C-Reactive Protein and Serum Amyloid A in Male Dogs after Orchiectomy

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Summary: The acute-phase response is considered part of the innate immune system. During acute phase response, concentrations of acute phase proteins occur. Therefore, acute-phase proteins are part of the innate immune response and its biological function, although variable, generally relate to defense to pathological damage and restoration of homeostasis. Their levels fluctuate in response to inflammation and tissue injury. The main acute-phase proteins in dogs are C-reactive protein (CRP) and serum amyloid A (SAA). Surgical trauma has effect on many parts of immunological and hematological profile. The aim of this study was to determine the perioperative dynamics of CRP and SAA in male dogs undergoing elective orchiectomy. Blood samples were collected by jugular venipuncture in the following order: before the surgery (day zero), on first (day one), third (day three) and seventh (day seven) postoperative day (POD). As markers of systemic inflammation, CRP and SAA levels were determined by ELISA using commercial kits.

The study showed that the CRP and SAA changes rapidly. The peak CRP and SAA concentrations were detected on the first day after surgery. Serum CRP concentration on the seventh postoperative day was within physiological ranges, while SAA concentration was significantly higher. In conclusion, SAA and CRP measurements above basal levels are clearly indicative of systemic inflammation in dogs.

Key words: CRP, dogs, orchiectomy, SAA

Introduction

The acute phase response is a non-specific reaction to any tissue stimulation disturbing the homeostasis e.g., surgery, trauma, infection, or neoplasia and plays an important role as part of the innate immune system (3). C-reactive protein (CRP) and serum amyloid A (SAA) are major positive acute phase proteins (APP) in dogs and humans that show marked increases in concentration during systemic inflammation (4). One definition of a "biomarker" is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention" (1). Studies on biomarkers have gained growing interest in both human and veterinary medicine for various diseases and conditions, with the goal to find suitable biomarkers for early detection and diagnosis. Compared to other biomarkers of inflammation like body temperature, leukocyte counts and erythrocyte sedimentation rate, CRP has been suggested to be a more sensitive and reliable marker of systemic inflammation following surgery in dogs (3). Good biomarker should be characterized by a rapid change in its levels when the disease develops, but also by rapid normalization during recovery, to allow its use-
fulness when monitoring the effects of therapeutic intervention. In humans, CRP is useful for prognostication, i.e. prediction of survival rate and duration of hospitalization and to evaluate the response of treatment (10). High levels of CRP were observed in dogs with pyometra, polyanarthitis, pancreatitis and panniculitis (5). However, many metabolic disorders were observed not to have significant increases. Consistent increase of acute phase proteins is observed with infectious diseases in dogs (2). Several investigations (2) have been conducted examining acute phase proteins increase in dogs with neoplastic disease. Research of dogs diagnosed with lymphoma, conducted by Nielsen et al. (8) showed 68% of dogs with abnormal levels of CRP. Studies have investigated SAA in various diseases, in humans and animals, including dogs (4,6,12). The changes in the concentrations of APPs are due largely to changes in their production by hepatocytes, which in turn are regulated by cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), which act in a complex network (9). Trauma, via surgery, also increases serum CRP and SAA concentration (5). Orchiectomy is used widely to modify undesirable behavior, prevent health problems, and control pet population, thus exposing a large number of male dogs to surgery each year. Orchiectomy in adult dogs is performed by a prescrotal incision (standard technique), scrotal ablation (pendulous scrotum), perineal approach (concurrent perineal surgery) or parapreputial incision (cryptorchid dogs). Serious complications after orchiectomy are rare, but may include scrotal swelling and bruising, hemorrhage, scrotal hematoma, abscess, granuloma, incisional problems (swelling, formation of seroma, infection), urinary incontinence, endocrine alopecia, behavioral changes (7). The aim of this study was to demonstrate and assess CRP and SAA concentrations before and after orchiectomy.

Material and Methods
Twelve clinically healthy male dogs with an age range of one–three years were admitted to elective orchiectomy. Dogs were client-owned dogs without history of clinical illness and no signs of illness on clinical examinations. The dogs were housed in individual cages, and were given commercial dry food twice a day and water ad libitum except for 24 h prior to general anesthesia. Surgical treatment was conducted in general anesthesia at Surgery Department of Faculty of Veterinary Medicine University of Sarajevo. Blood samples were collected from the distal cephalic vein into serum separating tubes (Vacutainer SST; Becton Dickinson, USA) for CRP and SAA measurement just before the orchiectomy (base values) as well as after 24 hours (day one), 72 hours (day three) and 168 hours (day seven). Serum samples were prepared by centrifugation (1.500 × g for 10 min) and stored in plain micro tubes (Eppendorf, Germany) at −20°C until CRP and SAA analysis. Serum CRP concentrations in the dogs were measured with ELISA microplate reader (Anthos 2001 ELISA-reader, Anthos Mikrosysteme, Krefeld, Germany) using a commercial canine CRP enzyme-linked immunosorbent assay kit (Tridelta PhaseTM Range CRP Assay, Tridelta Development Limited, County Kildare, Ireland). For SAA, the analyses were performed using also a commercially available ELISA (Tridelta PhaseTM Range SAA Assay, Tridelta Development Limited, County Kildare, Ireland). All statistical analyses were performed using the SPSS software package (for Windows, Version 11.5, SPSS Inc, USA). All data are shown as mean ± standard deviation. The results were analyzed using the independent t-test. A P-value less than 0.05 and 0.001 were considered statistically significant.

Results
The mean serum levels of CRP on day one (68.39 ± 7.87 μg/mL) and day three (29.67 ± 2.11 μg/mL) were significantly higher (P<0.001 for day one and P<0.05 for day three) than the base value (4.84 ± 0.73 μg/mL) (Figure 1). No statistical difference was found between the base value and day seven (7.95 ± 0.85 μg/mL). The mean serum levels of SAA on day one (103.32 ± 9.79 μg/mL), day three (87.30 ± 6.22 μg/mL) and day seven (46.11 ± 4.51 μg/mL) were significantly higher (P<0.001 for day one and day three; P<0.05 for day seven) than the base value (11.30 ± 2.49 μg/mL) (Figure 2).
Biomarkers have a key role in biomedical research as well as in clinical practice. Data obtained after orchidectomy indicate that CRP and SAA rise rapidly in the first 24 hours. Data collected over seven days following elective surgery showed that CRP rises very rapidly from normal concentrations of 4.84 ± 0.73 μg/mL to concentrations of 68.39 ± 7.87 μg/mL, peaking at day one, and then slowly decline. Serum amyloid A shows a slower initial rise, but does not return to a normal concentration on day 7, whereas CRP had returned to normal. Tvarijonaviciute et al. (11) showed that orchidectomy induce a short-term inflammatory process associated with the increase in serum levels of APPs. However, orchidectomy did not result in long-term changes of circulating concentrations of APPs. We have to underline that no post-operative complications were registered during our research. The first change during the acute phase response assume to be in cortisol concentration. Data from Northrop-Clewes (9), however, showed that the rise in concentration of cortisol peaks already at six hours after surgery. The study shows that the CRP and SAA changes rapidly. The maximum CRP and SAA concentrations were detected on the first day after surgery. Serum CRP concentration on the seventh postoperative day was within physiological ranges, while SAA concentration was significantly higher.

It is important to precisely understand the behavior of these inflammatory parameters which are induced by an elective complex surgery and expected in the course of "normal" postoperative evolution, and that it be routinely monitored in order to determine an abnormal response and prematurely detect complications or infection. C-reactive protein has peculiar characteristics potentially useful in clinical practice: rapid production in response to acute inflammatory processes and short half-life, which makes CRP better biomarker than SAA.

References
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