Plasma C-reactive protein concentration in dogs with experimentally-induced Staphylococcus aureus infection

T. M. GEORGIEVA¹, D. ZAPRYANOVA²*, I. NIKIFOROV², S. A. DENEV³

Introduction

The frequency of Staphylococcus aureus (S. aureus) infection is permanently increasing [22, 26]. Between one-third and one-half of all case of sepsis are caused by Gram-positive organisms and can induce septic shock and multiple organ failure [26]. Colonization was significantly more frequent in female (12%) than in male dogs (6%), and in adults than in puppies (<12 month old) [1]. It is often associated with purulent infections and is recognized as an inherent member of the microflora of the skin of humans and the dogs [10]. The interaction between Gram-positive bacteria (like Staphylococcus aureus) and immune system is still not fully understood. In contrast to infections caused by Gram-negative bacteria in which lipopolysaccharide (LPS) is well known to act as an immunostimulatory component [22], the possible roles in immune activation of some lipoproteins of Gram-positive bacterium wall and of teichoic acid (TA) and lipoteichoic acid (LTA) are only suspected [2, 22]. In case of Staphylococcus aureus LTA and lipoproteins would play a central role in immune activation [2, 22, 23] but HASHIMOTO et al. [8] suggest that not LTA but lipoproteins are the dominant immuno-active compound in Staphylococcus aureus. Other authors [5, 26] suggest that LTA and peptidoglycan (PepG) (which is a component of cell wall) synergize to cause release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-10, IL-12, IL-8, IL-6), shock and organ dysfunction.

The acute phase proteins (APPs) are reactants synthesized during an acute phase response (APR). This response can be due to infection, inflammation, stress, trauma or tissue...
damage [3, 21]. The synthesis and role of APPs may differ depending on the animal species. In the dog, the main positive APPs (whose circulating concentrations increase during APR) are CRP (C-reactive protein) and SAA (serum amyloid A), whereas AGP (α1-acid glycoprotein), Hp (haptoglobin) and ceruloplasmin (Cp) are moderate positive APPs and albumin and transferrin are negative APPs (whose circulating concentrations decrease during APR) [3, 21]. The maximal variation in serum APP concentration is typically reached within 24 to 48 hours after the initiation [11]. In the case of a positive APP, in particular CRP, a 100-1000-fold increase within 24-48 hours can be observed [17].

The APR, which the main role is to restore the normal homeostasis of the organism, is stimulated by the release of cytokines such as IL-1, IL-6 and TNF-α from macrophages and monocytes at the site of inflammatory lesions or infections. IL-6 is the major mediator for the liver secretion of most of the APPs [11]. IL-6 played a pro-inflammatory role (promoting cytokine and chemokine release) during infection induced by Staphylococcus aureus (Gram-positive bacteria) and an anti-inflammatory role in infection with Gram-negative bacteria [15]. CRP was originally discovered by Tillett and Francis in 1930 as a substance in the serum of patients with acute inflammation. This C-reactive protein is so named because it is able to affect precipitation of C-polysaccharide of Streptococcus pneumonia. CRP is a major APP and its serum concentration can increase rapidly from <1 mg/L to >100 mg/L as part of a number of infectious diseases [7].

Canine CRP would be considered as an almost “real-time” marker [12] during acute phase response in infection diseases because the increase in concentration corresponds to the active period of the diseases. CRP concentrations rise dramatically during inflammatory processes occurring in the body. For example, CRP concentrations rise to around 100 mg/mL in the first 24 hours after surgery, declining after that [11]. It is synthesized as a monomer or pentamer and assembly occurred in the endoplasmic reticulum (ER) [31]. It is also believed to play an important role in innate immunity, as an early defence system against infections. CRP is a member of the innate immune host defence system and has some functions as agglutination of foreign microbes, binding to phosphatidyl-choline on microbes, promotion of phagocytosis by macrophages and neutrophils and activation of the classic complement cascade [16]. The aim of the present study was to observe changes in the CRP concentration in dogs during an experimental infection caused by subcutaneous application of Staphylococcus aureus.

Material and methods

EXPERIMENTAL ANIMALS AND PROTOCOL DESIGN

The experiment was approved by the Ethic Committee at the Faculty of Veterinary Medicine, Stara Zagora. The study was performed on 15 mongrel male dogs, 2 years old, weighing 12-15 kg, provided by the municipality of Stara Zagora. Prior to the experiment, the animals were vaccinated with vaccine Nobivac® DHPPiLR (Intervet International B.V) and orally treated against internal parasites (Caniverm®, Bioveta, A. S. Czech Republic, 1 tablet/10 kg B.W.) and external parasites (Bolfo® Puder, Bayer, Germany). Dogs were housed in metal cages and exposed to a 12 hours light-dark cycle at room temperature (20-22°C). They were fed with a commercially available diet of dog pellet twice daily and had free access to water. Among them, 9 were inoculated in the lumbar region subcutaneously with a suspension of 24 hours broth culture of S. aureus strain (5 mL, density: 3.1x10⁸ c.f.u./mL) and constituted the experimental group, whereas the other dogs (n = 6) were injected with the same volume of saline solute and served as controls.

BIOCHEMICAL ANALYSES

Blood samples were collected from the puncture of the v. cephalica antebrachii. Blood samples were collected into heparinised tubes before inoculation (hour 0) then at 6, 24, 48, 72 hours and 7, 14 and 21 days after S. aureus inoculation or saline injection. Heparinised blood samples were centrifuged (1500g, 10 minutes, room temperature) within 30 minutes after collection. Plasma was immediately separated and stored at -20°C until analysis. Plasma C-reactive protein concentrations were assayed on a microplate reader (HP) with species-specific commercial kits produced by Tridelta Development Ltd, Ireland.

STATISTICAL ANALYSIS

The statistical analysis was performed using one way analysis of variance (ANOVA). The results were processed with software Statistica v.6.1 (StatSoft Inc., 2002). All results are presented as mean and standard deviation (Mean ± SD). The statistical significance of parameters was determined in the LSD test at p < 0.05.

Results

The changes in the CRP concentrations during the staphylococcal infection in the present study are shown in Table I. Blood C-reactive protein concentrations were significantly influenced by staphylococcal infection. In the control dogs (not inoculated with S. aureus), CRP concentrations have remained stable during the whole experimental period ranging from 6.2 ± 1.2 mg/L to 8.4 ± 2.3 mg/L. In the experimental group, initial concentrations (before inoculation) were 24.2 ± 19.3 mg/L and CRP concentrations began to rise 6 hours after S. aureus inoculation and remained significantly higher in inoculated dogs than in the controls until the end of the study (21 days) (at 6 and 72 hours, on days 14 and 21: p < 0.05; at 24 and 48 hours: p < 0.01 and on day 7: p < 0.001). In addition, CRP concentrations in this group peaked 2 times after bacteria inoculation, at 24 hours (p < 0.01 compared to the initial values) and 7 days (p < 0.001 compared to the initial values)
where culminated values were recorded. On day 21, CRP concentrations were still high and have not returned to the initial values.

The experimental staphylococcal infection in dogs was accompanied with swelling, painfulness and high temperature at the site of inoculation (Table II). Reduced appetite, impaired motor activity and enlargement of the inguinal lymphatic nodes in the limp in which bacteria were inoculated 24 hours after were recorded. Additionally, hair loss and tissue erosions were noticed at the inoculation site. Fever and skin abscesses were observed in inoculated dogs. Purulent conjunctivitis eye infection was also noticed in one dog. Taking into account 3 clinical parameters (body temperature, respiratory and heart rates), a strong and positive correlation was obtained only between the body temperature and the respiratory rate ($r = 0.84$, $p < 0.05$) whereas the CRP concentration was not significantly associated (Table III).

### Discussion

During the APR, concentrations of APPs may change, and measuring APPs concentrations is being used widely nowadays both in human and in veterinary medicine. C-reactive protein, an acute phase reactant protein, has no specificity for disease, but increases rapidly in the presence of inflammation or tissue destruction. Innate immunity is an early line of host defence against pathogens. In the case of Gram-positive infection (like *S. aureus* infection), a key bacterial element recognized by the innate immune system is

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**Table I**: Plasma CRP concentrations (mg/L) in dogs experimentally infected with *Staphylococcus aureus* (24 hours broth culture, density: $3.1 \times 10^9$ c.f.u./mL, 5 mL) ($n = 9$) compared to the control group (not inoculated) ($n = 6$). Results are expressed as mean $\pm$ standard deviation (SD).

<table>
<thead>
<tr>
<th></th>
<th>Control ($n = 6$)</th>
<th>Inoculated ($n = 9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour</td>
<td>6.9 $\pm$ 1.6$^a$</td>
<td>24.2 $\pm$ 19.3$^{bA}$</td>
</tr>
<tr>
<td>6 hours</td>
<td>7.2 $\pm$ 1.3$^a$</td>
<td>64.5 $\pm$ 42.0$^{bA}$</td>
</tr>
<tr>
<td>24 hours</td>
<td>7.6 $\pm$ 1.5$^a$</td>
<td>206.6 $\pm$ 102.0$^{bC}$</td>
</tr>
<tr>
<td>48 hours</td>
<td>7.1 $\pm$ 2.0$^a$</td>
<td>126.5 $\pm$ 63.0$^{bB}$</td>
</tr>
<tr>
<td>72 hours</td>
<td>6.2 $\pm$ 1.2$^a$</td>
<td>88.9 $\pm$ 27.9$^{bA}$</td>
</tr>
<tr>
<td>7 days</td>
<td>6.9 $\pm$ 1.1$^a$</td>
<td>363.0 $\pm$ 206.9$^{bC}$</td>
</tr>
<tr>
<td>14 days</td>
<td>8.4 $\pm$ 2.3$^a$</td>
<td>79.0 $\pm$ 92.2$^{bA}$</td>
</tr>
<tr>
<td>21 days</td>
<td>7.3 $\pm$ 1.4$^a$</td>
<td>77.3 $\pm$ 101.4$^{bA}$</td>
</tr>
</tbody>
</table>

Different superscripts $a,b$ indicate significant differences ($p < 0.05$ or more) between experimental and control groups. Different superscripts $A,B,C$ indicate significant differences ($p < 0.05$ or more) according to time within a same group.

**Table II**: Proportions (%) of dogs experimentally inoculated with *Staphylococcus aureus* (24 hours broth culture, density: $3.1 \times 10^9$ c.f.u./mL, 5 mL) ($n = 9$) showing at least 10 fold increase in plasma CRP concentrations (mg/L) on days 1 and 7 after injection according to the intensity of systemic (reduced appetite, impaired motor activity, enlargement of the inguinal lymphatic nodes, fever, purulent conjunctivitis) and local (inflammation, skin abscesses) clinical signs.

<table>
<thead>
<tr>
<th></th>
<th>On day 1</th>
<th>On day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic signs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intense</td>
<td>5 (55.55 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>Moderate</td>
<td>4 (44.44 %)</td>
<td>5 (55.55 %)</td>
</tr>
<tr>
<td>Absent</td>
<td>0 (0 %)</td>
<td>4 (44.44 %)</td>
</tr>
<tr>
<td><strong>Local signs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intense</td>
<td>1 (11.11 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>Moderate</td>
<td>2 (22.22 %)</td>
<td>5 (55.55 %)</td>
</tr>
<tr>
<td>Absent</td>
<td>6 (66.66 %)</td>
<td>4 (44.44 %)</td>
</tr>
</tbody>
</table>

**Table III**: Correlation coefficients between clinical parameters (body temperature, respiratory and heart rates) and plasma C-reactive protein concentrations in dogs experimentally infected with *Staphylococcus aureus* (24 hours broth culture, density: $3.1 \times 10^9$ c.f.u./mL, 5 mL) ($n = 9$).

<table>
<thead>
<tr>
<th></th>
<th>Body temperature (°C)</th>
<th>Respiratory rate (cycles / minute)</th>
<th>Heart rate (beats / minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rate (cycles / minute)</td>
<td>$r = 0.841$</td>
<td>$p &lt; 0.01$</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats / minute)</td>
<td>$r = 0.143$</td>
<td>NS</td>
<td>$r = 0.442$</td>
</tr>
<tr>
<td>C reactive protein (mg/L)</td>
<td>$r = 0.021$</td>
<td>NS</td>
<td>$r = 0.109$</td>
</tr>
</tbody>
</table>

Correlations were calculated from all time points (from 0 hour to 21 days) and significant associations ($p < 0.05$) were indicated in bold.
PLASMA C REACTIVE PROTEIN CONCENTRATIONS IN STAPHYLOCOCCUS AUREUS INFECTED DOGS

During the early stages of infection, the circulating CRP concentrations are reported to increase. The current study demonstrated that plasma CRP concentrations increased in dogs inoculated with S. aureus and that the peak was higher than 200 mg/L. An increase in CRP in dogs experimentally infected with Bordetella bronchiseptica [29] has been reported with peak values culminating at 498 ± 132 mg/L. A marked and significant increase in the plasma CRP concentrations (64.5 ± 42.0 mg/L) was observed as soon as 6 hours after the S. aureus inoculation in the present study and were closely related to those previously reported by LAZAROV et al. [14] in dogs with experimentally induced pancreatitis (75.4 ± 8.09 mg/L). Increases in plasma CRP concentrations were recorded since the 3rd hour in dogs infected with Staphylococcus intermedius [24], with experimentally induced pancreatitis [14] as well as in the present study (59.9 ± 41.4 mg/L, data not shown). However, the first peak in CRP concentrations observed here was at 24th hour (206.6 ± 102.0 mg/L) after inoculation with S. aureus. SLAVOV et al. [24, 25] also observed maximal CRP concentrations 24 hours after inoculation with S. intermedius in dogs but the culminated values were lower (81.83 mg/L) than in the present study. HAYASHI et al. [9] reported that the CRP concentrations ranged from 61.8 to 98.1 mg/L in dogs infected with S. aureus. Elevated CRP concentrations were reported by CHAPRAZOV et al. [4] in mongrel dogs with experimentally induced Pseudomonas aeruginosa infection: at 6 and 24 hours they observed values closely related to the present values (76.65 ± 14.39 and 176.91 ± 5.93, respectively). According to LAZAROV et al. [14], the maximal CRP concentrations (131.7 ± 6.86 mg/L) were also observed 24th hour after pancreas inflammation. In their study, between the 48th and the 96th hours, the CRP concentrations remained statistically significantly elevated and ranged between 112 and 124 mg/L, which matches almost with the present results (CRP concentrations at 48 hours and 72 hours were 126.5 ± 63.0 mg/L and 88.9 ± 27.9 mg/L, respectively).

Local and general systematic signs (fever, accelerated heart and respiratory rates at 24th hours) accompanied infection and are considered as non specific indicators of inflammation. DIMITROVA et al. [6] observed painfulness and oedema of the soft tissue 4 hours after S. aureus inoculation in dogs. In the present study these signs appeared at the 6th hour. Moreover, 24 hours after bacteria inoculation, enlargement of inguinal lymphatic nodes draining the limb in which bacteria were injected was noticed here and in the DIMITROVAs study [6] and scrotum oedema was also found in one dog. Reduced appetite and impaired motor activity in dogs were also observed between the 1st and the 2nd day post infection and on day 2, some dogs exhibited purulent conjunctivitis eye infection. In parallel, local severe signs, namely hair loss and skin ulcerations, were also found. During this time, plasma CRP concentrations remained strongly elevated in the experimental group compared to the initial values and to values recorded in the control group and the highest increases in CRP concentrations were obtained in dogs with severe inflammatory systemic or local clinical signs. Even though, in this experiment, significant associations between CRP concentration and the clinical parameters (body temperature, respiratory and heart rates) were not found but there is a significant and strong positive
correlation (r = 0.84, p < 0.05) between the body temperature and the respiratory rate (Table III). Nevertheless, a second important peak in plasma CRP concentrations were recorded in the experimental group in the present study whereas the clinical inspection revealed the lack of oedema and pain, recovered appetite and that dogs walked normally again and did not exhibit any acute local inflammatory sign.

As a conclusion, these results indicate that plasma CRP may be considered as an early positive acute phase protein and may be used as a helpful indicator for an early diagnostic of the staphylococcal infection slightly prior to evident clinical manifestations in *S. aureus* infected dogs.

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References


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