Original Study

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C-reactive protein, tumor necrosis factor α, and interleukin-6 in dogs with pyometra and SIRS

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Abstract

Objective: To determine the frequency of the systemic inflammatory response syndrome (SIRS) in canine pyometra and to evaluate the relationship between C-reactive protein (CRP), tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and SIRS.

Design: Prospective clinical study.

Setting: Veterinary teaching hospital.

Animals: Fifty-three clinical cases of canine pyometra and 19 healthy control bitches.

Interventions: Upon admission to the veterinary hospital, history and physical examination findings, including previously defined clinical SIRS parameters, were documented. Blood samples were obtained for hematology and biochemical tests and for CRP, TNFα, and IL-6 analysis. The diagnosis of pyometra was confirmed by histopathology of the uterus after ovariohysterectomy. After surgery, clinical SIRS parameters, length of hospitalization, and mortality were recorded.

Measurements and main results: Pyometra dogs were grouped as SIRS positive (30/53; 57%) or SIRS negative (23/53; 43%). Logistic regression showed that CRP was the only parameter that significantly related to SIRS apart from the clinical criteria that define this syndrome. The mortality rate was low (2/53; 3.8%), and conclusions regarding association with SIRS could not be drawn. A positive SIRS status, high plasma CRP concentration, and high body temperature were variables that related to increased morbidity reflected by the length of hospitalization.

Conclusions: SIRS was seen in 57% of canine pyometra cases and a positive SIRS status showed a positive association with prolonged hospitalization. The mortality rate was low (3.3%) among SIRS positive dogs, indicating that progression to multiple organ dysfunction syndrome (MODS) rarely occurs in surgically treated cases of pyometra. CRP was associated with SIRS and with prolonged hospitalization. Further studies of plasma CRP may be warranted in canine intensive care cases susceptible to development of SIRS and MODS.

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Introduction

Systemic inflammatory response syndrome (SIRS) is the clinical manifestation of the body's response to an inciting stimulus severe enough to cause systemic release of circulating inflammatory mediators.[1] Canine pyometra is a common disease in which dogs can manifest SIRS associated with bacterial infection.[2,3] However, the frequency with which dogs with pyometra display SIRS has not been determined.

The critically ill patient with SIRS is at risk for development of multiple organ dysfunction syndrome (MODS), which carries a high mortality rate despite recent advances in critical care.[2,4,5] Even the less severely affected patient with SIRS is at risk for MODS following reactivation of the inflammatory response. This second insult is often caused by a perceivable minor event such as a catheter site infection, and is the basis for the 'two-hit' theory of MODS.[6] An imbalanced immune response to pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor

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necrosis factor α (TNFα) has been considered the reason for progression of SIRS into MODS.[3,7] In humans, clinical criteria have been established to identify SIRS,[8,9] and these criteria have a significant association with mortality and/or morbidity as reflected by physiologic deterioration and increasing organ dysfunction.[9–16] The following clinical criteria used in humans have been adapted for use in dogs: fever or hypothermia, tachycardia, hypocapnea or tachypnea, increased or decreased white blood cell count (WBC), and/or increased percent band neutrophils (PBN). However, there has been no consensus in veterinary medicine determining the exact definition of these criteria in dogs. Purvis and Kirby[1] first presented definitions of the clinical criteria for SIRS and their limits in dogs (body temperature [BT] <37.8 °C/100 °F or >39.7 °C/103.5 °F, heart rate [HR] >160 beats per min [bpm], respiratory rate [RR] >20 breaths/min, and WBC $<4000/\mu L$, $>12{,}000/\mu L$, or PBN $>10\%$).[1,17] Hardie[3] suggested slight modifications of the limits for these criteria (BT <38.0 °C/100.4 °F or >40.0 °C/104.0 °F, HR >120 bpm, RR >20 breaths/min, and WBC $<5{,}000/\mu L$, $>18{,}000/\mu L$, or PBN $>5\%$), which led others to investigate the sensitivity and specificity of the different limits for detection of SIRS associated with infection (sepsis).[1,18] Hauptman et al.[18] found that the previously suggested limits showed a sensitivity of only 77–83% for the detection of sepsis.[18] With further modifications of the limits for these criteria (BT <38.1 °C/100.6 °F or >39.2 °C/102.6 °F, HR >120 bpm, RR >20 breaths/min and WBC $<6000/\mu L$, $>16{,}000/\mu L$ or PBN $>3\%$), a sensitivity of 97% was reached, minimizing the risk for the serious consequences that can result from failure to identify SIRS before the onset of organ dysfunction. However, with the limits defined to increase sensitivity, the specificity decreased, resulting in relatively high numbers of cases falsely identified as septic (specificity 64%).[18] In human critical care patients, plasma TNFα was increased in SIRS and MODS,[19] and increases in IL-6 and the acute phase protein, C-reactive protein (CRP), were detected in septic patients.[20] One goal of this study was to determine the frequency of SIRS in dogs with pyometra. A second purpose was to evaluate whether plasma TNFα, IL-6, or CRP concentrations correlate with, and therefore, can serve as a marker for SIRS, in dogs with a common etiology of SIRS (e.g., surgically treated pyometra). An additional aim was to determine whether clinical criteria or inflammatory markers can predict mortality or increased morbidity as reflected by prolonged hospitalization.

Material and Methods

Animals

This study was approved before onset by the board for ethical use of animals in research, Djurforsoksetiska Namnden, Tierp, Sweden. Client-owned dogs presenting with the presumptive diagnosis of pyometra and subsequently surgically treated by ovariohysterectomy were included in the study after informed consent by the owner. The presumptive diagnosis of pyometra was based on signalment, history, clinical signs such as vaginal discharge, polyuria/polydipsia, inappetance/vomiting, lethargy, fever, etc., and diagnostic imaging demonstrating an enlarged, fluid-filled uterus. The diagnosis of pyometra, according to a previously proposed definition,[21] was confirmed by gross and histopathologic examination of hematoxylin and eosin-stained sections of formaldehyde-fixated uteri.

The dogs in which pyometra was confirmed were further divided into 2 groups: SIRS positive and SIRS negative. Dogs were considered SIRS positive if at least 2 out of the following 4 criteria were present: (1) BT <38.1 °C (100.4 °F) or >39.2 °C (102.6 °F); (2) HR >120 bpm; (3) RR >20 breaths/min; and (4) WBC <6 or $>16\times10^3/\mu L$, or PBN $>3\%$.[18] Healthy adult bitches previously enrolled in the study and treated for pyometra were considered 1 subgroup of the control group. This group was chosen in order to minimize interference from possible individual variation of inflammatory mediator expression between bitches. The second subgroup consisted of healthy adult, intact bitches showing no signs of pro-estrus or estrus. None of the bitches in this study had been bred and no whelping occurred within at least 1 year after the study was completed in the control group. A history questionnaire was completed for all control bitches, ensuring that the owner considered the dog healthy for at least 8 weeks before examination. Physical examination was performed and documented by 2 of the co-authors (Drs. Bergstrom and Hagman). Only bitches with no abnormal findings in the history, on physical examination, or in hematology and biochemical panel were enrolled as control dogs.

Data collection

A questionnaire was completed by the pet owner before admission. The questionnaire documented the duration of clinical signs and the presence or absence of the following clinical signs: purulent vaginal discharge, inappetance or vomiting, lethargy, and polyuria/polydipsia. The owners were also asked to specify other observed clinical signs.

At admission, before any treatments, the clinician documented BT, HR, RR, mucous membrane color and capillary refill time, location of pain response during abdominal palpation, hydration status, and altered mentation. The altered mentation was subjectively recorded as either bright, alert and responsive; depressed (mildly, moderately, or severely); stuporous; or coma-

tose. Blood samples were thereafter obtained through venipuncture of the cephalic or jugular vein, and 5–10 mL was collected into 2 EDTA tubes and 1 tube without anticoagulant. Blood samples were immediately placed on ice and transported within 30 min to the Department of Clinical Chemistry, SLU, Uppsala, Sweden, where plasma and serum were separated without delay. Immediately thereafter, the plasma from one of the EDTA tubes was stored in 2 aliquots at $-70\ ^{\circ}C$ ($-94\ ^{\circ}F$). These samples were transported to Washington State University on dry ice within 24 h. A complete blood count and serum biochemical analysis (alanine aminotransferase, albumin, alkaline phosphatase [AP], blood urea nitrogen [BUN], cholesterol, glucose, total protein, sodium, potassium, chloride, and calcium) were performed on the remaining blood samples using routine methods.

After surgery, the BT, HR, and RR were recorded daily until discharge or death. Additional information on treatment, complications of treatment, and mortality were obtained from the medical records.

TNFα, CRP, and IL-6 assays

The TNFα concentration in plasma was determined by ELISA using a monoclonal human anti-TNFα-antibody, 61E71, and a polyclonal rabbit anti-hTNFα.[a] ELISA tests utilizing this human antibody experimentally in canine plasma have shown a detection limit of 15 pg/mL, which is 1/60 of the levels achieved after sub-lethal doses of endotoxin.[22] This ELISA test has shown a good correlation with the Walter and Eliza Hall Institute of Medical Research (WEHI) bioassay and is specific for biologically active TNFα.[23]

CRP was measured with a commercially available canine sandwich ELISA kit.[b] The activity of IL-6 in plasma samples was determined with a bioassay using an IL-6-dependent B-9 hybridoma cell.[c,24] This assay has previously been used experimentally in dogs.[25] The IL-6 assay was performed as previously described.[26] Briefly, diluted test samples and rIL-6 standard were incubated for 72 h with the indicator cell and then for 4 h with [^{3}H-methyl] thymidine. Absorbance was read at 560 nm (test wavelength) and 690 nm (reference wavelength). The detection limit of this assay is 0.1 pg/mL.

Statistical analysis

Descriptive statistics (mean and standard deviation) and 2-sample unequal variance Student's *t*-test with 2-tailed distribution were calculated for continuous variables.[d] Binary criteria were compared with Fisher's exact test. Univariate and multiple logistic regression[e,27] was used to evaluate the relationship between SIRS status, clinical criteria, blood parameters, and the length of hospitalization. A P value of <0.05 was considered statistically significant.

Results

A total of 71 dogs with the presumptive diagnosis of pyometra were initially enrolled in the study. In 53 bitches, the diagnosis of pyometra was confirmed through histopathologic examination and the data collection was considered adequate (<20% missing data). These 53 dogs were included in the study as the diseased group. The mean age of pyometra dogs was 8.4 years (range 10 months–12 years), and they represented 32 different breeds. Nineteen animals were included in the control group. Ten out of 19 were healthy spayed bitches that were recovered dogs from the 53 in the disease group retested a median of 64 days (range 57–111 days) after surgery. Nine of the 19 in the control group were healthy intact female dogs without a previous history of disease and without clinical signs of proestrus/estrus. The 19 control dogs had an average age of 7.3 ($\pm$ 2.4) years (range 3–12 years), and they represented 14 different breeds.

Clinical criteria

BT, WBC, PBN, HR, platelet count, RR, serum AP, and albumin were recorded in this study. The most common abnormality on physical examination of the diseased group was an altered mentation. This abnormality was noted in 87% of the cases and included moderate or severe depression in 30% of the cases. The following physical examination abnormalities were noted: abdominal pain on palpation (75%), RR > 20 breaths/min (41%), BT > 39.2 °C (30%), tachycardia HR > 120 bpm (23%), and pale or brick red mucous membranes (22%).

The WBC was $\geq$ 16,000/μL in 36 cases (68%), and PBN exceeded 3% in 44 (83%) cases. The platelet count was $\leq$ 200,000/μL in 19 (37%) of the dogs and AP exceeded the reference range (23–212 Iu/L) in 18 cases (34%). Albumin was decreased to $\leq$ 25 g/L in 15 cases (33%).

TNFα, CRP, and IL-6

The mean values for clinical criteria, hematology and biochemical parameters, and the inflammatory markers CRP, TNFα, and IL-6 in the diseased group and the control group are presented in Table 1.

The plasma concentration of CRP in the healthy animals ranged from 9.0 to 31.0 mg/L. In the pyometra group, the CRP concentrations ranged from 26.4 to 378.9 mg/L, with values from 7 animals below 100 mg/L and only 1 dog overlapping the range of the normal animals (26.4 mg/L).

Table 1: Mean and standard deviations for clinical parameters (body temperature, heart rate, respiratory rate), hematology (WBC, percent band neutrophils, platelet count), blood biochemistry (AP, albumin), and inflammatory markers (CRP, TNFα, and IL-6) in dogs with pyometra and in healthy control dogs

	Mean (± SD)		
	Pyometra group (*N*)	**Control group (*N*)**	***t*-test, *P* value***
Albumin (g/L)	26.4 (± 4.1) 45	33.9 (± 3.0) 10	**0.03**
AP (U/L)	369 (± 364) 53	82 (± 35) 10	<**0.0001**
Band neutrophils (%)	12.0 (± 9.5) 53	0.1 (± 0.3) 16	<**0.0001**
CRP (mg/L)	207.7 (± 92.5) 43	19.8 (± 8.2) 10	<**0.0001**
Heart rate (beats/min)	111 (± 25) 48	102 (± 53) 10	0.61
IL-6 (pg/mL)	172.6 (± 249.2) 46	167.6 (± 144.9) 19	0.98
Platelet count (10^9/L)	242 (± 121) 52	345 (± 112) 16	**0.04**
Respiratory rate (breaths/min)	25 (± 10) 39	22 (± 7) 10	0.23
Temperature (°C) [°F]	39.0 (± 0.5) [102.2 ± 1.0] 52	39.0 (± 0.3) [102.1 ± 0.5] 10	0.64
TNFα (ng/mL)	0.13 (± 0.073) 39	0.01 (± 0.012) 10	<**0.0001**
WBC (10^9/L)	23.3 (± 11.5) 53	9.0 (± 2.7) 16	<**0.0001**

Significant *P* values indicated by boldface numerals.
*Two-sample unequal variance Student's *t*-test (2-tailed test).
AP, alkaline phosphatase; CRP, C-reactive protein; IL-6, interleukin 6; TNFα, tumor necrosis factor α; WBC, white blood cell count.

The plasma concentration of TNFα in the healthy animals ranged from 0.0 to 0.03 ng/mL. In the pyometra group, the TNFα concentrations ranged from 0.0 to 0.26 ng/mL, with only 7 measurements below 0.10 ng/mL. These were not the same 7 animals with CRP concentrations below 100 mg/L. Five of the dogs with low CRP concentrations had TNFα concentrations within the range of the normal controls.

The plasma concentration range of IL-6 in the healthy animals ranged from 1.9 to 458.6 pg/mL. In the pyometra group, the IL-6 concentrations ranged from 1.1 to 847.7 pg/mL.

SIRS

Of the 53 dogs with pyometra, 30 (57%) were positive for 2 or more of the 4 SIRS criteria using limits proposed by Hauptman et al.[18] The mean values for clinical criteria, hematology, blood biochemical parameters, and plasma levels of TNFα, CRP, and IL-6 in the SIRS-positive group and the SIRS-negative group are presented in Table 2.

In order to develop a predictive model of SIRS status, the relationship between SIRS status and BT, WBC, PBN, HR, platelet count, RR, TNFα, CRP, and IL-6 was assessed using logistic regression. The results of logistic regression confirmed the relationship of an SIRS-positive status with BT ($P = 0.0005$, $\chi^2 = 12.11$, $df = 1$), HR ($P = 0.0003$, $\chi^2 = 13.07$, $df = 1$), and CRP ($P = 0.037$, $\chi^2 = 4.35$, $df = 1$) in the presence of the other predictors. Other predictor variables were not significantly related to SIRS status. Multiple logistic regression was performed to evaluate whether a combination of 2 or 3 of these variables would render a stronger relationship with SIRS status than each variable alone. The combination of BT, HR, and CRP showed higher significance ($P < 0.0001$) than any combination of 2 of these variables. Based on the logistic regression model, the estimated probability of SIRS-positive status is

$$\exp(-198.5 + 4.6971\,\mathrm{BT} + 0.0169\,\mathrm{CRP} + 0.1077\,\mathrm{HR})/[1 + \exp(-198.5 + 4.6971\,\mathrm{BT} + 0.0169\,\mathrm{CRP} + 0.1077\,\mathrm{HR})]$$

Classification of SIRS status using a cut-off probability of 0.5 resulted in a sensitivity of 79% and a specificity of 89%. Increasing the cut-off probability to 0.95 reduced the sensitivity to 63%, while the specificity increased to 100%. Figures 1 and 2 show, respectively,

Table 2: Mean and standard deviations for clinical parameters (body temperature, heart rate, respiratory rate), hematology (WBC, percent band neutrophils, platelet count), blood biochemistry (AP, albumin), and inflammatory markers (CRP, TNFα, and IL-6) in dogs with pyometra

	Mean (± SD)		
	SIRS group (*N*)	**No SIRS (*N*)**	***t*-test, *P* value***
Albumin (g/L)	26.2 (± 4.2) 24	26.6 (± 4.1) 21	0.77
AP (U/L)	400 (± 438) 30	339 (± 248) 21	0.53
Band neutrophils (%)	11.9 (± 9.59) 30	11.9 (± 9.6) 23	0.99
CRP (mg/L)	235.5 (± 79.8) 22	178.5 (± 97.6) 21	**0.04**
Heart rate (beats/min)	121 (± 27) 27	98 (± 13) 21	**0.0004**
IL-6 (pg/mL)	180.9 (± 265.5) 24	156.1 (± 232.1) 22	0.74
Platelet count (10^9/L)	240 (± 124) 29	236 (± 123) 23	0.91
Respiratory rate (breaths/min)	23 (± 7) 25	27 (± 13) 14	0.32
Temperature (°C [°F])	39.2(± 0.6) [102.6 ± 1.0] 30	38.7 (± 0.3) [101.7 ± 0.6] 22	**0.0002**
TNFα (ng/mL)	0.12 (± 0.06) 20	0.14 (± 0.09) 19	0.61
WBC (10^9/L)	24.0 (± 11.6) 30	22.5 (± 11.3) 23	0.63

The SIRS group consists of dogs with 2 or more positive SIRS criteria[16] and the No SIRS group of dogs with less than 2 positive SIRS criteria. Significant *P* values indicated by boldface numerals.

*Two-sample unequal variance Student's *t*-test (2-tailed test).

AP, alkaline phosphatase; CRP, C-reactive protein; IL-6, interleukin 6; TNFα, tumor necrosis factor α; WBC, white blood cell count, SIRS, systemic inflammatory response syndrome.

the contour plots for the 0.95 and 0.5 cut-off probabilities based on CRP, HR, and BT. If the observed BT exceeds the BT defined by the area of the graph outlined by plotting the values for the observed CRP and HR, then the probability for SIRS positive status exceeds 0.95 and 0.5, respectively (Figures 1 and 2).

Clinical signs

The 3 most commonly displayed clinical signs of pyometra were lethargy, polyuria/polydipsia, and vomiting/inappetance. At least one of these signs was observed in 70% of the cases. Vaginal discharge was noticed in 55% of cases. The duration of clinical signs before admission ranged from 1 to 28 days, with a mean of 6.5 (± 5.8) days. Dogs positive for SIRS showed an average onset of clinical signs of 8 (± 6.39) days, whereas SIRS-negative dogs showed a slightly shorter duration of clinical signs (4.5 ± 4.32 days). The difference in duration was notable, but did not reach statistical significance ($P = 0.055$).

The duration of clinical signs did not correlate with CRP levels above and below 200 mg/L or with TNFα levels above and below 0.10 pg/mL ($P = 0.54$ and 0.35, respectively).

Outcome

The length of hospitalization in the diseased group was noted for 45 dogs and showed a median of 1 day (range 1–5 days). A normal hospitalization stay was considered as 1–2 days and a hospitalization stay exceeding 2 days was considered a sign of increased morbidity. In SIRS-positive dogs, 6/26 dogs (23%) were hospitalized more than 2 days, whereas only 1/19 (5%) SIRS-negative dogs had an increased hospital stay. Logistic regression confirmed the significance of this positive relationship (i.e., a SIRS-positive status was predictive of prolonged hospitalization [1 tailed P value = 0.0471]). A similar relationship was shown between the hospitalization length and CRP (i.e., a high CRP was associated with prolonged hospitalization [$r = 0.318$, 1 tailed P value = 0.0314]). In addition, BT was correlated to hospitalization length ($r = 0.390$, $P = 0.0089$). Figure 3 depicts the regression analysis of hospitalization length versus plasma CRP concentration. Based on the regres-

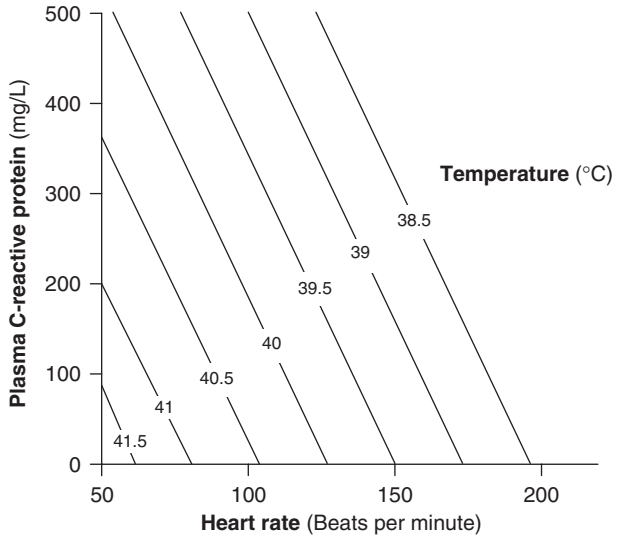

Figure 1: Estimated 0.95 probability of an animal being affected with systemic inflammatory response syndrome (SIRS) using values for body temperature (BT), heart rate (HR), and plasma C-reactive protein (CRP). Bisection of the values for CRP and HR to the right of the curve corresponding with observed BT in an animal indicates at least a 95% probability of SIRS. Alternatively, the observed values for CRP and HR can be plotted, creating a rectangular area, which then includes a range of BTs. If the observed temperature in the animal falls within this range, the probability for SIRS is at least 95%. Using this graph in the prediction of SIRS status was, in this study, associated with a sensitivity of 63% and a specificity of 100%.

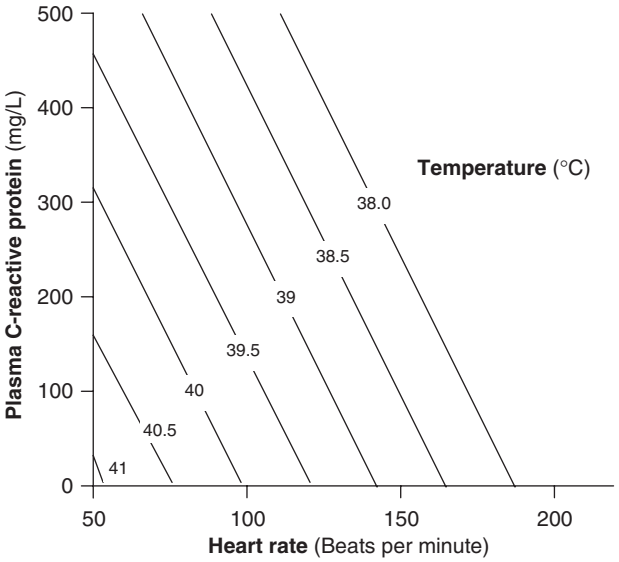

Figure 2: Estimated 0.5 probability of an animal being affected with systemic inflammatory response syndrome (SIRS) using values for body temperature (BT), heart rate (HR), and plasma C-reactive protein (CRP). Bisection of the values for CRP and HR to the right of the curve corresponding with BT in an animal indicates at least a 50% probability of SIRS. Using this graph in the prediction of SIRS status was, in this study, associated with a sensitivity of 79% and a specificity of 89%.

sion function, a plasma CRP $\geq$ 276.35 mg/L is associated with hospitalization exceeding 2 days.

Two of 53 dogs with medical records available for complete evaluation died (3.8%). One dog died 4 days after surgery and was considered SIRS positive; the other dog died on the day following surgery and was included in the SIRS-negative group. The owners of these 2 dogs declined necropsy and the reason for death was not determined. CRP levels were 370 and 183 mg/L in the 2 dogs that died.

Discussion

This study found that dogs with pyometra are affected by SIRS in 57% of the cases based on sepsis/SIRS criteria as defined by Hauptman et al.[18] These criteria were used here due to the demonstrated high sensitivity (97%)[18] for detection of sepsis as compared with other described limits (sensitivity ranging from 77% to 83%).[1,3] However, these criteria were also not highly specific (specificity 64%) and sepsis could be overdiagnosed.[18] The limits suggested in previous studies were not associated with greatly improved specificity (74–77%)[1,3] over the Hauptman and co-workers' study. Thus, increased specificity could not justify use of the limits as defined by Hardie[3] or Purvis and Kirby.[1] The low specificity of SIRS criteria reflects that these criteria can be caused by physiologic events such as pain or anxiety rather than by a systemic response to inflammatory mediators. However, the dogs in this study fulfill previously used diagnostic criteria for sepsis, i.e., histological and/or gross confirmation (purulent exudate) of infection in combination with systemic illness.[18] Bacteriologic culture for confirmation of infection was not performed, but histological verification of pyometra corresponds with a positive bacterial culture from the uterus in 98% of cases.[28] The vast majority of the pyometra cases presented here were systemically ill, as noted by the owner and/or the admitting clinician. Only 3 (6%) cases presented with a bright and alert mentation; in these cases, vaginal discharge was the sole complaint from the owner. The high frequency of systemic clinical signs, blood abnormalities, and the histological verification of an ongoing suppurative process makes systemic inflammatory response a likely explanation for the abnormal clinical criteria.

In this study, the variables investigated included BT, WBC, PBN, HR, platelet count, RR, serum AP, and albumin. The first 5 of these criteria have previously been shown to differ significantly between dogs with and without sepsis.[18] RR was studied as constituting one of the classical SIRS criteria,[1,2] and albumin was included due to

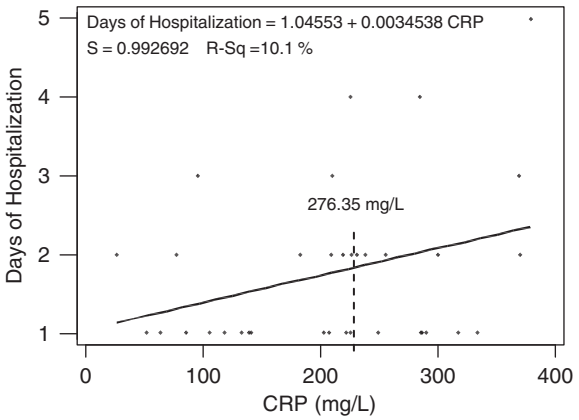

Figure 3: Length of hospitalization versus plasma C-reactive protein (CRP) in 53 dogs with pyometra. A plasma CRP exceeding 276.35 mg/L predicts a hospitalization length exceeding what is considered normal, i.e., 1–2 days.

the tendency for decreased production and increased loss of this protein during systemic inflammation.[29] AP was studied based on previous findings of this enzyme being significantly increased in pyometra cases and possibly related to the magnitude of hepatic involvement.[28]

Of the variables (albumin, AP, PBN, platelet count, TNFα, WBC, and CRP) that showed differences between diseased and healthy animals in this study, only CRP was able to distinguish SIRS status in the diseased animals. Also, when evaluating a possible relationship to a positive SIRS status among variables (BT, WBC, PBN, HR, RR, platelet count, CRP, TNFα, and IL-6) in order to create a sensitive predictive model of SIRS, only BT, HR, and CRP were statistically related to SIRS. Of these 3 variables, CRP was the only factor outside the standard clinical SIRS criteria that related to SIRS ($P = 0.0372$). CRP showed a less significant relationship with SIRS than BT ($P = 0.0005$) and HR ($P = 0.0003$) alone, but the latter 2 criteria are part of the definition of SIRS. Interestingly, the mean BT and the mean HR did not significantly differ between the pyometra group and the healthy control animals showing the limitations of these criteria as indicators of disease. The graphs in Figures 1 and 2 provide an alternative way to estimate the 95% versus the 50% probability of SIRS in an animal given the values for CRP, HR, and BT as compared with the classically grouped SIRS criteria. The RR was not related to SIRS in this study, which is consistent with a previous study.[18] Surprisingly, neither the WBC nor the PBN were significantly different between SIRS-positive and -negative dogs. This finding indicates that most bitches with pyometra, regardless of SIRS status, did have an elevated WBC (68%) or PBN (83%).

These results clearly demonstrate the inherent problems with the SIRS criteria specificity when applied to clinical cases. This study is the first attempt to find a more specific alternative, i.e., a marker for SIRS, for use in veterinary medicine. The ideal parameter would enable clinicians to clearly predict increased morbidity and mortality in a variety of cases susceptible to development of SIRS and MODS. In this study, CRP was the only inflammatory marker that related to SIRS and morbidity. CRP is mainly synthesized by the liver as part of the acute-phase response after hepatocyte stimulation with pro-inflammatory cytokines such as IL-6, IL-1, and transforming growth factor β.[30] CRP enhances inflammation, in part, by activating the classical complement pathway[31] and by compounding the effects of endotoxin,[32] but it also shows anti-inflammatory properties.[33,34] Alone, or together with procalcitonin, CRP has been shown to be a valuable marker for sepsis in critically ill humans.[35,36] In addition to its value in sepsis, CRP has diagnostic and predictive value in atherosclerotic cardiovascular disease, a condition that is now widely accepted as a chronic inflammatory disorder.[32] In experimentally induced inflammation in dogs, CRP increases above the baseline value.[37–39] However, limited clinical studies of CRP in dogs are available. In a report from Poland, levels of CRP were increased in bitches with pyometra[40] and another report showed CRP elevations in dogs with various disorders and surgical trauma.[41] In addition, elevated CRP has been associated with acute pancreatitis in dogs.[42] CRP has not been previously used to predict severity of disease in animals or to predict outcome. A possible limitation of CRP as a predictor of outcome and severity of disease is the high individual variability,[35] which was also reflected here by standard deviations exceeding 40% of the average concentrations in both healthy and sick animals. This variability makes certain conclusions based on an individual value more difficult. At present, access to CRP analysis is limited primarily to experimental studies. The availability of a recently validated human turbidimetric CRP assay may increase clinical use of CRP levels in dogs.[43]

The IL-6 values were not significantly different between healthy and diseased dogs, but IL-6 concentrations in the healthy dogs were much higher than expected. The activities of cytokines such as TNFα and IL-6 are normally tightly regulated; only if a loss of regulation occurs or a massive overproduction or release takes place can they be detected systemically.[44] The bioassay used in this study for IL-6 determination has been used with success experimentally,[22,26] but it is not entirely specific for IL-6. The B9 cells have been reported to respond to other cytokines, but with a lower sensitivity than for IL-6.[26] It is possible that the heterogenous population of dogs used in this study might have had circulating cytokines, hormones, or other humoral factors that interfered with the assay.

The plasma TNFα concentrations were significantly different between dogs with pyometra and healthy dogs. The high TNFα concentrations were slightly surprising, considering that in experimental studies, this cytokine has a short half-life[44] and tends to precede the acute-phase response reflected by increased CRP and decreased albumin.[45] The dogs in this study showed a mean onset of disease of approximately 1 week. This is a relatively long time, considering that experimental studies have been unable to detect TNF activity 6–24 h after induction of inflammation.[45] The explanation for this phenomenon may be either a continuous or intermittent systemic release of inflammatory mediators or bacterial components such as endotoxin into the circulation from the infected uterus. However, TNFα was not able to differentiate between SIRS-positive versus -negative dogs and, therefore, does not seem to be related to severity of disease.

The morbidity of diseased animals was assessed in this study by the length of hospitalization. Length of hospitalization is a nonspecific indicator of severity of disease in veterinary medicine. Factors such as convenience for the owner to pick up their pet from the hospital can falsely increase the number of hospitalization days. However, the vast majority of the cases at Klinikcentrum, SLU, are hospitalized only the minimum number of days. In the cases presented here, there was no indication in the medical record that any of the dogs were hospitalized longer than medically indicated or that any animal was discharged earlier than recommended. Thus, hospitalization appeared to be based on each dog's need for veterinary care. Length of hospitalization has also been a factor used in human clinical studies when associating outcome with SIRS.[13] When evaluating the relationship between all individual variables with length of hospitalization, only the body temperature (2 tailed $P = 0.0089$) and CRP showed significant association. CRP was significant if a positive relationship was presumed, i.e., high CRP-longer hospitalization (1 tailed $P = 0.0314$). Logistic regression showed that the ability of a high CRP concentration to predict increased hospitalization was similar to the predictive ability of a positive SIRS status (1 tailed $P = 0.0471$). However, both a high CRP value and a positive SIRS status could predict increased hospitalization only when a positive relationship was presumed. It seems reasonable to report on this positive relationship as there is a solid body of evidence of a similar relationship in humans, i.e., that a positive SIRS status and increased CRP in humans are associated with increased morbidity.[4–14,18] These factors have not been related to decreased morbidity nor has a negative SIRS status or a decreased CRP ever been reported associated with increased morbidity. None of the other factors investigated were valuable as predictors of hospitalization length.

The mortality rate in this population was less than 4% (2/53), and only 1 of the deaths (1/30; 3%) occurred in a SIRS-positive dog. This rate is slightly lower than the previously reported mortality rate (6–7%) among human patients with SIRS.[16] However, in the 2 cases that died in this study, the lack of necropsy precludes determination of whether the mortality was related to SIRS. The small number of cases also precludes conclusions regarding CRP levels as a predictor of mortality. One of the cases that died was considered SIRS positive before and after surgery and showed a very high CRP level (370 mg/L). The other dog showed only a moderately elevated CRP concentration (183 mg/L) and was considered SIRS negative.

In conclusion, CRP, despite notable individual variation, was the only parameter that was significantly related to SIRS, with the exception of the clinical criteria that define this syndrome. Multiple logistic regressions showed that a combination of body temperature, HR, and CRP was most strongly related to SIRS as compared with all other variables. TNFα and IL-6 were not able to distinguish dogs with or without SIRS. Furthermore, a positive SIRS status, high plasma CRP concentration, and high body temperature were predictors of increased morbidity as reflected by length of hospitalization. The mortality rate among the dogs in this study was low, indicating a low rate of progression of SIRS into MODS in canine pyometra. However, under conditions with a higher risk for progression of SIRS into MODS, early recognition and treatment of SIRS may decrease mortality rates. Further investigations of plasma CRP in a variety of such conditions seem warranted.

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Footnotes

[a] Dr. WA Buurman, University of Maastricht, Maastricht, the Netherlands.
[b] Canine CRP ELISA kit, TriDelta Diagnostics Inc., Plains, NJ.
[c] Dr. Boon Chew, Department of Animal Science, WSU, Pullman, WA.
[d] Microsoft® Excel 97, Microsoft, Redmond, WA.
[e] SAS® system, Proc Logistic, SAS System, Cary, NC.

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