally and is referred to as foreign body syndrome (1, 2). A disease that is prevalent among cows is traumatic reticuloperitonitis (TRP), which occurs when sharp, pointed, and piercing foreign objects

enter the reticulum with feeds. These foreign bodies cause damage and inflammation to various organs after piercing and traversing the reticulum (3, 4). Because cows have backward-facing tongue papillae, a wide esophagus, and a reticulum mucosa resembling a honeycomb, foreign objects can easily be swallowed and become lodged in these honeycomb-like structures. Ruminants do not have a strong sense of taste and

swallow their food without chewing, making it easy for them to consume foreign objects such as wires and nails. Situations that contribute to intra-abdominal pressure, e.g., pregnancy and bloating, can cause objects to sink into tissues such as the reticulum or diaphragm. These objects can cause peritonitis by puncturing the wall of the reticulum or damage organs by sinking into the liver, kidney, spleen, lungs, or heart (5). Symptoms include fever, loss of appetite, bloatedness, repeated stomach swelling, discomfort in the abdomen, moaning due to pain, overall weakness, rapid breathing and heart rate, a noticeable pulse in the jugular vein, abnormal heart sounds, swelling in the lower front part of the body due to poor circulation, a dull sound when tapping the heart, and an irregular heartbeat (6).

Traumatic pericarditis (TP) is caused by an object passing through the diaphragm and puncturing the heart, which can cause inflammation in tissue around the heart. Initially, inflammation occurs in the sac around the heart and is referred to as pericarditis sicca. Afterwards, fluid accumulates in the sac, which can become infected with bacteria, and thus pus and gas accumulate in the sac, causing a condition known as exudative pericarditis (7). Treating TP in animals is challenging (6). Clinical signs, ferrosopic examination, radiographic examination, pericardiocentesis, and ultrasonography are used to diagnose the disease (6, 8). Furthermore, diagnosis and treatment depend on biomarkers, i.e., natural substances that indicate organ damage (9, 10, 11, 12).

Neopterin is synthesized by monocytes and macrophages through the stimulative effects of endotoxin and interferon- γ . This process can result in elevated neopterin levels in the blood due to macrophage or T-cell activation. Such increases have especially been observed in cases of viral infections, inflammatory conditions, autoimmune diseases, neurodegenerative disorders, and some types of cancer. As a diagnostic tool, measuring neopterin levels can be useful for evaluating the prognosis of various pathological conditions and assessing disease activity (9, 10, 11, 12). Additionally, procalcitonin is an important protein for diagnosing inflammatory conditions. Bacterial endotoxin and inflammatory cytokines lead to procalcitonin production during inflammation. Procalcitonin is usually secreted by the thyroid gland but is also synthesized by neuroendocrine cells in the lungs and intestines, adipocytes, and liver, kidney, and muscle cells during inflammation (12, 13). Procalcitonin is released within 2–4 h in response to bacterial toxins and has a biological half-life of 22–26 h (12,14). Furthermore, endothelin-1 is a strong blood vessel constrictor released by endothelial cells. During endotoxemia, prepro-endothelin-1 is released from the heart and lungs and is correlated with the mortality rate (15,16). The aim of this study was to determine and compare serum neopterin, procalcitonin, endothelin-1 levels and various blood parameters in cows with TP and TRP.

Materials and methods

Ethical Statement

This study was approved by the Local Ethics Board for Animal Experiments of Kafkas University (KAU-HADYEK, Research Code: KAU-HADYEK/2020-110, Kars, Turkey).

Animals

This study included 70 Simmental cows, aged 2–7 years, of which 35 had TRP and 35 had TP, and 20 age-matched healthy cows. Cows with clinical symptoms of anorexia, pain, depression, and a hunched posture were admitted to the Veterinary Faculty Hospital of Kafkas University and diagnosed with TRP or TP based on clinical and radiographic examination. These cows grazed for 4 months in a closed system and for 8 months on pastures in Kars province and surrounding villages. Livestock farming in this area tends to consist of small family farms that primarily feed their animals with grass found on pastures. The control group was selected to match the TRP and TP groups, of which 60% of the cows were pregnant for at least 6 months. Rectal temperatures (measured with a digital thermometer), pulse rates, and respiratory rates per minute were recorded for all cows.

Radiographic Imaging

The radiological assessment was conducted at the Department of Radiology, Kafkas University School of Veterinary Medicine. Definite diagnosis of suspected TRP and TP patients was made using radiographic images. Ferroscopy and x-ray exams of the control cows yielded negative findings. A dynamic ceiling-mounted x-ray device (Rotanode, Toshiba Electron Tubes and Devices, Japan) and imaging unit (Fujifilm FCR Prima T2 Veterinary Set, Medical Technology, Turkey) were used for the radiological examination that assessed the reticulum and diaphragm border. The irradiation doses were tailored to fit each cow's size, with ranges of 30–40 mA and 80–90 kW. Radiographic images were captured through irradiation at a distance of 75 cm between the tube and cassette (35×43). The reticulum, diaphragm edge, and thoracic cavity were then thoroughly examined to identify any foreign bodies.

Blood Samples

Blood samples (10 mL) were collected from all cows via the jugular vein and placed into serum tubes containing gel (BD Vacutainer®, BD, USA) and tubes containing K2EDTA (BD Vacutainer®, BD, USA). Complete blood analyses (VG-MS4e®, Melet Schloesing, France) were conducted within 15 min of sample collection. Blood samples were collected for serum, kept at room temperature for approximately 1 h, and centrifuged at 3000 rpm for 10 min (Hettich Rotina 380R®, Hettich, Germany). Serum samples for biochemical analysis were stored at –20 °C until the time of measurement.

Hematological and Biochemical Analyses

Total leukocyte ($\times 10^3/\mu\text{L}$), lymphocyte ($\times 10^3/\mu\text{L}$), monocyte ($\times 10^3/\mu\text{L}$), granulocyte ($\times 10^3/\mu\text{L}$), and red blood cell counts ($\times 10^6/\mu\text{L}$); mean red cell volume (fL); hematocrit (%); and hemoglobin (g/dL) were measured using a complete blood count device (VG-MS4e[®], Melet Schloesing, France). Serum levels of alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase, glucose (mg/dL), cholesterol (mg/dL), creatinine (mg/dL), urea (mg/dL), total bilirubin (mg/dL), lactate dehydrogenase, total protein, and creatine kinase were quantified using a fully automatic biochemistry analyzer (Mindray BS120[®], Mindray Medical Technology, Turkey). Albumin was measured using a commercial test kit (Biolabo, France) on the microplate spectrophotometer (Biotek, Epoch, USA) by the colorimetric method. Bovine endothelin-1, neopterin, and procalcitonin were measured using ELISA kits (Bovine Endothelin-1 ELISA Kit, Cat No: E0122Bo; Bovine Neopterin ELISA Kit, Cat No: E0121Bo; Bovine Procalcitonin ELISA Kit, Cat No: E0085Bo; BT Lab, China) according to the manufacturer's instructions. The optical density was then measured at a wavelength of 450 nm using a microplate reader (Epoch[®], Biotek, USA).

Glutaraldehyde Test

The test was performed by mixing 2 mL of blood with 2 mL of 1.4% glutaraldehyde stock solution. The resulting mixture was then placed in a 10 mL glass tube and inverted every 30 s. A positive result was recorded if coagulation occurred within 15 min. Clotting times were classified as severe (0–5 min), moderate (5–10 min), and mild inflammation (10–15 min). Coagulation times were considered normal if > 15 min (17).

Statistical Analysis

The statistical program SPSS 25 (IBM Corp[®], 2017, Armonk, NY, USA) was used to evaluate the data. The Shapiro–Wilk test was used to determine normality of the variables and homogeneity of variance. Data were presented as mean $\pm$ standard error of the mean (SEM). The one-way analysis of variance test was used for multiple comparisons, and the Tukey honestly significant difference test was used for post-hoc comparisons. Pearson correlation coefficients were calculated to determine the relationship between certain parameters and variables. Receiver operating characteristic (ROC) analysis was performed to determine the prognostic cut-off, sensitivity, and specificity of variables to discriminate between TP and TRP. The differences between the groups were considered significant if $p < 0.05$.

Results

Clinical, hematological, and biochemical findings

In TRP cows, objects traversed the reticulum toward the heart or abdominal cavity (Figure 1A). Although the diaphragm border appeared normal in acute cases, severe adhesions were evident. In cases for which the glutaraldehyde test revealed severe inflammation, the adhesions were observed to be very severe, depending on the degree of coagulation. Furthermore, in a TP cow, the puncturing body completely left the reticulum and punctured the heart (Figure 1B).

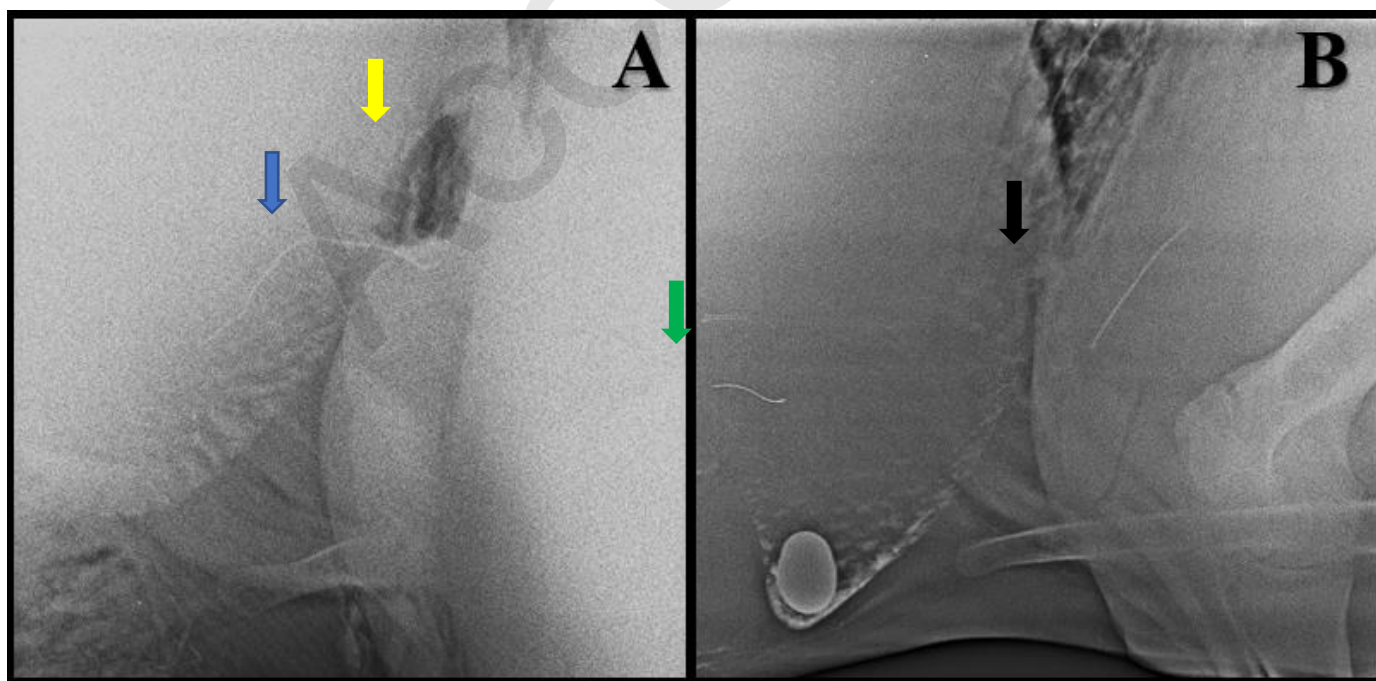


Figure 1: Radiographic images of cows with traumatic reticuloperitonitis and traumatic pericarditis. **A.** A foreign body (blue arrow) penetrating the reticulum towards the heart and an abscess (yellow arrow). **B.** A foreign body (black arrow) penetrating the heart by passing into the thoracic cavity. A foreign body (green arrow) floating freely in the reticulum

Table 1: Clinical findings and hematology of cows with TRP and TP

Parameters	Groups (mean $\pm$ SEM)			P value
	TRP (n=35)	TP (n=35)	Control (n=20)	
Rectal temperature ($^{\circ}$ C)	38.77 $\pm$ 0.23 ^{ab}	39.18 $\pm$ 0.35 ^b	38.42 $\pm$ 0.16 ^a	0.012
Breaths/min	24.68 $\pm$ 1.40	26.20 $\pm$ 1.29	23.30 $\pm$ 2.52	0.474
Heart beats/min	73.82 $\pm$ 2.57 ^b	62.11 $\pm$ 2.95 ^a	67.60 $\pm$ 2.36 ^{ab}	0.008
Total leukocyte count ($\times 10^3/\mu$ L)	15.78 $\pm$ 1.11 ^b	21.54 $\pm$ 1.53 ^c	8.35 $\pm$ 0.34 ^a	<0.001
Lymphocyte count ($\times 10^3/\mu$ L)	3.97 $\pm$ 0.35 ^a	6.78 $\pm$ 1.05 ^b	4.03 $\pm$ 0.38 ^a	0.011
Monocyte count ($\times 10^3/\mu$ L)	0.32 $\pm$ 0.02 ^a	0.76 $\pm$ 0.09 ^b	0.59 $\pm$ 0.10 ^{ab}	<0.001
Granulocyte count ($\times 10^3/\mu$ L)	8.51 $\pm$ 0.69 ^b	13.42 $\pm$ 1.71 ^c	3.73 $\pm$ 0.39 ^a	<0.001
Red blood cell count ($\times 10^6/\mu$ L)	7.72 $\pm$ 0.33 ^b	6.23 $\pm$ 0.27 ^a	7.76 $\pm$ 0.33 ^b	<0.001
Mean red cell volume (fL)	52.77 $\pm$ 0.98 ^b	47.38 $\pm$ 1.07 ^a	43.50 $\pm$ 1.97 ^a	<0.001
Hematocrit (%)	39.20 $\pm$ 1.79 ^b	30.28 $\pm$ 1.20 ^a	32.20 $\pm$ 1.30 ^a	<0.001
Hemoglobin (g/dL)	11.68 $\pm$ 0.42 ^c	8.71 $\pm$ 0.35 ^a	10.20 $\pm$ 0.33 ^b	<0.001

^{a-c}: Different letters in the same row indicate significant differences between groups ($p < 0.05$). n: the numbers of cows; SEM: standard error of the mean; TRP: traumatic reticuloperitonitis; TP: traumatic pericarditis

Table 2: Biochemical parameters and glutaraldehyde test values of cows with TRP and TP

Parameters	Groups (mean $\pm$ SEM)			P value
	TRP (n=35)	TP (n=35)	Control (n=20)	
Alanine aminotransferase (IU/L)	22.70 $\pm$ 1.35	25.30 $\pm$ 1.12	18.01 $\pm$ 1.26	0.057
Aspartate aminotransferase (IU/L)	109.35 $\pm$ 8.83 ^a	296.86 $\pm$ 67.11 ^b	73.04 $\pm$ 10.02 ^a	0.004
Gamma glutamyl transferase (IU/L)	54.52 $\pm$ 10.35 ^{ab}	87.25 $\pm$ 20.42 ^b	24.44 $\pm$ 0.79 ^a	0.030
Alkaline phosphatase (IU/L)	71.90 $\pm$ 9.85	84.39 $\pm$ 11.45	67.87 $\pm$ 10.83	0.550
Glucose (mg/dL)	55.51 $\pm$ 2.86	65.13 $\pm$ 3.83	57.68 $\pm$ 3.49	0.101
Cholesterol (mg/dL)	53.52 $\pm$ 3.33 ^a	65.32 $\pm$ 4.12 ^a	121.79 $\pm$ 6.92 ^b	<0.001
Creatine (mg/dL)	1.70 $\pm$ 0.12 ^b	2.54 $\pm$ 0.18 ^c	1.14 $\pm$ 0.41 ^a	<0.001
Urea (mg/dL)	41.85 $\pm$ 3.47	46.01 $\pm$ 3.59	37.36 $\pm$ 4.14	0.315
Total bilirubin (mg/dL)	0.45 $\pm$ 0.05 ^b	0.76 $\pm$ 0.08 ^c	0.15 $\pm$ 0.01 ^a	<0.001
Lactate dehydrogenase (IU/L)	386.54 $\pm$ 61.95 ^a	1795.28 $\pm$ 108.93 ^b	128.05 $\pm$ 14.14 ^a	<0.001
Total protein (g/dL)	8.32 $\pm$ 0.17	8.42 $\pm$ 0.26	8.92 $\pm$ 0.17	0.213
Albumin (g/dL)	2.72 $\pm$ 0.06 ^a	2.76 $\pm$ 0.06 ^a	3.06 $\pm$ 0.08 ^b	0.007
Creatine kinase (IU/L)	226.36 $\pm$ 51.49 ^a	659.28 $\pm$ 125.09 ^b	130.73 $\pm$ 14.58 ^a	<0.001
Glutaraldehyde (coagulation/min)	4.98 $\pm$ 0.68 ^a	3.75 $\pm$ 0.73 ^a	>15.00 ^b	<0.001

^{a-c}: Different letters in the same row indicate significant differences between groups ($p < 0.05$). n: the numbers of cows; SEM: standard error of the mean; TRP: traumatic reticuloperitonitis; TP: traumatic pericarditis group

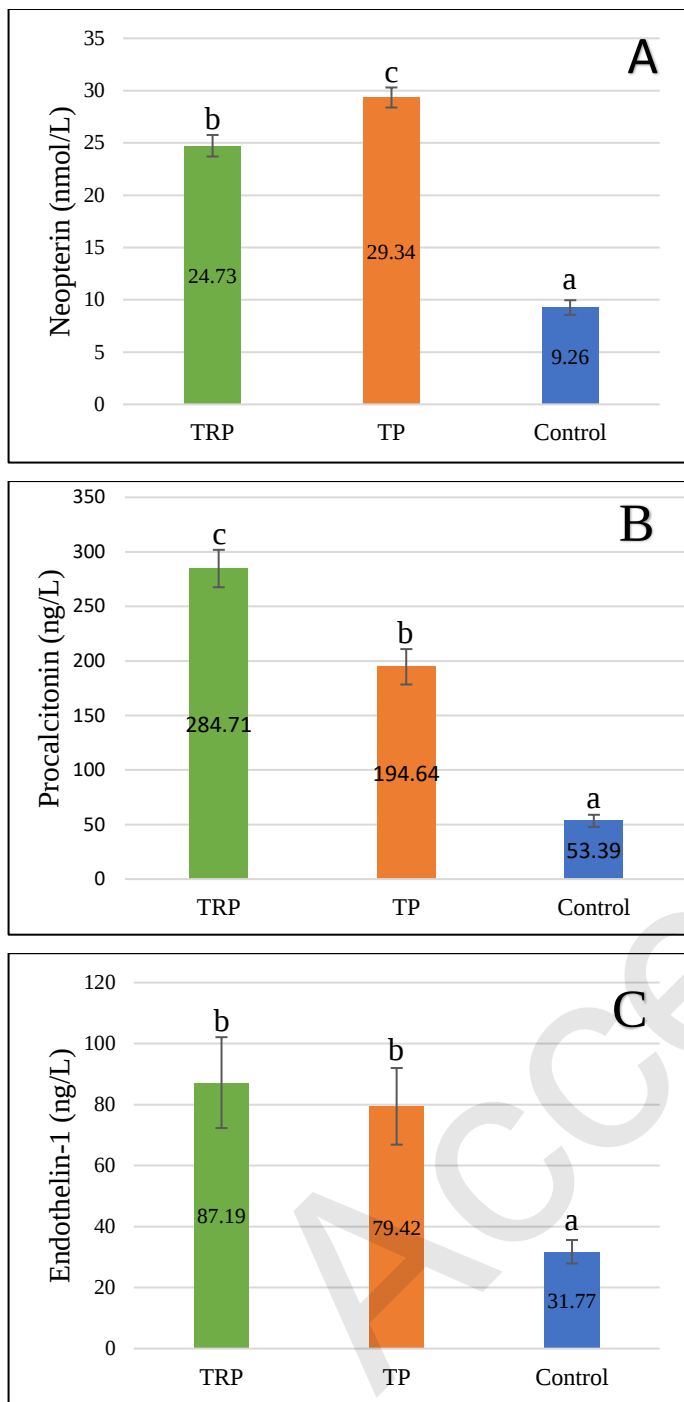


Figure 2: Serum neopterin, procalcitonin, and endothelin-1 levels. Serum levels of neopterin ($P < 0.001$; **A**), procalcitonin ($P < 0.001$; **B**), and endothelin-1 ($P < 0.021$; **C**). TRP: traumatic reticuloperitonitis; TP: traumatic pericarditis. ^{a-c}: denote significant differences between patient and control groups ($p < 0.05$).

Fever, anorexia, atonia, recurrent tympany of the rumen, abdominal tension, moaning attributable to pain, cachexia, tachypnea, and tachycardia were evident in both the TRP and TP groups. Furthermore, TP cows exhibited abdominal distension, a positive venous pulse in the jugular vein, whooshing and rasping sounds in the heart, edema in the ventral body region associated with circulatory failure, a dull percussion sound from

the heart, and a rhythm disorder in the heart. Rectal temperature was statistically higher in the TRP and TP groups than that in the control group ($P = 0.005$), and the heart rate was the lowest in the TP group and the highest in the TRP group ($P = 0.008$, Table 1). Hematological analyses indicated that total leukocyte, monocyte, lymphocyte, and granulocyte counts were significantly higher in the TP group than those in the other groups ($P < 0.05$, Table 1). Conversely, hematocrit values were significantly higher in the TRP group than those in the other groups ($P < 0.001$, Table 1). Hemoglobin was significantly lower in the TP group than that in the other groups ($P < 0.001$, Table 1). Additional hematological parameters are shown in Table 1.

Among the serum biochemical parameters, aspartate aminotransferase was significantly higher in the TP group than that in the other groups ($P = 0.004$, Table 2), whereas gamma glutamyl transferase was significantly higher in the TP group compared to that in the control group ($P = 0.030$, Table 2). Compared to the control group, the TP and TRP groups had significantly lower cholesterol, glutaraldehyde ($P < 0.001$), and albumin levels ($P = 0.007$) (Table 2). The TP group had significantly higher levels of creatinine, creatine kinase, total bilirubin, and lactate dehydrogenase compared to the other groups ($P < 0.001$, Table 2). Moreover, the correlations of some important parameters in our study are presented in Table 3.

Neopterin concentration was significantly higher in the TP group compared to the other groups ($P < 0.001$, Figure 2A). Procalcitonin concentration was significantly higher in the TRP group compared to the other groups ($P < 0.001$, Figure 2B). Endothelin-1 concentration was significantly higher in the TRP and TP groups compared to the control group ($P = 0.021$, Figure 2C).

Receiver operating characteristic analysis showed that procalcitonin was the most reliable biomarker to distinguish between TP and TRP at the cut-off concentration of 236.48 pg/mL, area under the curve of 0.739 (95% confidence interval: 0.617–0.861; $P < 0.001$), and with 77% sensitivity and 75% specificity (Table 4, Figure 3).

Discussion

The primary risk factors for TRP in cows are improper housing, indiscriminate feeding habits, and substandard management (8). Additionally, pregnancy, tenesmus, and intense reticular contractions increase the susceptibility to developing foreign body syndrome in cows (2). Studies found that most cows diagnosed with TRP and TP were either in advanced pregnancy or had recently calved, suggesting that pregnancy and calving increase the incidence of TRP and TP by facilitating the penetration of foreign bodies (3,18). Our present study found clinical signs such as fever, anorexia, atony and recurrent tympany in the rumen, abdominal tension, groaning due to pain, and cachexia, and similar findings in animals with TRP have been reported previously (7,18, 19).

Table 3. Pearson correlation coefficients for numerous parameters

Parameter	Endothelin-1	Neopterin	Procalcitonin	Albumin	Glutaraldehyde
Total leukocyte count	0.153	0.505**	-0.050	-0.206	-0.237*
Lymphocyte count	0.048	0.158	-0.084	-0.078	-0.089
Monocyte count	-0.005	0.051	-0.233*	-0.013	0.035
Granulocyte count	0.137	0.455**	0.001	-0.178	-0.207
Red blood cell count	-0.031	-0.101	-0.043	0.139	0.113
Mean corpuscular volume	-0.023	0.130	0.435**	-0.144	-0.242*
Hematocrit	-0.093	0.039	0.226*	0.018	-0.061
Hemoglobin count	-0.072	-0.089	0.220*	0.020	0.056
Alanine aminotransferase	-0.163	-0.193	-0.103	-0.041	-0.021
Aspartate aminotransferase	0.085	0.034	-0.011	-0.300**	-0.191
γ -glutamyl transferase	0.205	0.171	0.100	-0.170	-0.037
Alkaline phosphatase	0.067	0.052	0.077	-0.068	-0.016
Glucose	-0.051	-0.003	0.044	0.072	-0.087
Cholesterol	-0.183	-0.614**	-0.438**	0.264*	0.440**
Creatinine	-0.001	0.258*	0.086	-0.335**	-0.332**
Urea	-0.047	0.012	0.072	0.034	-0.076
Total bilirubin	0.125	0.285**	0.139	-0.192	-0.344**
Total protein	-0.045	-0.151	-0.081	0.077	0.151
Albumin	-0.136	-0.465**	-0.327**	0.218*	0.467**
Lactate dehydrogenase	0.114	0.393**	0.044	-0.204	-0.348**
Creatine kinase	-0.056	0.176	0.016	-0.114	-0.250*
Body temperature (°C)	0.226*	0.228*	0.185	-0.271**	-0.055
Heart beats/min	0.027	-0.003	0.032	-0.084	-0.125
Breaths/min	0.042	0.001	0.037	-0.023	-0.022
Endothelin-1	1	0.275**	0.253*	-0.053	-0.166
Neopterin	0.275**	1	0.445**	-0.218*	-0.507**
Procalcitonin	0.253*	0.445**	1	-0.081	-0.376**
Albumin	-0.053	-0.218*	-0.081	1	0.260*
Glutaraldehyde	-0.166	-0.507**	-0.376**	0.260*	1

**.: correlation is significant at the 0.01 level (two-tailed); *: correlation is significant at the 0.05 level (two-tailed)

Table 4. The area under the curve (AUC), standard error, confidence interval (95%), cut-off values, respective sensitivity, and specificity of selected biomarkers for distinguishing between traumatic reticuloperitonitis and traumatic pericarditis

Parameters	AUC	Std. error	Asymp. Sig	Asymp. 95% CI		Cut-off	Sensitivity %	Specificity %
				Lower Bound	Upper Bound			
Endothelin-1	0.623	0.068	0.076	0.490	0.756	63.48	57	52
Neopterin	0.387	0.068	0.103	0.253	0.520	26.68	42	38
Procalcitonin	0.739	0.062	0.001	0.617	0.861	236.48	77	75

Asymp: Asymptotic significance

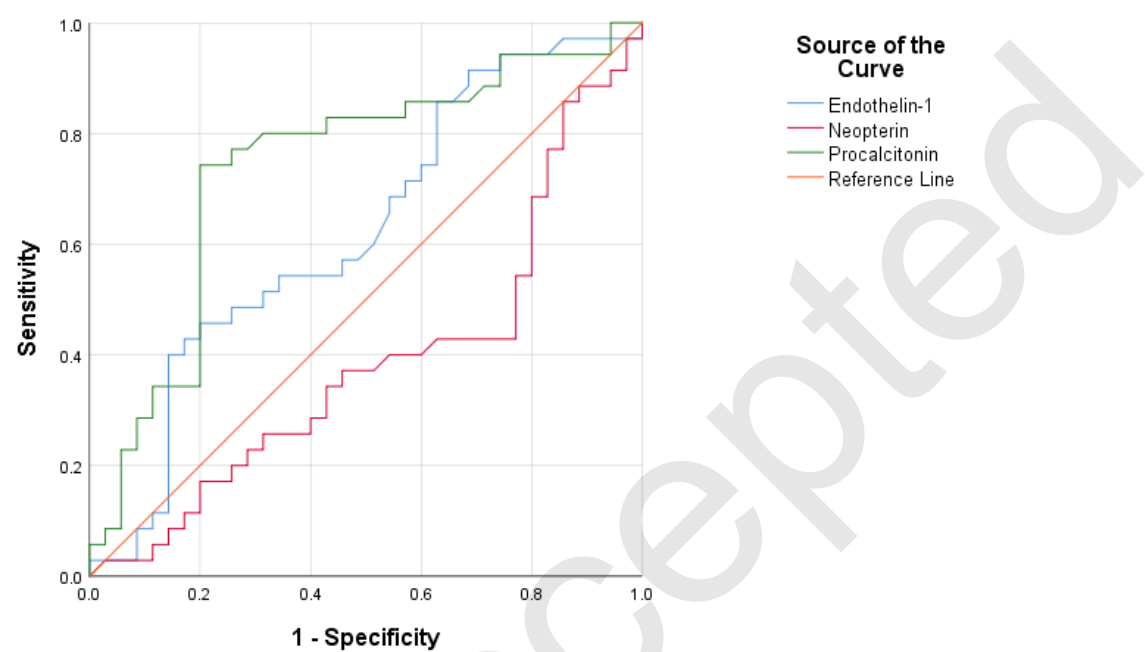


Figure 3: Receiver operating characteristic curve (ROC) analysis for differentiating between traumatic reticuloperitonitis and traumatic pericarditis based on serum endothelin-1, neopterin, and procalcitonin concentrations

Hematological variations in animals with TRP include altered leukocyte counts and erythrocytopenia (3). Hemograms of affected animals show anemia and a neutrophilia-predominant increase in white blood cells (20). Our study also revealed anemia and increased white blood cell counts in the TRP and TP groups. Inflammation, secondary infections, and foreign body-induced trauma can trigger anemia (4).

TRP and TP can alter biochemical parameters, such as increasing levels of serum total bilirubin, urea, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase and decreasing albumin levels (3, 19, 21). The biochemical measurements in our current study yielded similar results. Previous studies have reported that severe hypoalbuminemia in animals suffering from TRP and TP could be the result of acute inflammation (22). The underlying cause of hypoalbuminemia could be post-traumatic inflammation and disrupted liver function.

The glutaraldehyde test is commonly used to assess TRP and TP patients and provides information on the levels of fibrinogen and gamma globulin (23, 24). The test results are useful for diagnosis and treatment decisions. Fibrinogen is a protein produced by the liver in response to inflammation and represents a nonspecific marker for tissue damage and inflammation (25). It is commonly observed in infectious, purulent, and traumatic conditions in cows. Glutaraldehyde test findings showed a statistically significant decrease in the TP and TRP groups compared to the control group. The rapid completion of the glutaraldehyde test within 5 min in both the TRP and TP groups strongly suggests the presence of severe inflammation.

Neopterin is synthesized by monocytes and macrophages after endotoxin and interferon-gamma stimulation. Neopterin levels can be used for prognosis of different pathological conditions and disease activity (9, 10, 11). In a study of calves with aspiration pneumonia, neopterin levels increased due to severe inflammation (26). Similarly, in a study of neonatal calves with sepsis, the pre-treatment neopterin level was higher than that of the control group (12). In our current study, neopterin levels were significantly higher in the TRP and TP groups compared to the control group, which may be attributed to severe diffuse inflammation and increased white blood cell counts due to secondary infection. The TP group is presumed to have the most severe inflammation and the highest white blood cell and monocyte counts based on the glutaraldehyde test, and thus the highest neopterin levels. Few studies have assessed the correlation between neopterin levels and TP. However, the higher neopterin levels in the TP group observed in our study support the use of neopterin as a potential cardiac biomarker for TP.

Procalcitonin levels increase during infection or inflammation because of the immune system's response to endotoxins, proinflammatory cytokines, and chemokines released by microorganisms (27). Procalcitonin is recognized as an acute-phase reactant that is specific to infections, especially bacterial

infections. Measuring procalcitonin levels is useful for clinical follow-up because these levels do not increase in local infections but only in systemic infections (28). Furthermore, procalcitonin levels increase in response to both inflammation and infection in calves affected by sepsis and pneumonia (12, 25). Severe inflammation and secondary infections likely increased procalcitonin levels in the TRP and TP groups in our study. This idea is supported by the increased white blood cell count and glutaraldehyde test used to evaluate severe diffuse inflammation. Furthermore, the abscesses formed in the areas that the foreign body punctured may have spread diffusely through the bloodstream, contributing to inflammation. As such, the increased levels of procalcitonin, an acute-phase biomarker, could be considered a response to endotoxins, proinflammatory cytokines, and chemokines released by microorganisms.

Endothelin-1, a potent vasoconstrictor, is secreted by endothelial cells, and its precursor is released by the heart and lungs. Elevated endothelin-1 levels are associated with mortality rates of various diseases resulting from endothelial damage (15, 16). Our study found a significant increase in epithelial damage in the TRP and TP groups, which may be attributed to trauma-causing objects and foreign bodies that penetrate the diaphragm and cause lung damage in the TRP group and heart damage in the TP group. Furthermore, our study found a significant positive correlation between neopterin, procalcitonin, and endothelin-1 levels. Neopterin levels increase during sepsis, inflammation, and various infections (10,12). Similarly, procalcitonin levels increase during inflammation and infection (27,28), and endothelin-1 levels increase in response to inflammation (15,16). These biomarkers are thus indicative of acute immune responses, and their elevated levels in the TP and TRP groups suggest peritonitis and severe inflammation.

Conclusions

Procalcitonin exhibits the greatest sensitivity and specificity in discriminating between TRP and TP cases. Furthermore, increased neopterin, procalcitonin, and endothelin-1 levels are diagnostically significant in TP and TRP cows.

Acknowledgments

We thank dr. Eva Lasic for reviewing a draft of this manuscript.

Conflicts of interest. The authors declare that there are no conflicts of interest associated with this study or its results.

Funding information: This study was supported by the Scientific Research Projects Coordinatorship of Kafkas University with the approval code 2021-TS-48.

Author contributions: EA conceived and supervised the study. EA, UA, GG, AN, MS, and YUB collected and analyzed data. OM analyzed biochemical measurements. UA, ET, and EK performed

radiology. MK diagnosed cow pregnancy. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

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