



Association between Systemic Inflammatory Response Syndrome and Canine Pyometra

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ABSTRACT

The present study was conducted on 75 bitches affected with pyometra. Majority (70.67%) of them were positive for SIRS. The criteria used in the present study to evaluate SIRS in pyometra-affected bitches were temperature, heart rate, respiratory rate, TLC, percentage of band neutrophils, albumin, C- reactive protein (CRP) and Serum amyloid A (SAA). Among these parameters temperature (102.57 ± 0.15 vs 102.01 ± 0.11 °F), heart rate (111.06 ± 1.92 vs 104.59 ± 1.46 bpm), TLC (23.34 ± 1.33 vs $14.98 \pm 1.56 \times 10^3/\mu\text{l}$), percentage of band neutrophils (22.57 ± 1.07 vs 11.00 ± 1.59) and SAA (67.45 ± 2.49 vs 44.36 ± 2.64 mg/L) were significantly ($P < 0.01$) higher in SIRS positive bitches compared to SIRS negative bitches.

HIGHLIGHTS

- Study on Systemic Inflammatory Response Syndrome (SIRS) in canine pyometra.
- Temperature, heart rate, TLC, percentage of band neutrophils and SAA were found to be useful for identification of SIRS in canine pyometra.

Keywords: Canine pyometra, C- reactive protein, Serum amyloid A, SIRS

Uterine bacterial infection generally accompanied by endotoxemia, sepsis, Systemic inflammatory response syndrome (SIRS) is a common outcome in canine pyometra (Hagman *et al.*, 2006 and Fransson *et al.*, 2007). SIRS is the clinical manifestation of the body's response to an inciting stimulus, severe enough to cause systemic release of circulating inflammatory mediators (de Laforcade, 2015). It is a common outcome in canine pyometra due to the response of the body to bacterial infection. The critically ill patient with SIRS is at risk for development of multiple organ dysfunction syndrome (MODS), with high mortality rate (Pelander *et al.*, 2008). Hauptman *et al.* (1997) suggested the criteria adopted for diagnosing patients with SIRS had 97.0 per cent sensitivity and 64.0 per cent specificity. The criteria included were respiratory rate more than 20 breaths/min, heart rate above 120 bpm,

temperature below 38.1°C or above 39.2°C, total leukocyte count of $>16000/\mu\text{L}$ or $<6000/\mu\text{L}$ or band neutrophils count of more than 3.0 per cent. The percentage of bitches with pyometra positive for SIRS ranged between 57.0 to 82.5 (Fransson *et al.* (2007), Pelander *et al.* (2008), Karlsson *et al.* (2012), Jitpean *et al.* (2014b), Sant'Anna *et al.* (2014), Jitpean *et al.* (2017) and was more common in dogs with closed cervix pyometra (77.0%) than open cervix pyometra (51.0%) (Jitpean *et al.* (2017). CRP and SAA are useful diagnostic markers for SIRS, however, SAA had higher diagnostic capacity for over CRP (Christensen *et al.* (2014). Van der Geest *et al.*, (2014) and Mare *et al.*,

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(2015) opined that raised levels of band cells in blood have a diagnostic significance for sepsis and prognostic significance in septic human patients. The present study was aimed to study the incidence of SIRS and usefulness of biomarkers *viz.*, CRP and SAA in identifying SIRS in pyometra affected bitches.

MATERIALS AND METHODS

The study involved 75 bitches aged between 2 to 15 years with the presumptive diagnosis of pyometra presented to the Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram. All the dogs were subjected to a detailed clinical examination and the data regarding the breed, age, parity and clinical signs were collected. The preliminary diagnosis of pyometra was made based on case history, findings of physical examination and diagnostic imaging by either abdominal ultrasonography or radiography, or both. All the dogs were subjected to Ovariohysterectomy. The diagnosis was confirmed by postoperative macroscopic examination of the uterus and histopathological examination of uterine tissue.

SIRS Categorization

The 75 dogs affected with pyometra were further divided into 2 groups, SIRS positive and SIRS negative by following the criteria suggested by Hauptman *et al.* (1997). Dogs were considered SIRS positive if at least 2 out of the following 4 criteria were present: Body temperature (BT): $< 38.1^{\circ}\text{C}/100.6^{\circ}\text{F}$ or $> 39.2^{\circ}\text{C}/102.6^{\circ}\text{F}$, Heart Rate (HR): > 120 bpm, Respiratory Rate (RR): > 20 breaths/min and WBC: $< 6000/\mu\text{L}$ or $> 16,000/\mu\text{L}$ or percentage of band neutrophils (PBN): $> 3\%$.

Collection of samples

Blood samples were collected aseptically using sterile EDTA vacutainers from cephalic and/or saphenous vein. Further 5 ml of blood was collected in a sterile vial with clot activator. The serum was separated by centrifugation at 3000 rpm for 7 min. The blood samples were processed within 2 hours of collection for the haematological parameters. An aliquot of serum was utilized for estimation of biochemical parameters while the remaining portion was preserved at -20°C for estimation of biomarkers.

After ovariohysterectomy, the uterus was subjected to macroscopic evaluation and the representative tissue samples of the uterus were collected for histopathology and stored in 10 per cent neutral buffered formal saline until further processing.

Laboratory tests

Haematological (Differential leucocyte count, total leucocyte count, Hemoglobin, total erythrocyte count) and biochemical tests (Alanine aminotransferase, Aspartate amino transferase, total protein, albumin, globulin, A:G ratio, Blood urea nitrogen, creatinine) were performed by adopting standard methods. C-reactive protein estimation was done by using a latex agglutination kit, used for humans, as per the manufacturer's recommendations (C-Reactive Protein, BioSystems, Barcelona, Spain). Canine Serum amyloid A, (GENLISATM, Krishgen Biosystems, Mumbai, India) sandwich ELISA commercial kit was used for the quantitative determination of Canine Serum amyloid A.

STATISTICAL ANALYSIS

The data generated in the study was tabulated and was subjected to Statistical analysis by using SPSS (Software Packages for Social Sciences) 20.0 version as per the methods described by Snedecor and Cochran (1994). The mean values of physiological, hemato-biochemical and serum biomarker values were compared by students t-test between SIRS positive and SIRS negative bitches.

RESULTS AND DISCUSSION

Association of SIRS with pyometra

In the present study, out of 75 bitches with pyometra 70.67 percent ($n=53$) of the bitches were positive for SIRS while 29.33 percent ($n=22$) bitches were negative for SIRS (Table 1). Earlier studies reported that, SIRS occurred with a variable percentage from 57.00 to 82.50 per cent of canine pyometra (Fransson *et al.*, 2007; Pelander *et al.*, 2008; Hagman *et al.*, 2009; Karlsson *et al.*, 2012; Jitpean *et al.*, 2014b; Sant'Anna *et al.*, 2014 and Singh *et al.*, 2020). In the present study, 68.33 per cent of open cervix pyometra cases and 80.00 per cent of the closed cervix pyometra cases had positive SIRS status. The present finding was in

agreement with the report of Jitpean *et al.* (2017) who also recorded SIRS in 51.00 per cent of open cervix pyometra and 77.00 per cent of closed cervix pyometra cases.

Table 1: Occurrence of SIRS in bitches affected with pyometra

Sl. No.	SIRS status	Open	Closed	Total
1	SIRS Positive	41 (68.33)	12 (80.00)	53 (70.67)
2	SIRS Negative	19 (31.67)	3 (20.00)	22 (29.33)
Total		60 (100.00)	15 (100.00)	75 (100.00)

Figures in parenthesis indicate percentage.

Clinical criteria for SIRS

To assess the SIRS status physiological parameters like temperature ($^{\circ}$ F), heart rate (bpm) and respiratory rate (breaths/min) were considered, while the hematological considerations included were TLC and percentage of band neutrophils. The concentration of biomarkers like CRP, SAA and albumin were also taken into consideration to assess the severity of SIRS in pyometra (Table 2).

Table 2: Clinical criteria to assess SIRS in pyometra affected bitches (Mean \pm SE)

Sl. No.	Parameter	SIRS positive (n=53)	SIRS negative (n=22)
1	Temperature ($^{\circ}$ F)	102.57 \pm 0.15**	102.01 \pm 0.11
2	Heart rate (bpm)	111.06 \pm 1.92**	104.59 \pm 1.46
3	Respiratory rate (breaths/min)	27.32 \pm 1.27	22.86 \pm 1.81
4	Total leucocyte count ($\times 10^3/\mu$ l)	23.34 \pm 1.33**	14.98 \pm 1.56
5	Band neutrophils (%)	22.57 \pm 1.07**	11.00 \pm 1.59
6	Albumin (mg/dl)	2.62 \pm 0.08	2.88 \pm 0.11
7	CRP (mg/L)	72.57 \pm 7.21	67.64 \pm 12.26
8	SAA (mg/L)	67.45 \pm 2.49**	44.36 \pm 2.64

Values within a row differ significantly between groups (**P<0.01).

The mean temperature, heart rate recorded in SIRS positive bitches was significantly (P<0.01) higher than SIRS negative bitches. The result was in accordance with the findings of Fransson *et al.* (2007), Hagman *et al.* (2009), and Singh *et al.* (2020). However, Jitpean *et al.* (2014b) reported no significant difference in the heart rate between SIRS positive and negative groups. In the present study,

no significant difference was observed in mean respiratory rate between SIRS positive and negative groups. The result was in concurrence with the reports of Fransson *et al.* (2007) and Jitpean *et al.* (2014b). However, Hagman *et al.* (2009) and Singh *et al.* (2020) reported a significant increase in the respiratory rate of SIRS positive group than SIRS negative group. The increase in temperature, heart rate and respiratory rate in SIRS positive group might be due to the presence of *E. coli* cell wall endotoxins in the systemic circulation from the infected uterus (Fransson *et al.*, 2007 and Krekeler *et al.*, 2012).

The mean total leucocyte count in SIRS positive group ($23.34 \pm 1.33 \times 10^3/\mu$ l) was significantly (P<0.01) higher than SIRS negative group ($14.98 \pm 1.56 \times 10^3/\mu$ l). The result was in accordance with the reports of Singh *et al.* (2020). However, Fransson *et al.* (2007) and Jitpean *et al.* (2014b) reported no significant difference in the total leucocyte count between SIRS positive and negative groups.

The percentage of band neutrophils were significantly higher in SIRS positive (22.57 ± 1.07) group compared to SIRS negative (11.00 ± 1.59) group. Similar findings were recorded by Hagman *et al.* (2009) and Jitpean *et al.* (2014b). On the contrary, Fransson *et al.* (2007) reported no significant difference in the percentage of band neutrophils between SIRS positive and negative groups. The storage pool of neutrophils could be quickly depleted if tissue demand for neutrophils was high (Burton *et al.*, 2014). In such cases, particularly in conditions associated with an acute systemic inflammatory response syndrome (SIRS), band neutrophils would be released from the bone marrow. In human medicine, an increased percentage of band neutrophils was often considered as a marker for acute and severe infections (Van der Geest *et al.*, 2014) and was associated with sepsis, early deterioration and poor outcome in septic patients (Mare *et al.*, 2015).

Albumin was considered to be a negative acute phase protein because serum concentrations decreased in inflammation and/or infection. In the present study, the mean albumin concentration was not significantly different in bitches with or without SIRS. The finding was in accordance with the earlier reports of Fransson *et al.* (2007) and Jitpean *et al.* (2014b). The results of the present study suggest that albumin could be clinically used as an adjunct marker for the diagnosis of pyometra, but albumin does not seem to be a valuable maker in the detection of sepsis.

The mean CRP concentration did not differ between SIRS positive and SIRS negative bitches. Previous studies reported that elevated CRP concentrations had been associated with SIRS in pyometra bitches (Fransson *et al.*, 2007) and mortality in dogs with SIRS and sepsis (Gebhardt *et al.*, 2009). However, in the present study higher CRP concentrations were not associated with SIRS status which was in accordance with the reports of Jitpean *et al.* (2014b) who also reported no significant variation in CRP concentration between septic and non-septic bitches with pyometra.

The mean SAA concentration was significantly ($P < 0.01$) higher in SIRS positive bitches (67.45 ± 2.49 mg/L) than SIRS negative bitches (44.36 ± 2.64 mg/L). Similarly, Jitpean *et al.* (2014b) reported that SAA concentration was significantly higher in septic bitches with pyometra than non-septic bitches. Various other studies had shown that SAA was more promising as a diagnostic marker than CRP for systemic inflammation in dogs (Christensen *et al.*, 2013 and Christensen *et al.*, 2014). SAA might have a clinically higher diagnostic or prognostic value than the more frequently used CRP. The results of present study suggest that CRP and SAA levels have different diagnostic abilities in spite of both being major acute phase proteins in dogs and was in agreement with the findings of previous study in which CRP concentrations were moderately positively correlated with SAA concentrations (Jitpean *et al.*, 2014a).

CONCLUSION

Present study reports that 70.67 per cent of bitches affected with pyometra were SIRS positive. The mean temperature, heart rate, TLC, percentage of band neutrophils and SAA were significantly higher in SIRS positive bitches compared to SIRS negative bitches. The present study concludes that a higher percentage of pyometra affected bitches were positive for SIRS and biomarker like SAA could be used in identification of SIRS in pyometra affected bitches.

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