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Evaluation of Serum Amyloid A and Procalcitonin in Some Inflammatory Diseases of Cattle

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Abstract

Recent study in humans and animal has been focused on inflammatory biomarkers that infectious diseases, such as serum amyloid A (SAA), procalcitonin (PCT), that may more accurately and efficiently diagnose inflammation. The aim of this study was to evaluate SAA and PCT levels in the diagnosis of cattle with inflammatory disease. Ten healthy control cattle and 64 patients with systemic inflammatory response syndrome (SIRS) were included in cattle. Inflammatory disease in cattle was diagnosed based on clinical signs and the laboratory examination in clinically suspected cases. SAA and PCT concentrations were measured with a commercial ELISA assay for cattle. SAA and PCT concentrations in cattle with inflammatory disease were significantly higher than in the healthy controls (respectively, $P<0.001$, $P<0.008$). Concentrations of SAA and PCT at admission were significantly ($r = 0.376$, $P<0.01$) correlated with outcome in cattle with inflammatory conditions. The cut-off value of SAA and PCT for healthy and inflammatory cattle was determined 28.52 µg/mL and 149.55 pg/mL. In conclusion, PCT levels may be used as an alternative to serum SAA measurement as an indicator of acute inflammation in cattle. Serum PCT concentrations were ~9 times higher in the cattle with peritonitis than in the healthy cattle, suggesting that PCT could be a useful marker of peritonitis in cattle.

Keywords: *Cattle, Marker, Procalcitonin, Serum amyloid A*

Sığırların Bazı İnflamatuar Hastalıklarında Serum Amiloid A ve Prokalsitoninin Değerlendirilmesi

Öz

İnsanlarda ve hayvanlarda yapılan son çalışmalar, enfeksiyöz hastalıkları daha doğru ve etkili şekilde teşhis edebilmek için serum amiloid A (SAA) ve prokalsitonin (PCT) gibi infamasyon biyobeliteçlerine odaklanmıştır. Bu çalışmanın amacı, inflamatuar hastalığı olan sığırların tanısında SAA ve PCT seviyelerinin değerlendirilmesidir. On sağlıklı kontrol sığır ve sistemik inflamatuar yanıt sendromlu (SIRS) 64 hasta sığır dahil edildi. Sığırlarda inflamatuar hastalık klinik olarak şüpheli vakalarda, klinik bulgular ve laboratuvar incelemelerine dayanarak teşhis edildi. SAA ve PCT konsantrasyonları, sığırlar için ticari bir ELISA ile ölçüldü. Enflamatuar hastalığı olan sığırlarda SAA ve PCT konsantrasyonları sağlıklı kontrollerden anlamlı olarak daha yüksekti (sırasıyla, $P<0.001$, $P<0.008$). SAA ve PCT'nin başvuru sırasındaki konsantrasyonları, enflamatuar koşulları olan sığırlarda, sonuçla anlamlı derecede ($r = 0.376$, $P<0.01$) ilişkiliydi. Sağlıklı ve inflamatuar hastalıklı sığırlar için SAA ve PCT'nin cut-off değeri 28.52 µg/mL ve 149.55 pg/mL olarak belirlendi. Sonuç olarak; PCT düzeyleri, sığırlarda akut enflamasyonun bir göstergesi olarak serum SAA ölçümüne alternatif olarak kullanılabilir. Serum PCT konsantrasyonları, peritonitisli sığırlarda sağlıklı sığırlara göre yaklaşık 9 kat daha yüksekti; bu, PCT'nin sığırlarda faydalı bir peritonit belirteci olabileceğini düşündürmektedir.

Anahtar sözcükler: *Prokalsitonin, Serum amiloid A, Sığır, Belirteç*

INTRODUCTION

One of the most important aspects of the complete evaluation and follow-up of the diseases in the veterinary medicine is to determine the inflammatory condition. Early detection of systemic inflammatory status is indispensable for an effective treatment plan [1]. Infections and inflammatory events cause a systemic response in the organism, which is

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calledtheacutephaseresponse(APR).APRisanaturaldefense mechanism that stimulates healing following trauma, infection, or inflammation for limiting tissue damage [2,3]. APR ensures the initiation of repair process which is necessary; for preventing further damage to the organs, for isolation and destruction of infectious agents, for removal of harmful molecules and residues and for the organs to regain its functions. This response of the organism also includes changes in plasma protein concentrations, which are synthesized by the liver and called its APRs [1,2]. Different inflammatory molecules introduced into circulation with the effects of immune system mechanism against infection in organism. These molecules are thought to be diagnostic markers and can be used to monitor infections [4]. Serum amyloid A (SAA), which is an important acute phase protein in cattle, is one of the markers used for the diagnosis of infections, the presence of bacteremia, the course of the disease and mortality [5,6]. SAA, which is used as markers of inflammation or infection, has been used in many studies in this context. SAA is an acute phase protein produced by the liver. The plasma concentrations of SAA are normally very low, but it has been reported that the blood values increase after trauma, inflammation and tissue damage. Especially infectious agents lead to a sudden rise in SAA values [1,2,4].

Procalcitonin (PCT) is a prohormone of calcitonin and is a 116 amino acid protein with a molecular mass of 13 kDa [7]. In a normal metabolic state, hormonal active calcitonin is produced and secreted by C-cells of the thyroid gland after proteolytic treatment processing of PCT intracellularly. It has been reported that in infective cases PCT is secreted from the extrathyroidal source such as liver and lung [8-10]. The mediators produced in response to endotoxins or bacterial infections are said to induce the production of PCT. It was reported that PCT started to increase 4 h after inflammation, peaked about 6 h later, and rapidly returned to normal values after the inflammation was controlled by the organism [11]. Serum PCT level is associated with the prevalence and severity of bacterial infections. It has also been reported that increased interferon gamma (INF-γ) during viral infections suppresses PCT production and may be useful in differentiating viral and bacterial infections [12,13]. It has also been stated that PCT levels above 0.5 ng/mL ay be an indicator of diseases in human medicine [14].

This study examines the levels of SAA, which has recently become a common practice for following up infections, and PCT, which is recently used in veterinary medicine, in some important diseases in cattle, and the correlations between them.

MATERIAL and METHODS

Ethics

This study was approved by the Local Ethics Committee for Animal Experiments (Approval number: 2017/117), Sivas Cumhuriyet University.

Animals

The study animals consisted of 74 cattle referred to Sivas Cumhuriyet University Veterinary Teaching Hospital (Sivas, Turkey) for various diseases. In the study, there were the Holstein (n=12), the Simmental (n=32), and the Swiss Brown (n=20) cattles, different sex (52 females, 12 males), ranging in age from 90 day to 3 years. The study included acute diseased animals. Septic patients were not included in the study. Among the diseases included in the study, respiratory (pneumonia, 10), reproductive systems (retentio secundinarum, 6; metritis, n=10), mastitis 10, omphalitis (n=10), arthritis (n=10), peritonitis (n=8), control (n=10). Similarly, the cattle included in the control group were also of different sex (8 females, 2 males) and breed (2 Holstein, 5 Brown Swiss, and 3 Simmental cattle), age (90 days-3 years).

After clinical examinations, blood samples were collected from the *v. jugularis* to sterile test tubes for laboratory analysis. After centrifugation of test tubes at 3000 rpm for 15 min, serum samples were stored at -80°C for evaluation PCT and SAA.

SAA Measurement

The SAA concentration was measured by the solid phase sandwich ELISA method (Tri-Delta Phase SAA, Tri-Delta Diagnostic, Boonton Township, NJ). Samples were analyzed by diluting 1/500. The serum or plasma analytical sensitivity of this test in cattle was determined as 1.5 ug/mL by the manufacturer. The intra and inter assay precision-reproducibility (CV%) of the test was 7.5% and 12.1% for cattle, respectively.

PCT Measurement

Serum PCT concentration was determined by ELISA method using a commercial kit (Bovine procalsitonin ELISA Kit, SunRed, Ltd. Shanghai, China). This ELISA kit is based on the principle of double-antibody sandwich technique to detect bovine. The analytical sensitivity of this test in cattle was determined as 8.775 ng/mL by the manufacturer.

Statistical Analysis

Statistical analyses were performed using the 15.0 SPSS package programme (Statistical Package for Social Sciences, Chicago, IL). The variables were tested for normal distribution with the Kolmogorov-Smirnov test.Comparisons between groups were made by use of non-normal distribution were analysed with the Mann-Whitney U test. Furthermore, correlations between SAA and PCT levels were assessed with Spearman's correlation coefficients. Receiver operating characteristic (ROC) curves and the area under these curves were used to assess the diagnostic potential of SAA and PCT levels. ROC analyses were performed for both the healthy and cattle with inflammatory disease. To assess the

diagnostic potential of SAA and PCT levels in the diagnosis of the cattle with inflammatory disease, the area under curve (AUC) and some cut-off values were analyzed. The results were assessed at a 95% confidence interval and at a significance level of P<0.05.

RESULTS

A total of 74 cattle, including healthy (n=10) and sick cattle (n=64), were included to the study. Clinically, general findings such as increased body temperature, fatigue, loss of appetite, reduction in mobilization, lameness, pain in the flexion of the relevant joint, local temperature increase, swelling and sensitivity at varying levels were identified in all arthritis calves. The calves with omphalitis had swelling and tenderness in the umbilical cord, increased body temperature and weakened suction reflex. Mastitis was diagnosed with clinical examination and California Mastitis Test. Animals without systemic clinical symptoms and with a purulent or mucopurulent discharge from the uterus were evaluated with clinical endometritis 21 days or more after birth. Increased respiratory rate, tracheal tenderness, cough and nasal discharge and high fever were observed in the pneumonia. At the 12th h following the delivery, those who were not able to expel some or all of the offspring placenta were considered as retentio secundinarum. In diagnosis of peritonitis, reduction in cattle appetite, increase in body temperature in heart rate, respiration and ultrasonography was used for diagnosis of peritonitis. Pericarditis traumatica not included.

When SAA and PCT levels of healthy and sick cattle were compared, statistically significant difference (P<0.001) was found *(Table 1)*. Cattle in the patient group were grouped according to their disease. The grouping is shown in *Table 2*. Cattle were divided into groups according to their diseases and their importance levels among themselves and healthy cattle were show in *Table 3*. While the highest significance values (P<0.001) were found in arthritis group and omphalitis group in SAA, it was found that P<0.001 level in peritonitis group according to PCT levels. A significant correlation was found between SAA and PCT

Table 1. *Serum amyloid A and procalcitonin determined in diseases and control cattle*

Parameters	Control Group (n=10; mean±SE)	Diseased Group (n=64; mean±SE)	P Value
SAA (µg/mL)	21.66±9.40	152.84±9.19	0.001
PCT (pg/mL)	149.84±65.71	352.41±65.71	0.008

SAA: *Serum amyloid A;* ***PCT:*** *Procalcitonin*

Table 2. *Serum serum amyloid A and procalcitonin values of diseases and control animals*

Disease	SAA (µg/mL) (mean±SE)	PCT (pg/mL) (mean±SE)
Arthritis (n=10)	194.76±8.99	151.93±12.44
Omphalitis (n=10)	160.21±20.96	273.54±45.50
Mastitis (n=10)	83.36±28.63	160.25±18.05
Metritis (n=10)	118.95±24.61	207.15±21.30
Pneumonia (n=10)	160.62±14.66	231.47±32.82
Retentio secundinarum (n=6)	205.72±5.61	430.47±134.89
Peritonitis (n=8)	171.07±29.00	1215.97±407.53
Control (n=10)	12.49±2.35	139.26±12.36

SAA: *Serum amyloid A;* ***PCT:*** *Procalcitonin*

Table 3. *Importance between sick and healthy animals of serum amyloid A and procalcitonin*

Diseases	SAA (µg/mL)	PCT (pg/mL)
Arthritis-Omphalitis	0.290	0.041
Arthritis-Mastitis	0.013	0.094
Arthritis-Metritis	0.041	0.041
Arthritis-Pneumonia	0.096	0.016
Arthritis- Retentio secundinarum	0.745	0.005
Arthritis-Peritonitis	0.722	0.000
Omphalitis-Mastitis	0.034	0.034
Omphalitis-Metritis	0.226	0.406
Omphalitis-Pneumonia	0.597	0.650
Omphalitis-Retentio secundinarum	0.278	0.233
Omphalitis-Peritonitis	0.722	0.026
Mastitis-Metritis	0.406	0.07
Mastitis-Pneumonia	0.059	0.041
Mastitis-Retentio secundinarum	0.03	0.007
Mastitis-Peritonitis	0.076	0.001
Metritis - Pneumonia	0.151	0.705
Metritis-Retentio secundinarum	0.051	0.083
Metritis-Peritonitis	0.155	0.003
Pneumonia - Retentio secundinarum	0.039	0.193
Pneumonia - Peritonitis	0.214	0.003
Retentio secundinarum - Peritonitis	0.796	0.121
Arthritis - Control	0.000	0.327
Omphalitis - Control	0.000	0.011
Mastitis - Control	0.41	0.41
Metritis - Control	0.003	0.018
Pneumonia - Control	0.000	0.003
Retentio secundinarum - Control	0.000	0.002
Peritonitis - Control	0.009	0.000

SAA: *Serum amyloid A;* ***PCT:*** *Procalcitonin*

Table 4. *Correlations between serum amyloid A and procalcitonin*

Parameters	SAA (µg/mL)	PCT (pg/mL)
SAA (µg/mL)	-	0.376**
PCT (pg/mL)	0.376**	-

*** P<0.01,* ***SAA:*** *Serum amyloid A,* ***PCT:*** *Procalcitonin*

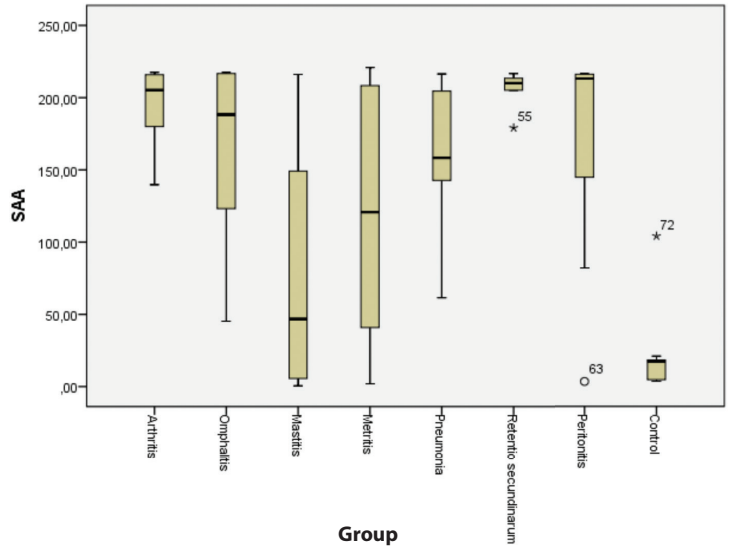

Fig 1. Distribution of serum amyloid A (SAA) among diseased and control cattle

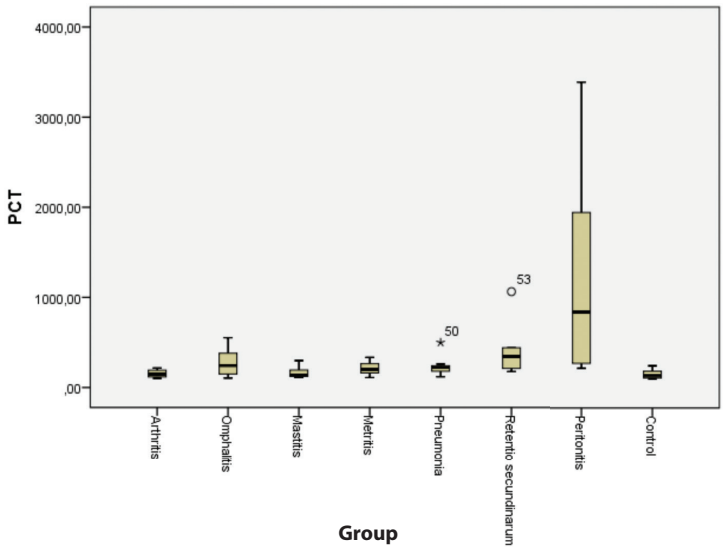

Fig 2. Distribution of procalcitonin (PCT) among diseased and control cattle

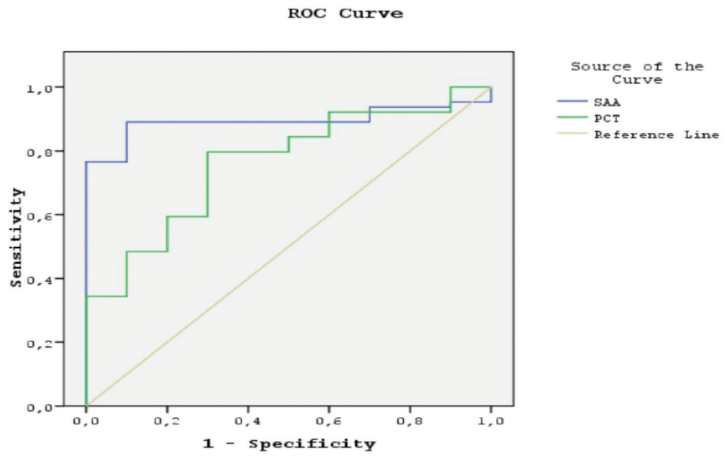

Fig 3. The receiver operating characteristic (ROC) curves used to assess the diagnostic potential of SAA and PCT levels

levels of all cattle (r = 0.376, P <0.01) *(Table 4)*. The distribution between SAA and PCT is shown in the *Fig. 1* and *Fig. 2*.

Receiver Operating Characteristic (ROC) analysis was conducted on the SAA and PCT levels in the healthy and cattle with inflammatory disease. The ROC curves used to assess the diagnostic potential of SAA and PCT levels, are presented in *Fig. 3*. *Table 5* shows the cut-off, sensitivity, specificity, the areas under the curves (AUC) of the SAA and PCT levels of the cattle with inflammatory disease. The cut-off values for the SAA and PCT levels were determined as 28.52 µg/mL and 149.55 pg/mL respectively.

DISCUSSION

Diseases associated with inflammation such as arthritis, metritis, omphalitis, pneumonia and peritonitis may cause significant economic losses or deaths in cattle. In veterinary medicine, the tests used to determine the presence of inflammation in the acute inflammation of cattle are valuable in terms of its contribution to the determination of clinic, diagnosis and prognosis of diseases [2,6,15,16].

Glycoproteins whose blood concentrations change rapidly after tissue damage are defined as acute phase proteins (APP), and the resulting response is called as APR [1,2]. It is considered to be the first condition for maintaining physiological homeostasis and sustaining life after tissue damage. APR is a part of the nonspecific immune response, and some components vary due to the wide distribution of stimulatory conditions. The synthesis of these proteins, whose concentrations vary positively or negatively, usually occurs in the liver. In this context, acute phase proteins have been used in veterinary clinical biochemistry as nonspecific variables for monitoring inflammation activity [4,17].

It is stated that the clinical value of APPs varies according to animal, species and this should be taken into account in the evaluation of APPs. In particular, SAA is considered to be one of the most important APPs for cattle because there are low levels of SAA in healthy cattle, yet there are high levels of SAA in blood during acute phase response [6,18]. Therefore, the level of SAA was determined in order to assess significance of the value in PCT levels.

Serum amyloid A frequently preferred as inflammatory marker on different clinical pathologies and patient groups in recent years.

Table 5. *The cut-off, sensitivity, specificity, the areas under the curves values of the serum amyloid A and procalcitonin levels in diseases and control cattle*

Parameters	Cut-off	Sensitivity (%)	Specificity (%)	AUC	P Value
SAA (µg/mL)	28.58	89	90	0.894	0.000
PCT (pg/mL)	149.55	70	70	0.763	0.008

SAA: *Serum amyloid A;* ***PCT:*** *Procalcitonin;* ***AUC:*** *The areas under the curves*

SAA is from the apolipoprotein family of high density lipoprotein. It is an acute phase protein expressed at different levels in inflammatory reactions [1]. SAA is a rapidly reacting APP, which shows a high level of inflammation in cattle. For example, it has been stated that the mean of SAA levels in cattle with acute diffuse peritonitis is 312.4 µg/mL, and that a statistically significant increase can be useful in clinical medicine [19]. Especially in the diagnosis of mastitis, the determination of SAA levels in blood serum and milk has been reported to be useful in identifying acute, chronic and subclinical conditions of the disease, as well as in distinguishing between mild and moderate conditions [20,21].

In this study, SAA levels in healthy cattle are consistent with previous studies. The 4-9 fold increase in SAA in cows with arthritis, pneumonia, mastitis, retained placenta compared with healthy cattle, were similar in magnitude to that reported previously in cows with traumatic reticuloperitonitis, mastitis, metritis, pododermatitis, and abdominal infection [22,23].

The significance level for the comparison between study group and healthy cattle was determined to be $P<0.001$. In addition, the highest increases in SAA levels were observed in patients with retentio secundinarum, arthritis, peritonitis and pneumonia, while the largest statistical significance was found in arthritis, omphalitis and pneumonia groups when compared with healthy cattle ($P<0.001$). In addition, these results suggest that clinical use of SAA may be beneficial in inflammatory disease in cattle

Procalcitonin is an acute phase reactant which has been studied frequently in medicine in recent years. Viral diseases and autoimmune diseases do not cause increases in PCT. Therefore, in human medicine PCT is most commonly used to distinguish between bacterial diseases and non-bacterial diseases [24,25]. In addition, serum PCT levels were found to be high in sepsis, bacteremia, meningitis and fungal infections causing serious systemic infection [24,26,27]. This observation is consistent with the findings of previous researches and may be explained by the fact that IFN-γ inhibits IL-1 beta-induced calcitonin mRNA expression and PCT secretion [12,13,28]. All values of PCT above 0.5 ng/mL are considered important and indicate that patients are in life-threatening conditions [9]. In addition, it has been reported that it is a significant advantage that PCT has a long serum half-life and is stable when kept at room temperature [28].

Veterinary research has reported that PCT has shown significant increases in septic calf infections [28,29]. No study on the diagnosis of large-scale inflammatory diseases has been found as a result of literature review although previous studies in human medicine have also used C reactive protein (CRP) and PCT level to identify of some inflammatory diseases [24-26].

In this study, the difference between SAA and PCT levels of healthy and infected cattle was determined as significant ($P<0.001$). It is noteworthy that PCT is higher when compared to SAA in patients with the diagnosis of peritonitis. Therefore, it can be concluded that PCT is more suitable for use in the diagnosis of peritonitis than SAA. In addition, a significant correlation between SAA and PCT indicates that PCT can be a valuable marker of infection. The study is supported by another recent study of neonatal calves with septicemic colibacillosis, which concluded that PCT concentrations were significantly higher, when compared to healthy subjects, and that a positive correlation was found between PCT and proinflammatory cytokines [28-30].

In conclusion, it was determined that SAA and PCT concentrations were significantly increased in the acute phase of the disease in cattle with inflammatory infection and there was a positive correlation between them. In addition, it was concluded that further studies on animals should be carried out to reveal the clinical significance of PCT.

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