

Changes in CRP, SAA and haptoglobin produced in response to ovariectomy in healthy bitches and those with pyometra

Roman Dąbrowski^{a,*}, Władysław Wawron^a, Krzysztof Kostro^b

^a Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine,
Agricultural University, 30 Głęboka St., 20-612 Lublin, Poland

^b Sub-department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine,
Agricultural University, 30 Głęboka St., 20-612 Lublin, Poland

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Abstract

The aim of the study was to assess changes in serum C-reactive protein (CRP), serum amyloid A component (SAA) and haptoglobin (Hp) concentrations in healthy bitches and in those with pyometra undergoing ovariectomy, and to establish the usefulness of such determinations for monitoring the postoperative period. Our results indicate that CRP and SAA determinations serve to evaluate the severity of the inflammatory process in pyometra since the concentrations of these acute phase proteins were increased immediately after surgery and diminished thereafter. The CRP and SAA response was rapidly produced while Hp concentrations increased in a more gradual manner. Thus, postoperative concentrations of CRP and SAA provide valuable information on the subsidence of the inflammatory response during the uneventful postoperative period. Our findings also suggest that acute phase proteins might be useful diagnostic markers of early postoperative complications.

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1. Introduction

Pyometra is a common reproductive disorder affecting female dogs that manifests as an accumulation of pus in the uterine lumen [1–3]. Pyometra mainly affects bitches over the age of 6 years, although recent data from the literature and our own observations indicate that the disease is becoming increasingly common in bitches as young as 4 months [1,4–6]. Dogs most susceptible to pyometra are those who have never given birth [7]. The etiology of the disease involves

hormonal changes, which are its primary cause, and secondary infectious factors, which complicate the process [1,7–12].

Pyometra leads to intoxication of the organism due to secondary infection of the uterus with microorganisms. *Escherichia coli* is the most common bacterium isolated from the diseased uterus [1,4,8,13–17], and progesterone causes the increased adherence of these bacteria to the microvilli of the uterine serous membrane [8,18,19]. The release of lipopolysaccharide (LPS) from the cell walls of *E. coli* results in dysfunction of neutrophils and macrophages leading to inhibition or excessive synthesis and release of proinflammatory cytokines (TNF, IL-1, IL-6) as well as secondary inflammatory mediators (oxygen free radicals, nitrous oxide, prostaglandin). This is one of the

* Corresponding author. Tel.: +48 81 445 61 68;
fax: +48 81 445 61 15.

E-mail address: roman.dabrowski@ar.lublin.pl (R. Dąbrowski).

main mechanisms for the increased inflammatory and immune response [10,20–25]. Moreover, Gram-positive *Staphylococcus* and *Streptococcus* spp. producing enterotoxins with superantigenic properties have been isolated from the inflamed uterus [25]. It, therefore, seems that the uncontrolled production of mediators of inflammation in pyometra complicated by *E. coli*, *Streptococci* and *Staphylococci* is the main factor provoking irreversible damage to internal organs or septic shock, which in some cases may lead to death [10,22].

The proinflammatory cytokines that regulate the immune response also induce the biosynthesis of positive acute phase proteins (APP) in hepatocytes [26]. In dogs, the main APPs are C-reactive protein (CRP), serum amyloid A component (SAA) and haptoglobin (Hp) [26].

Ovariohysterectomy is the current treatment of choice for pyometra in bitches [27,28]. It is also widely used as a method of contraception in dogs. Preventing postoperative complications largely depends on proper monitoring of the health condition of bitches. Serum APP concentrations reflect the severity of inflammation and this marker enables the early detection and restoration of abnormal homeostasis (particularly when clinical symptoms are absent) [26]. Indeed, the determination of APP concentrations in humans and animals has proved extremely useful in postoperative monitoring [29–36].

Unfortunately, the usefulness of APP determinations as prognostic markers of the post-ovariohysterectomy course in bitches has been scarcely addressed and this makes the interpretation of our results much more difficult. The aim of the present study was to examine the changes produced in serum CRP, SAA and Hp concentrations in bitches undergoing ovariohysterectomy and to establish the usefulness of such determinations for monitoring the postoperative period.

2. Materials and methods

The study population was comprised of 20 bitches of several breeds and mongrels aged 3–12 years divided into two experimental groups:

- Group I: clinically healthy bitches ($n = 10$) from the Animal Shelter in Lublin. Prior to the surgical procedure, the bitches underwent standard gynecological-obstetric examinations and complementary tests (blood tests, cytology, ultrasound, vaginoscopy). According to these findings, healthy bitches in anestrus were selected to undergo surgical sterilization (ovariohysterectomy).

- Group II: bitches suspected to suffer pyometra ($n = 10$) attending the Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine, University of Agriculture in Lublin. In the majority of cases (70%), their medical histories revealed that hormonal contraception had been used and that 60% of bitches had never given birth. Moreover, 90% of the dogs showed polydipsia and a reduced or complete lack of appetite (anorexia). Physical examinations revealed asthenia, apathy, enlarged abdominal integuments, and an increased pulse and respiration rate in most of the bitches. In all animals, morphological observations indicated leukocytosis with a left image shift. Abdominal ultrasound using the Pie Medical Scanner 200 and the linear head 5/7.5 MHz revealed an enlarged uterus of diameter ranging from 3 to 6 cm with a hypoechogenic content. Vaginoscopy indicated congested serous membranes of the vaginal vestibule and vagina, which could be seen to be covered with purulent secretions in bitches with patent pyometra. On the basis of these findings, the bitches were assigned to the pyometra group and then subjected to ovariohysterectomy.

Blood samples were collected from the v. saphena lateralis of all bitches prior to the procedure (sample 0), and 24 h (sample 1), 72 h (sample 2), 120 h (sample 3), 240 h (sample 4) and 408 h (sample 5) after ovariohysterectomy. Blood was collected into aseptic centrifugation and silicone (vakuette-9 ml-Greiner Labortechnik Gm-bH, Austria) tubes containing 20 i.u. of heparin per 1 ml of blood. Serum was prepared by 10-min centrifugation ($3000\text{--}4000 \times g$) of full blood, frozen at -76°C (Polar 110H) and stored. The following serum determinations were made: (1) CRP levels using the ELISA method and a commercial CRP kit (Tridelta, Ireland); (2) SAA levels using the ELISA method and a commercial SAA kit (Tridelta, Ireland); and (3) Hp levels using the colorimetric method and Haptoglobin Assay kit (Tridelta, Ireland).

Data were compared by calculating means, standard deviations and the significance of differences. Intragroup means were compared with baseline values using the Student's *t*-test. The level of significance was set at the p -values ≤ 0.05 and ≤ 0.01 .

3. Results

Fig. 1 and Table 1 provide the blood CRP concentrations determined in the two groups of animals. The mean preoperative CRP concentration for group I

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