SHORT COMMUNICATION

Evaluation of Prognostic Biomarkers and Acute Phase Proteins to Predict Survivability in Acute Abdomen Dogs

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Abstract

Acute abdomen is characterized by sudden onset of abdominal pain which develops due to multiple etiologies. It is a life-threatening emergency that requires rapid recognition of disease severity and early prognosis prediction for timely treatment. This study was aimed to assess the prognostic utility of acute phase proteins and biomarkers including lactate, C-reactive protein, procalcitonin, D-dimer and interleukin-6 in dogs presented with acute abdomen. In this study, 52 dogs presented to critical care unit of Madras Veterinary College Teaching Hospital, Chennai with the history of depression, abdominal pain, vomiting and distended abdomen were subjected to a standard diagnostic protocol. Comprehensive assessments including physical examination, vital sign monitoring, haematology, serum biochemistry, blood gas analysis and imaging studies were performed in all the dogs. Out of which 36 (69.23%) dogs were diagnosed with acute abdomen and acute phase protein analysis were performed in those dogs. Statistical analysis of biomarkers lactate, C-reactive protein, procalcitonin, D-dimer and IL-6 highlighted the significance of acute phase proteins and biomarkers in predicting outcome in dogs with acute abdomen.

Key words: Acute abdomen dogs, Acute phase proteins, Biomarkers, Prognosis.

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Introduction

Acute abdomen refers to a clinical syndrome characterised by sudden onset of severe abdominal pain, often associated with systemic illness, requiring immediate diagnostic evaluation and therapeutic intervention. Acute abdomen is considered to be one of the real emergency condition as it may arise from a wide spectrum of underlying etiologies such as gastrointestinal, hepatobiliary, splenic, urinary, or reproductive tract disorders to traumatic, infectious or metabolic causes (Mazzaferro, 2003). Rapid diagnosis and reliable prognosis are essential for timely management and improve the survivability in affected dogs.

Biomarkers and acute phase proteins reflecting tissue hypoxia, inflammation, coagulation dysfunction and systemic inflammation are valuable for assessing the patient status. Acute phase proteins like procalcitonin, C-reactive protein (CRP), interleukin-6 are plasma proteins whose concentration markedly increase in response to systemic inflammation, tissue injury and infection in dogs with acute abdomen (Montagnana *et al.,* 2018). Blood lactate concentration is widely used as a marker of tissue hypoperfusion and hence serves as a predictor for outcome in dogs with shock, Gastric dilatation and volvulus (GDV), sepsis and other severe abdominal emergencies. The serial evaluation of lactate and CRP help to assess the treatment response and outcome of acute abdomen dogs (Mooney *et al.,* 2014). This study was aimed to elucidate the prognostic significance of acute phase proteins and biomarkers in predicting survival in dogs with acute abdomen and to improve treatment strategies accordingly.

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Materials and Methods

The present study was conducted in the clinical cases (n=52) presented to the critical care unit of Department of Veterinary Clinical Medicine, Madras Veterinary College,

Vepery, Chennai, over a period of one year. Six apparently healthy adult dogs of varying breeds, age and sex were selected as control dogs (Group I). In the current study, out of 52 examined, 36 (69.23%) dogs with acute abdomen were included under Group II, which was further subdivided based on etiology into Group II-a (gastrointestinal disease), Group II-b (peritoneal cavity disease) and Group II-c (urogenital disease).

All the selected dogs underwent detailed clinical, physical examination, haematology, serum biochemistry, blood gas analysis, radiography, ultrasonography for the diagnosis of acute abdomen. Vital parameters such as temperature, heart rate, respiratory rate, ECG, SpO_2 and systolic blood pressure were recorded. Arterial blood or venous blood was collected in heparinized syringe for blood gas analysis (Epoc®, Siemens 0L085- RAPIDLAB 348). The dogs were subjected to radiography as per standard protocol. Abdominal ultrasonographic (AFAST) imaging was carried out using basic ultrasound scanner (Aeroscan CD 25). The underlying etiology of acute abdomen was confirmed by detailed diagnostics and imaging.

Approximately 3 mL of blood was collected in a clot activating vial for analysis of acute phase proteins and biomarkers (CRP, procalcitonin, IL-6 and D-dimer). The serum was separated by centrifuging for 20 min and analysed using the i-Chroma III analyser, which is based on principle of fluorescence immunoassay (FIA) using a sandwich immune-detection method. The test procedure followed as per manufacturer's protocol**.**

All the cases of acute abdomen were medically managed with fluid therapy, antibiotic and pain management. In this study, 7 (19.4%) cases with medical emergencies like acute pancreatitis were managed with intensive medical stabilisation**.** Out of remaining 29 (80.55%) cases of acute abdomen dogs with surgical emergency, 13 dogs collapsed before surgery and only 16 dogs underwent immediate surgical correction after medical stabilisation**.** The cases underwent surgical correction were GDV, intussusception, septic peritonitis, uroabdomen and splenic torsion. Post-operatively cases were intensively managed until stabilisation. Lactate and C-reactive protein were measured at 0, 24 and 48 h post-stabilisation. Statistical analyses compared acute phase proteins and biomarkers between survivors and non-survivors.

Results and Discussion

In the present study, a total of 52 cases were selected based on history and clinical signs. Of which 36 (69.23%) cases were confirmed with acute abdomen by detailed clinical examination, diagnostic imaging and laboratory evaluation and were taken up for present study. The cases were further divided into three categories, based on the system affected, gastrointestinal diseases (Group II-a, 17 cases), peritoneal cavity diseases (Group II-b, 9 cases) and urogenital diseases (Group II-c, 7 cases) and splenic diseases (2 cases) and extra-abdominal cause (1 case)**.**

In Group II-a dogs with gastrointestinal disease, acute pancreatitis, foreign body, intussusception and haemorrhagic enteritis were included, whereas, in Group II-b with peritoneal cavity disease uroabdomen and septic peritonitis were recorded. Under Group II-c dogs with urogenital disease pyometra, urethral caliculi, prostate abscess and urethral rupture were included. Under splenic diseases, splenic tumour and splenic torsion were included and extra abdominal case, umbilical hernia was included. The GDV and foreign body were diagnosed by radiography and acute pancreatitis, intussusception, uroabdomen, hemoabdomen, septic peritonitis were diagnosed via abdominal ultrasonography as suggested by MacPhail (2014).

The total number of survivor in this study were 8 dogs (22.22 %) only (2/7- medical emergency and 6/16 in surgical emergency). The mean ± SE values of biomarkers are shown in Table 1. All the acute abdomen dogs showed significant increase in lactate, CRP, D-dimer, IL-6 and procalcitonin when compared to control dogs. The significant increase in plasma lactate concentration in dogs with acute abdomen was associated with inflammatory activation and aerobic glycolysis (Manosalva *et al*., 2022).

There was significant increase in CRP concentration in the present study. CRP concentration was increased in cases like GI perforation, peritonitis, pancreatitis or splenic torsion. In these cases due to tissue necrosis, bacterial contamination or ischemia happens thereby resulting in activation of macrophages and monocytes, which release cytokines especially interleukin-6 which acts on hepatocytes thereby

Table 1: Biomarkers (Mean ± SE values) in dogs with acute abdomen

Parameters	Control (n=6)	Group II (n=33)			F value
		Group II-a (n= 17)	Group II-b (n=9)	Group II-c (n=7)	
Lactate (mmol/L)	0.65^{a}±0.12	4.83^{b}±0.74	4.51^{b}±1.48	4.72^{b}±0.87	2.92*
C-reactive protein (mg/L)	4.78^{a}±0.44	58.45^{b}±6.31	64.54^{b}±14.15	57.81^{b}±15.09	5.61**
D- dimer (µg/dL)	0.15^{a}±0.02	0.51^{b}±0.06	0.72^{b}±0.09	0.53^{b}±0.13	5.58**
Interleukin- 6 (pg/mL)	5.65^{a}±0.50	101.07^{c}±11.15	53.57^{b}±5.82	81.13^{bc}±18.26	10.2**
Procalcitonin (pg/mL)	19.20^{a}±0.94	77.08^{b}±6.82	27.36^{a}±2.22	24.38^{a}±2.90	16.3**

The values bearing same superscript did not differ significantly, *p <0.05 - significant, **p <0.01.

Table 2: Prognostic biomarkers (Mean ± SE value) in dogs with acute abdomen

Parameters	Survivor n=8	Non Survivor n=28	t value	P value
Lactate (mmol/L)	3.50 ± 1.64	4.95 ± 0.50	- 1.13	0.26^{NS}
C- Reactive Protein (mg/L)	40.03 ±2.44	65.69 ± 6.52	-2.07	0.05^{*}
D- dimer (µg/dl)	0.67 ± 0.15	0.71 ± 0.07	0.24	0.80^{NS}
Interleukin – 6 (pg/mL)	55.37 ± 13.06	86.9 ± 7.96	-1.91	0.06^{NS}
Procalcitonin (pg/mL)	35.20 ± 5.88	60.85 ± 5.71	-2.17	0.03^{*}

The values bearing same superscript did not differ significantly, *p <0.05 - significant, **p <0.01.

inducing production of CRP. Thus CRP serves as a biomarker in case of severe inflammation as suggested by Galezowski *et al.* (2010). In this study there was highly significant increase in D-dimer concentration in dogs with acute abdomen. D-dimer concentration was increased in response to systemic inflammation, tissue necrosis and sepsis which was observed in septic peritonitis and acute pancreatitis as reported by Wan *et al.* (2019)

As reported by Silverstein (2015) the LPS -LBP of the Gram negative bacteria of sepsis bind to membrane bound CD14 on macrophage leading to release of anti-inflammatory cytokine like IL-6. In this study majority of cases of acute abdomen had sepsis, and hence, IL-6 was significantly increased in all groups. There was significant increase in level of procalcitonin in acute abdomen dogs with gastrointestinal disease when compared to other causes of acute abdomen. In GDV, peritonitis, intestinal perforation, tissue ischemia resulted in secretion of procalcitonin by leukocytes, which occur in response to sepsis leading to cytokine stimulation regulated by pathogen recognition and cell-cell interaction via up regulation of CALC-I gene (Guinto *et al*., 2010).

In this study in dogs with various etiology of acute abdomen, only eight dogs survived. In non-survivor dogs irrespective of underlying etiology there was significant increase in acute phase proteins such as CRP, procalcitonin and non-significant increase in interleukin-6 was recorded when compared to survivor dogs.

The mean ± SE values of biomarkers in survivor and non-survivor dogs are shown in Table 2. As reported by Brunner *et al.* (2021) CRP and procalcitonin concentration was progressively increased in non-survivor dogs with SIRS and sepsis indicating severity of inflammation in dogs with acute abdomen. Simeonova *et al.* (2013) and Walters (2003) opined that acute abdomen comprises multiple diseases often life threatening and require immediate decision for medical or surgical treatment and any delay could result in fatal outcome due to vital organ dysfunction. Further in this study poor outcome recorded in majority of cases was mainly due to the severity of condition at the time of presentation, which were evident on elevated acute phase proteins such as CRP and procalcitonin in non-survivor dogs.

In this study serial evaluation of CRP and lactate were performed at 0, 24 and 48 h of stabilisation in all the cases. At 0 h, non-survivor dogs showed elevated lactate level when compared to survivors. Over a time, survivors showed a progressive decline in lactate concentration from 0 to 48 h, indicating an improvement in tissue perfusion and response to therapy, whereas non-survivors maintained persistently elevated lactate level at 0 and 24 h, indicating failure of lactate clearance associated with poor prognosis in dogs with acute abdomen. Bonczynski *et al.* (2003) reported that serial evaluation of lactate was helpful to detect hypoperfusion and severity of systemic shock in dogs with acute abdomen.

The plasma CRP value was elevated at the time of presentation in non-survivors when compared to survivors. At 24 and 48 h, survivors showed decrease in CRP indicating resolution of inflammation, whereas persistently high CRP level in non-survivors indicate ongoing inflammation, sepsis. In this study it was found that CRP concentration was progressively declined in acute abdomen dogs in response to therapy and hence the serial evaluation of CRP concentration in acute abdomen dogs was helpful in assessing clinical response to treatment and predicting the outcome as suggested by Galezowski *et al.* (2010).

In summary, the acute phase proteins and biomarkers like CRP, procalcitonin, IL-6, D-dimer and lactate were elevated in all dogs presented with acute abdomen. Procalcitonin was significantly elevated in gastrointestinal cause of acute abdomen than other causes. CRP and procalcitonin level and IL-6 were higher in non-survivor dogs whereas no change in lactate was seen between survivors and non-survivors. However, there was significant decrease in blood lactate in the survived dog. Thus the acute phase proteins like CRP and procalcitonin were considered to be predictors of survivability in acute abdomen dogs.

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