Serum insulin-like growth factor-I, iron, C-reactive protein, and serum amyloid A for prediction of outcome in dogs with pyometra

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ABSTRACT

Pyometra, accumulation of pus in the uterus, is a bacterial infection that frequently initiates systemic inflammation. The disease may have lethal consequences when the systemic effects are severe or complications occur. Markers for identifying high-risk patients and predicting outcome are therefore in high demand. The objective of this study was to measure serum concentrations of insulin-like growth factor-I (IGF-I), iron, C-reactive protein (CRP), and serum amyloid A (SAA) in bitches with pyometra and to explore the possible value of these variables for detection of increased morbidity. In total, 31 bitches were diagnosed with pyometra and destined for surgical treatment (ovariohysterectomy) and 17 healthy bitches were included in the study. Concentrations of IGF-I and iron were lower in the pyometra group (mean concentration 221.2 ± 22.5 ng/mL and 16.9 ± 1.6 μmol/L, respectively) compared with the healthy control group (mean concentration 366.7 ± 46.2 ng/mL and 38.1 ± 2.7 μmol/L, respectively). In contrast, concentrations of CRP and SAA were significantly higher in bitches with pyometra (mean concentrations 212.9 ± 17.3 mg/L and 119.9 ± 8.5 mg/L, respectively) compared with the control group (<5 mg/L and <10 mg/L, respectively). None of the explored variables were associated with morbidity as measured by duration of postoperative hospitalization. In conclusion, IGF-I and iron concentrations were decreased in pyometra, whereas SAA and CRP concentrations were increased in the disease. Although unspecific, measurement of these variables may be valuable as adjunctive markers for prognosis in cases of pyometra.

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1. Introduction

Pyometra, one of the most common pathologies in intact female dogs, is induced by a hormonal imbalance followed by an opportunistic uterine bacterial infection [1]. Escherichia coli, Gram-negative bacteria, known to cause endotoxemia, are most commonly isolated from the infected uterus in dogs with the disease [2–5]. Early diagnosis and treatment are essential for increased chance of survival because pyometra may have deadly consequences when complications such as endotoxemia or sepsis develop. Sepsis, defined as systemic inflammatory response syndrome caused by infection, is identified in most bitches with the disease [5,6]. Surgical ovariohysterectomy is regarded as a safe treatment and also prevents recurrence of the disease [7].
Recently, some studies on biomarkers that may be useful as diagnostic or prognostic tools in veterinary medicine have been published. Such possible biomarkers are the acute phase proteins (APPs), C-reactive protein (CRP) and serum amyloid A (SAA), and also insulin-like growth factor-I (IGF-I) [8–13]. Insulin-like growth factor-I is an anabolic peptide mediated by growth hormone, which has many roles such as controlling cell proliferation, cell differentiation, and anti-apoptosis [14,15]. Several studies on humans have reported that circulating IGF-I concentrations are decreased in response to infection and inflammatory processes [15,16]. Studies on rat, pigs, and dogs have reported that IGF-I secretion is downregulated in response to endotoxin insult and also inhibited in chronic inflammation [18–21]. However, few studies have been done on dogs concerning IGF-I in bacterial infection [22]. Iron is an important cofactor for the function of the immune system and is involved in many biological processes, for example, production of enzymes and metabolism [23,24]. The blood concentration of iron is known to decrease in response to infection in several animal species [25,26]. Studies evaluating iron concentrations in response to infection in dogs are rare [27].

After an infection, inflammation, or trauma, cytokines that are released from leukocytes trigger the hepatic APP production [28]. In dogs, the two major APPs are CRP and SAA, with CRP so far most often assessed in clinical studies [9,29–32]. Blood concentrations of APPs have been widely used as diagnostic and prognostic tools in human medicine [33–37] and are increasingly used also in veterinary medicine as indicators of inflammation and to follow treatment response in inflammatory diseases [10,29,36,38,39]. C-reactive protein has previously been shown to increase in pyometra with higher concentrations detected in dogs with sepsis [5,40]. Several studies have reported that SAA concentrations increase in the circulation during inflammation and/or infection in dogs [10,41–43]. Serum amyloid A and CRP concentrations have also been shown to increase in trauma, infectious diseases, and tumor malignancy; hence, the analyses are not specific for pyometra or bacterial infection. The diagnostic and prognostic value of these variables for pyometra in clinical practice thus remains to be determined. The aims of the present study were to investigate concentrations of IGF-I, iron, CRP, and SAA in bitches with pyometra and to explore the possible value of these variables for estimation of morbidity as measured by postoperative hospitalization.

2. Materials and methods

2.1. Ethical approval

The study was approved by the Uppsala Local Ethical Board (permission number C413/12), and an informed consent was obtained from the owner before inclusion of the dog in the study.

2.2. Animals

Thirty-one client-owned dogs with pyometra admitted to the University Animal Hospital (UDS), Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, during 2011, were included in the study before surgical treatment (ovariohysterectomy). The diagnosis pyometra was confirmed by postoperative macroscopic and histopathological examination of the uterus and ovariess performed at the Department of Biomedical Sciences and Veterinary Public Health, SLU, Uppsala, Sweden. The bitches had no previous history of pyometra. Additionally, 17 healthy female staff-owned dogs of similar weight and in a comparable stage of the estrus cycle (metoestrus, progesterone concentrations exceeding basal concentrations 0.5 nmol/L) were enrolled as the control group. The estrus cycle stage was defined by vaginal cytology and progesterone analysis using an enhanced chemiluminescence immunoassay (ImmuliTE, Diagnostic Products Corporation, Los Angeles, CA, USA).

2.3. Hospitalization

In general, bitches subjected to ovariohysterectomy due to pyometra at UDS are hospitalized one to two days. Prolonged hospitalization (defined as more three days) is only warranted when specific complications occur or when the general condition of the bitch is depressed and it requires additional veterinary care and monitoring.

2.4. Laboratory tests

2.4.1. Hematology, biochemistry, and hormone analyses

Blood samples for hematology and biochemistry were collected from the distal cephalic vein and transferred into EDTA and nonadditive collection tubes (Vacutainer, Becton-Dickinson, Stockholm, Sweden), respectively. The nonadditive tubes were centrifuged and sera-separated before analysis of biochemistry parameters and progesterone. Hematological (total white blood cell count, including differential counts, hemoglobin (Hb)), biochemical (albumin, creatinine, bile acids, and alanine aminotransferase) and progesterone analyses were performed according to routine methods at the Clinical Pathology Laboratory, UDS, SLU, Uppsala, Sweden. After centrifugation, sera not used for biochemistry or hormone analyses were directly transferred in aliquots of 200 μL to cryogenic vials (NuncCryo Tubes, VWR International, Stockholm, Sweden), and freeze-stored at −80 °C until analysis of IGF-I, CRP, SAA, and iron within 8 months. The samples were not hemolytic or lipemic.

2.4.2. Inflammatory markers

For CRP measurement a human immunoturbidimetric CRP test previously validated for dogs (Randox Laboratories Ltd., Crumlin, UK) was used [8,9]. The analyses were performed on Abbot Architect (Abbott Architect c4000, Abbott Park, IL, USA) and the method was calibrated with canine CRP (Life Diagnostics canine CRP, West Chester, USA). Values below the lowest measurable concentration (5 mg/L) were reported as less than 5 mg/L. The intra- and interassay variations were 1.4% and 1.1%–3.7%, and samples with CRP concentrations above the highest measurable concentration (217 and 225 mg/L for the two lots used) were autodiluted 1:3 with 0.9% NaCl and reanalyzed to obtain exact values. Analysis of SAA was performed with a commercially available ELISA (Tridelta Development
Limited, County Kildare, Ireland), with intra- and interassay coefficients of variation of 4.5%–5.0% and 6.2%–11.4%, respectively, and detection limit 10 mg/L. An IGFBP-blocked ELISA (Mediagnost, Reutlingen, Germany), previously validated for use in dogs, was used for evaluation of IGF-I concentrations, with intra- and interassay coefficients of variation of less than 10%, and the lowest measurable concentration was 22 ng/mL [44]. Measurement of iron concentrations was performed by using a direct colorimetric determination (Abbott Laboratories Inc., IL, USA) with detection limit 0.9 μmol/L. All laboratory tests for inflammatory parameters were performed according to the manufacturer’s instructions by trained laboratory staff.

2.5. Statistical analyses

The program SAS 9.2 for Windows version 6.1.7601 (SAS Institute Inc. Cary, NC, USA) was used for statistical analyses. Student’s t-test and ANOVA were used for normally distributed variables, and Van der Waerden two-sample test was used for variables when most healthy bitches had concentrations below the lower limit of detection (i.e., CRP and SAA levels set to half the lowest measurable concentrations (5 and 10 mg/L, respectively) for the analyses). Pearson’s product moment correlation coefficient was used for analyzing the association of variables including length of postoperative hospitalization. Significance level was set to P < 0.05 for all tests in the study.

3. Results

3.1. Comparisons between bitches with pyometra and healthy bitches

In the pyometra group, the mean age was 7.7 ± 2.4 years with a range from 9 months to 12.7 years. In the control group, the mean age was 5.1 ± 2.9 years with a range from 1.0 to 10.2 years. The age differed significantly between the two groups (higher in dogs with pyometra) (P = 0.001). The mean body weight in the pyometra group was 27 ± 12 kg (range 11–64 kg) and 21 ± 9 kg (range 10–41 kg) in the control group. The body weight did not differ significantly between the two groups (P = 0.09).

C-reactive protein and SAA concentrations were significantly higher in the pyometra group compared with the control group (P < 0.0001). In contrast, IGFB-I and iron concentrations were significantly lower in the pyometra group compared with the control group (P = 0.003 and P < 0.0001, respectively) (Table 1).

In 12 of the 31 bitches (39%) with pyometra, the postoperative hospitalization was prolonged (>3 days). No significant association was found between concentrations of CRP, SAA, iron, and IGFB-I and prolonged postoperative hospitalization. The results of hematology and biochemistry analyses are given in Table 2.

3.2. Associations of inflammatory variables

C-reactive protein concentrations were positively associated with SAA (rP = 0.543, P = 0.002) and hemoglobin (rP = 0.416, P = 0.020). Insulin-like growth factor-I concentrations were positively associated with weight in all dogs (rP = 0.449, P = 0.011). Iron concentrations were negatively

<table>
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<th>Table 1</th>
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<tr>
<td>Mean ± standard error (SE) concentrations of IGF-I, iron, CRP, and SAA measured in bitches with pyometra and healthy bitches.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (±SE) Pyometra group (n = 31) (range)</th>
<th>Healthy group (n = 17) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I (ng/mL)</td>
<td>211.2 ± 22.5 (45.2–628.5)</td>
<td>366.7 ± 46.2 (120.4–748.5)</td>
</tr>
<tr>
<td>Iron (μmol/L)</td>
<td>16.9 ± 1.6 (3.0–40.0)</td>
<td>38.1 ± 2.7 (19.0–69.0)</td>
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<tr>
<td>CRP* (mg/L)</td>
<td>212.9 ± 17.3 (&lt;5–362.0)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>SAA* (mg/L)</td>
<td>119.9 ± 8.5 (&lt;10–180.0)</td>
<td>&lt;10</td>
</tr>
</tbody>
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Abbreviations: CRP, C-reactive protein; IGF-I, insulin-like growth factor-I; SAA, serum amyloid A.

* P-value <0.05, ANOVA, or Van der Waerden two-sample tests.

<table>
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<tr>
<td>Hematology and blood chemistry variables measured in bitches with pyometra (pyometra group) and healthy bitches (control group).</td>
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<tbody>
<tr>
<td>Hematocrit* (%)</td>
<td>36 ± 0 (31) (25–50)</td>
<td>47 ± 0 (12) (39–57)</td>
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<tr>
<td>Hemoglobin* (g/L)</td>
<td>128 ± 4 (31) (90–173)</td>
<td>160 ± 3 (17) (137–189)</td>
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<td>Neutrophils* (×109/L)</td>
<td>22.3 ± 3.2 (31) (7.1–106.4)</td>
<td>7.1 ± 0.5 (17) (3.9–11.1)</td>
</tr>
<tr>
<td>Eosinophils* (×109/L)</td>
<td>13.4 ± 2.6 (31) (1.4–85.1)</td>
<td>4.1 ± 0.3 (12) (2.7–5.6)</td>
</tr>
<tr>
<td>Band neutrophils* (×109/L)</td>
<td>4.6 ± 0.8 (31) (0.3–18.1)</td>
<td>0.2 ± 0.07 (12) (0.0–0.7)</td>
</tr>
<tr>
<td>Lymphocytes* (×109/L)</td>
<td>1.7 ± 0.2 (31) (0.0–4.9)</td>
<td>2.5 ± 0.2 (17) (1.3–5.3)</td>
</tr>
<tr>
<td>Monocytes* (×109/L)</td>
<td>2.3 ± 0.3 (31) (0.2–6.4)</td>
<td>0.4 ± 0.04 (17) (0.2–0.8)</td>
</tr>
<tr>
<td>Serum albumin* (g/L)</td>
<td>25 ± 1 (31) (16–33)</td>
<td>28 ± 1 (12) (27–28)</td>
</tr>
<tr>
<td>Serum creatinine* (μmol/L)</td>
<td>4.6 ± 1.0 (31) (0.3–20.4)</td>
<td>7.3 ± 2.8 (12) (0.8–29.4)</td>
</tr>
<tr>
<td>ALT* (μkat/L)</td>
<td>0.4 ± 0.1 (30) (0.0–0.7)</td>
<td>0.3 ± 0.0 (17) (0.0–0.1)</td>
</tr>
<tr>
<td>Serum creatinine (g/L)</td>
<td>64 ± 2 (30) (41–100)</td>
<td>76 ± 4 (12) (53–94)</td>
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Abbreviations: ALT, alanine aminotransferase; WBC, white blood cell.

* P-value <0.05, ANOVA, or Van der Waerden two-sample tests.

Reference range (at the Clinical Pathology Laboratory, University Animal Hospital, Swedish University of Agricultural Sciences, Sweden).
associated with SAA concentrations ($r_p = -0.427, P = 0.016$). Creatinine concentrations were positively associated with SAA ($r_p = 0.621, P = 0.000$) and CRP ($r_p = 0.529, P = 0.003$) and negatively associated with iron ($r_p = -0.374, P = 0.042$).

4. Discussion

The results from this study show for the first time that IGF-I concentrations are lower in bitches with pyometra than in healthy bitches. These findings are in agreement with the results of a previous study, which reported that the systemic IGF-I concentrations decreased as a response to chronic inflammation in piglets and during recurrence of infection in transgenic mice [45]. A study has also shown that administration of IGF-I in mice with sepsis increases survival [17]. Because sepsis is common in pyometra, possible effects of IGF-I administration in dogs with the disease could be worth investigating. However, several studies on humans have reported that blood concentrations of IGF-I can be influenced by other factors such as age, gender, nutrition status, and disease [14,15,46,47]. This indicates that such factors also have to be considered when using this parameter in clinical practice to detect inflammation or infectious disease. Similarly, a study in dogs reported that body size and age were associated with the IGF-I levels, that is, a positive correlation with body size and negative correlation with age was shown [48,49]. In the present study, the body weight was similar in the disease and control groups allowing for comparison. However, the age difference cannot be excluded as possible confounder for the difference in IGF-I levels between the two groups. Moreover, IGF-I concentration in dogs has been shown to decrease with restricted energy intake [50]. Most pyometra dogs had decreased appetite at admission, which also may have contributed to decreased serum IGF-I concentrations. The iron concentrations were lower in dogs with pyometra compared with the control group. This is in agreement with several other studies showing decreased iron concentrations in response to infection [51,52]. However, there are no previous studies of iron concentrations in dogs with pyometra. In the present study, neither IGF-I nor iron were specific for diagnosing pyometra because of the overlap between the two groups. Interestingly, iron concentrations were negatively associated with SAA concentrations but not with CRP. The reason for this is unclear but the observation may reflect the notion that SAA could be a more sensitive marker to detect inflammation compared with CRP, as was indicated in an earlier study where SAA less frequently exceeded the clinical determination limit in healthy dogs than CRP [53]. Assessment of several inflammatory markers may thus be clinically advantageous for diagnostic purposes in inflammatory diseases due to differences in the mechanisms of induction or action.

C-reactive protein and SAA concentrations were significantly increased in bitches with pyometra compared with healthy dogs. Similarly, several studies have reported that serum CRP can be useful as a marker for diagnosis and prognosis in various diseases [5,11,29,30,39,54]. However, CRP is not specific for detection of bacterial infections because it is known to increase in a variety of conditions such as trauma, viral infections, pancreatitis, and immune-mediated hemolytic anemia [11,29,54–56]. Previously, CRP concentrations have been shown to be associated with the duration of hospitalization and sepsis in dogs with pyometra [5,39]. In the present study, CRP concentrations in the bitches that stayed longer in the animal hospital after surgical treatment did not differ significantly from those with normal postoperative hospitalization. This difference may be due to the lower number of dogs included in the present study or the selection of dogs studied. Only dogs with pyometra had SAA and CRP concentrations over the lowest measurable concentration (5 and 10 mg/L, respectively). It was therefore possible to differentiate dogs with pyometra from healthy dogs by using either CRP or SAA.

C-reactive protein was moderately, but not strongly, positively correlated with SAA. This is probably due to different induction or mechanisms of action of the two APPs. Analysis of a panel of inflammatory markers including both CRP and SAA might increase the possibility for diagnosis of bacterial diseases such as pyometra or sepsis.

In this study anemia, leukocytosis, and presence of band neutrophils were common findings in bitches with pyometra. This agrees with most studies of the disease [2,39,57,58]. Renal dysfunction is also a common feature in the disease [59–61]. Interestingly, increased creatinine concentrations were positively associated with CRP and SAA concentrations and negatively associated with iron concentrations. Increased creatinine concentrations in pyometra may reflect dehydration, anemia, or anorexia which are common signs of the disease [62–64].

4.1. Conclusions

The inflammatory response induced by pyometra led to decreased systemic concentrations of IGF-I and iron. In contrast, serum concentrations of CRP and SAA increased in bitches with the disease and were below the limit of detection of assays in all healthy bitches. These results indicate a possible value of these inflammatory variables as adjunctive prognostic markers in pyometra and/or other diseases leading to systemic inflammation. None of the investigated inflammatory variables were associated with morbidity as measured by duration of postoperative hospitalization.

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