Plasma protein profiles and fibrinogen concentrations in dogs with experimentally induced \textit{Staphylococcus aureus} infection

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\begin{abstract}

The aim of the present study was to define changes in concentrations of total proteins, albumin (as a negative acute phase protein), globulins and fibrinogen (as a positive acute phase protein) in dogs with experimentally-induced \textit{Staphylococcus aureus} (\textit{S. aureus}) infection. For that, 9 male mongrel adult dogs were subcutaneously inoculated with \textit{S. aureus} suspension (5 mL, 10^9 cfu/mL) while 6 control dogs were injected with saline solution and the plasma concentrations of total proteins, albumin, globulins and fibrinogen as well as the albumin/globulins (A/G) ratios were determined immediately before infection (0 hour), 6 hours and 1, 2, 3, 7, 14 and 21 days after. Whereas biochemical parameters remained stable in the controls, proteinemia slightly and significantly increased on day 7 in inoculated dogs. In parallel, globulin concentrations markedly increased and albuminaemia as well as the A/G ratios dramatically declined compared to the controls from day 3 to day 14 and maximal variations were also observed on day 7. On the other hand, the staphylococcal infection induced gradual and marked increases in fibrinogen concentrations since the 6th hour until the 3rd-7th days (maximal values) and thereafter this marker slowly declined. Strong positive correlations were recorded between proteinemia, globulin and fibrinogen concentrations in one hand, and between albuminaemia and A/G ratios in the other hand, and negative associations were evidenced between the 2 groups of parameters in infected dogs. These results confirm that albumin may be considered as a negative acute phase protein and fibrinogen as a positive acute phase protein and show that the fibrinogen concentrations may be useful for the diagnosis and the follow up of a bacterial infection in dogs.

\textbf{Keywords:} \textit{Staphylococcus aureus}, dog, total protein, albumin, globulins, Albumin/globulins ratio, fibrinogen.

\end{abstract}

\begin{acknowledgments}

SUMMARY

The changes in blood protein concentrations and in the fibrinogen concentrations in dogs with experimentally induced \textit{Staphylococcus aureus} infection.

\textbf{RESUME}

Concentrations plasmatiques des protéines et du fibrinogène chez des chiens expérimentalement infectés par \textit{S. aureus}

L’objectif de cette étude a été de définir les modifications des concentrations plasmatiques des protéines totales, de l’albumine (en tant que protéine négative de la phase aiguë), des globulines et du fibrinogène (en tant que protéine positive de la phase aiguë) chez des chiens expérimentalement infectés par \textit{Staphylococcus aureus} (\textit{S. aureus}). Pour cela, 9 chiens mâles adultes ont subi une injection sous-cutanée d’une suspension de \textit{S. aureus} (5 mL, 10^9 cfu/mL) alors que 6 autres ont reçu une injection de soluté salé et les concentrations plasmatiques des protéines totales, de l’albumine, des globulines, du fibrinogène ainsi que le rapport albumine / globulines ont été déterminés immédiatement avant l’injection (0 heure), 6 heures et 1, 2, 3, 7, 14 et 21 jours après. Alors que les paramètres biochimiques sont restés stables chez les contrôle, la protéinémie a légèrement mais significativement augmenté chez les chiens inoculés le 7ème jour. Parallèlement, les concentrations des globulines ont nettement augmenté tandis que l’albuminémie et le rapport A/G ont considérablement diminué par rapport aux valeurs obtenues chez les témoins du 3ème au 14ème jour et dans tous les cas, les variations maximales ont été observées au 7ème jour. D’autre part, l’infection par le staphylocoque a induit une augmentation progressive et nette des concentrations de fibrinogène dès la 6ème heure jusqu’aux 3ème et 7ème jours (concentrations maximales) puis ce marqueur a lentement décliné. Des corrélations positives et fortes ont été obtenues entre la protéinémie, les concentrations de globulines et celles de fibrinogène d’une part, et entre l’albuminémie et le rapport A/G d’autre part alors que des corrélations négatives ont été mises en évidence entre les 2 groupes de paramètres chez les chiens infectés. Ces résultats confirment que l’albumine peut être considérée comme une protéine négative de la phase aiguë et le fibrinogène comme une protéine positive et montrent que la fibrinogénémie peut être utile au diagnostic et au suivi des infections bactériennes chez le chien.

\textbf{Mots-clés :} \textit{Staphylococcus aureus}, chien, protéines totales, albumine, globulines, rapport albumine / globulines, fibrinogène.

\end{acknowledgments}

Introduction

\textit{Staphylococcus aureus} (\textit{S. aureus}) is a medically important bacterial pathogen that is responsible for a broad and divergent range of human and animal infections. Toxin-mediated diseases caused by \textit{S. aureus} include range from cutaneous infections to infections of wounds, osteomyelitis, endocarditis, bacteraemia with metastatic complications, toxic shock syndrome [19]. The bacterial components and secreted products that affect the pathogenesis of \textit{S. aureus} infections are numerous and include surface-associated adhesins, exoenzymes, exotoxins, and capsular polysaccharide [15, 19]. This infection was chosen because \textit{Staphylococcus aureus} is commonly found in various animal species including dogs, horses, cats and pigeons. It is established, that this is mostly 100% pathogenic cause for pyodermitis and this is the most described skin disease in these species.

The changes in blood protein concentrations and in the respective proportions are resulting from different clinical signs and are related to a number of pathologies as well as
to synthesis of acute phase proteins (APP) [11]. APPs are synthesized during an acute phase response (APR). The source of the response can be due to infection, inflammation, stress, trauma or tissue damage [2, 16]. The APR, which is the major mediator for the hepatocyte secretion of most of the APPs [8]. As one of the multiple effects of these cytokines is the dramatic change in hepatic protein synthesis and especially in fibrinogen production [12], the blood fibrinogen concentrations would be secondary greatly enhanced, creating an amplification pattern of the positive APP synthesis. Several hours after inoculation of S. aureus, the ratio between different proteins produced by liver, changes. Plasma concentration of some proteins increases and these proteins are called positive acute-phase proteins (fibrinogen) whereas plasma concentration of others decreases and these proteins are called negative acute-phase proteins (albumin). The serum optimal concentration of APPs is typically reached within 24 to 48 h after the initiation [8].

Fibrinogen (Fb) is an APP, a β-globulin, present in the plasma of all vertebrates [2], which is also produced in the liver. It is a moderate (2 to 10-fold increase) positive acute phase protein in dogs [13], which is often elevated in inflammatory conditions. The biochemistry laboratory routinely measures “total protein” and “albumin” concentrations, usually in a serum specimen, and reports the “globulin” fraction as the difference between the first results. Albumin (Alb) is synthesized in the liver as all other plasma proteins except immunoglobulins, and is catabolised by all metabolically active tissues. Its molecular weight is 69 kDa and its half-life is 8.2 days in dogs. It is a negative acute phase protein, which concentrations decrease during infection or inflammation processes. Furthermore, it transfers many organic and inorganic compounds. The usual range of plasma Alb concentrations in animals is between 29 and 36 g/L [11]. Globulin (Glb) concentrations in dogs vary from 15 to 35 g/L, according to KANEKO [9] and from 18 to 39 g/L according to HINES [7]. Globulins have molecular weights ranging from 90 000 Daltons to 156 000 Daltons (γ-globulins). The ratio albumin to globulins (A/G ratio) is often considered as more informative than the respective concentrations in various disorders that could not be identified only by total protein determination. The normal A/G ratios in dogs are from 0.7 to 2.00 [9] or from 0.8 to 2.2 [7].

The present study aimed to establish the alterations occurring in blood circulating proteins, i.e. total proteins, albumin (as a negative APP), globulins, the albumin/globulin ratio and fibrinogen (as a positive APP) during experimental Staphylococcus infection, using dogs as model.

Materials and Methods

EXPERIMENTAL ANIMALS AND PROTOCOL DESIGN

The experiment was approved by the Ethic Committee at the Faculty of Veterinary Medicine, Stara Zagora. The experimental animals were provided by the municipality of Stara Zagora. The study was conducted on 15 mongrel male dogs, 2 years old and weighing 12-15 kg, divided into the experimental group (n = 9) and the control group (n = 6). Prior to the experiment the animals were vaccinated with vaccine Nobivac® (Intervet International B.V) and treated per oral against internal parasites with Caniverm® (Bioveta, A. S. Czech Republic, 1 tablet/10 kg B.W) and external parasites with Bolfo® Puder (Bayer, Germany). The 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. The infection was reproduced by subcutaneous inoculation of 5 mL of 24 hours broth culture of S. aureus strain with density of 3.1x10⁹ c.f.u./mL in experimental animals whereas the control dogs were injected with the same volume of saline solution.

BIOCHEMICAL ANALYSES

Blood samples were collected from the puncture of the v. cephalica antebrachii into heparinised tubes before inoculation (hour 0) then 6 hours after and on days 1, 2, 3, 7, 14 and 21 after S. aureus or vehicle injections. Heparinised blood samples were centrifuged (1500g, 10 minutes, at room temperature) within 30 minutes after collection. Plasma was immediately separated and stored at -20°C until analysis.

The total protein (TP) concentrations were determined with commercial kits (Human-GmbH, Germany). Determination of albumin concentrations was based on the bromocresol green assay, after mixing blood plasma (10 μL) with the reactive buffer. After 3 minutes, the absorption was measured at 546 nm and the sample albumin concentration was calculated against a standard (40 g/L). Globulins were determined by subtracting albumin concentration from total protein concentration. The concentration of fibrinogen was estimated by the method of PODMORE by nephelometric determination (in [18]).

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 error of the mean (Mean ± SEM). The statistical significance of parameters was determined in the LSD test at p < 0.05.
Results

The experimental staphylococcal infection in dogs was accompanied with swelling, painfulness and high temperature of the tissues at the site of the injection since the 1st day post-inoculation. At the site of bacteria injection, hair loss and tissue erosions occurred on day 14. Skin abscesses were evidenced at the 7th day in 5 inoculated dogs. A reduced appetite, impaired motor activity and enlargement of the inguinal lymphatic nodes in the limp which was injected was also noted the 1st day post staphylococcal inoculation. Furthermore, fever and purulent conjunctivitis eye infection were also recorded in 9 and 1 infected dogs, respectively.

Changes in the blood protein profiles during the staphylococcal infection are shown in Table I. The total protein, albumin and globulin concentrations as well as the albumin/globulins ratios remained stable according to time in the control dogs. By contrast, the total protein and the globulin concentrations gradually increased in S. aureus inoculated dogs and peaked on day 7: at this date, proteinemia significantly differed between infected and healthy dogs (p < 0.05) while highly significant differences in globulin concentrations between the experimental and control groups (p < 0.01) were found on days 3, 7 and 14. In addition, the mean globulin concentration measured on day 7 in infected dogs was significantly lowered compared to the initial value determined on day 0 (p < 0.05). In parallel, staphylococcal infection caused marked fall in serum albumin concentrations: they were significantly decreased compared to the mean control values on days 3, 7 and 14 (p < 0.05) and significantly lower concentrations compared to the initial value (p < 0.05) were recorded on days 7 and 14. Additionally, the albumin/globulin (A/G) ratio progressively declined in the experimental group, reaching significantly low values on day 7 (0.8) (p < 0.05) and on days 3 and 14 (0.9) compared to the mean basal ratio (1.2). Compared to the control group, this biochemical parameter was markedly diminished in the experimental group on days 3, 7 (p < 0.05) and 14 (p < 0.01).

As shown in Table II, the plasma fibrinogen concentrations were significantly increased since the 6th hour after S. aureus inoculation compared to the control values and remained significantly enhanced until the 21th day (p < 0.05 at the 6th hour, on day 1 and day 21; p < 0.01 on days 2, 3, 7 and 14). Peak fibrinogen concentrations, significantly higher than basal values, were recorded on days 3 (p < 0.05) and 7 (p < 0.01) and thereafter, the APP concentrations gradually declined.

As reported in Table III, strong positive associations were observed between proteinemia and globulin concentrations (r = 0.80, p < 0.05) or fibrinogen concentrations (r = 0.80, p < 0.05) and between albuminaemia and the A/G ratio (r = 0.98, p < 0.05). In addition, fibrinogen and globulin concentrations were highly positively associated (r = 0.86, p < 0.05), whereas albuminaemia was significantly and negatively coupled to the globulin concentrations (r = -0.95, p < 0.05) and to the fibrinogen concentrations (r = -0.76, p < 0.05). The A/G ratios were also negatively coupled to globulin concentrations (r = -0.99, p < 0.05) and to A/G ratios (r = -0.83, p < 0.05).

Discussion

In this study, changes in blood protein profiles and fibrinogen concentrations were observed in dogs in response to Staphylococcus aureus infection. Whereas blood total protein concentrations were slightly affected by bacteria inoculation, the experimental staphylococcal infection has lead to significant increases in globulins on days 3, 7 and 14, as well as to significant decreases in albumin concentrations, considered as a negative APP, in parallel and all dogs responded by markedly and significantly enhanced fibrinogen concentrations. These results matched with those previously observed in dogs infected with Staphylococcus intermedius [5].

In bacterial or viral infections, the blood fibrinogen concentration is elevated. In the present study, it was observed that the experimental infection with Staphylococcus aureus have induced marked increases in the fibrinogen concentrations compared to initial values and to the control values in dogs: the positive APP concentrations were significantly raised in dogs since the 6th hour until the 14th day after bacterial inoculation for reaching maximal value. The increases in circulating fibrinogen concentrations were likely caused by increased rate of synthesis probably stimulated by cytokines, growth factors, hormones, and other cellular effectors [1]. In this way, fibrinogen concentrations were rapidly increased as previously reported [3, 4, 17]. In agreement, plasma fibrinogen concentrations in the present study significantly increased within the first day after staphylococcal infection. Some authors reported that in experimental infection and acute inflammation (acute pancreatitis) in dogs, high circulating fibrinogen concentrations reached maximal values at the 72nd hour and persisted elevated for 8 days [3, 4, 21]. SLAVOV et al. [17] observed in dogs infected with Staphylococcus intermedius, maximal fibrinogen concentrations on day 2 (4.72 ± 0.80 g/L). In the present experiment, maximal concentrations (4.29 ± 0.26 g/L) were recorded on day 3 and then fibrinogen concentrations started to decline.

In healthy dogs for the whole experimental period and in dogs before inoculation, the plasma total protein concentrations were closely related to the upper limits of the usual ranges defined in ambulatory adult dogs, as indicated by MARTINEZ-SUBIELA and CERON [10] (48-66 g/L) and by VALLADARES et al. [20] (54-73 g/L). In the present study, after bacteria inoculation, proteinemia slightly and gradually increased since the first day according to time for peaking on day 7 but remaining in the upper limits [20]. During the whole post inoculation period, proteinemia in inoculated dogs generally appeared higher than in the controls,
although not significantly. DIMITROVA et al. [4] observed slight increases in proteinemia induced by staphylococcal infection in dogs but they have also described a transient decrease during the first day which was not evidenced here. In this way, proteinemia was strongly and positively coupled to globulin and to fibrinogen concentrations in S. aureus inoculated dogs in the present study.

Hypoalbuminaemia is very common in many diseases and results from disorder in liver synthesis, reduced absorption of amino acids or increased catabolism linked to the turn off amino acids for the synthesis of other proteins (like positive acute phase proteins) in liver, or a combination of these factors [5]. The reduction in the plasma concentration of negative APP is probably caused by a preferential synthesis in liver of positive APPs which are important components of systemic defence mechanisms [6], the partial deficiency of α-amino acids induced by increased production of APP being compensating by the strong reduction of the synthesis of the negative APPs, including albumin. The changes in albuminaemia could also be interpreted as a possible disorder in liver synthesis. The decreases in albuminaemia after staphylococcal infection in the present study confirm that albumin may be considered as a negative APP and

For a given biochemical parameter: * (p < 0.05) and ** (p < 0.01) indicate significant differences between S. aureus inoculated and control dogs. Different superscripts a,b indicate significant difference (p < 0.05) according to time within the experimental group (S. aureus inoculated dogs).

**Table I:** Plasma concentrations (g/L) of total protein (TP), albumin (Alb) and globulins (Glb) and albumin/globulin (A/G) ratios in healthy dogs (n = 6) and in dogs with experimental Staphylococcus aureus infection (n = 9) according to time after subcutaneous inoculation. Results are expressed as means ± standard errors of the means (SEM).

<table>
<thead>
<tr>
<th>Time</th>
<th>Control group</th>
<th>Inoculated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour</td>
<td>1.40 ± 0.10</td>
<td>1.90 ± 0.32*</td>
</tr>
<tr>
<td>6 hour</td>
<td>1.42 ± 0.09</td>
<td>2.59 ± 0.30*ab</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.47 ± 0.10</td>
<td>2.87 ± 0.27*ab</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.64 ± 0.08</td>
<td>3.26 ± 0.28*ab</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.33 ± 0.20</td>
<td>4.29 ± 0.26*cc</td>
</tr>
<tr>
<td>Day 7</td>
<td>1.35 ± 0.21</td>
<td>4.17 ± 0.19*cc</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.74 ± 0.19</td>
<td>3.22 ± 0.18*ab</td>
</tr>
<tr>
<td>Day 21</td>
<td>1.64 ± 0.18</td>
<td>2.47 ± 0.27*ab</td>
</tr>
</tbody>
</table>

For a given biochemical parameter: * (p < 0.05) and ** (p < 0.01) indicate significant differences between S. aureus inoculated and control dogs. Different superscripts a,b indicate significant difference (p < 0.05) according to time within the experimental group (S. aureus inoculated dogs).

**Table II:** Plasma fibrinogen concentrations (g/L) in healthy dogs (n = 6) and in dogs with experimental Staphylococcus aureus infection (n = 9) according to time after subcutaneous inoculation. Results are expressed as means ± standard errors of the means (SEM).

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th></th>
<th>Glb</th>
<th></th>
<th></th>
<th>A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alb</td>
<td>r = 0.57</td>
<td>NS</td>
<td>r = 0.80</td>
<td>&lt; 0.05</td>
<td>r = 0.80</td>
<td>r = -0.69 NS</td>
</tr>
<tr>
<td></td>
<td>r = 0.95 NS</td>
<td></td>
<td>r = 0.76</td>
<td>&lt; 0.05</td>
<td>r = 0.98</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>r = 0.86 NS</td>
<td></td>
<td>r = 0.86</td>
<td>&lt; 0.05</td>
<td>r = 0.99</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>r = 0.83 NS</td>
<td>&lt; 0.05</td>
<td></td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

NS: not significant

**Table III:** Correlations between proteinemia (TP), albuminaemia (Alb), globulin (Glb) and fibrinogen (Fib) concentrations, and albumin / globulin (A/G) ratios in dogs experimentally inoculated with Staphylococcus aureus (n = 9). Correlations were calculated from all time points (from 0 h to the 21th day) and significant associations (p < 0.05) were in bold.

albuminaemia was significantly and negatively coupled to globulin and to fibrinogen concentrations. In support of these findings, GEORGIEVA et al. [5] found out decreased albuminaemia in dogs from 35.74 g/L to 32.69 g/L, 3 days after staphylococcal infection whereas this parameter returned to initial values one week after inoculation. In the present study, albuminaemia was significantly decreased in *S. aureus* infected dogs on Day 3 compared to the control ones and remained dramatically lowered until the 14th day after inoculation while DIMITROVA et al. [4] reported low albuminaemia in *S. aureus* infected dogs only until the 8th day. However, ORHUE et al. [14] found a statistically significant fall in serum albumin concentrations 14 days after infection with *Trypanosoma brucei* in rabbits and they attributed it to a decline in liver protein synthesis (impaired synthesis) or to increased protein loss through the gut or the kidney. Plasma proteins (albumin and globulin) in dogs have half lives varying from 8 to 10 days, and therefore a decrease in albuminaemia is usually not apparent early in the infection.

Globulins are divided into three main groups, the α-, β-, and γ-globulins. Globulins are divided into three main groups, the α-, β-, and γ-globulins. Immunoglobulins (antibodies) are produced by the plasma cells as part of the immune system in response to inflammation, which increased in both acute and chronic infection [5]. Likewise, some authors [10] reported strong increase in γ-globulin concentrations in naturally diseased dogs. In the present study, globulin concentrations significantly increased after *S. aureus* inoculation and reached maximal values on day 7. These results were corroborated by those of GEORGIEVA et al. [5] who reported increases in the globulin fractions 7-14 days after *Staphylococcus intermedius* infection in dogs. The rising of globulins in the infected animals may be caused by bacteria induced liver damage, by antibody production towards the bacterial infection or by increased production of positive APPs such as fibrinogen. In the present study, a highly positive correlation between proteinemia and globulin concentrations was found in *S. aureus* inoculated dogs.

The normal albumin / globulins (A/G) ratios in dogs are from 0.7 to 2.00 according to KANEKO [9] or from 0.8 to 2.2 according to HINES [7]. In healthy dogs and in experimental dogs before inoculation the A/G ratios determined in the present study were included in these usual ranges. On days 3, 7 and 14 after inoculation this parameter was markedly lowered, indicating globulin overproduction or albumin reduction or both. Similarly, ZAPRYANOVA et al. [21] observed declined in the A/G ratios 3 days after induction of an acute pancreatitis in dogs. Furthermore, the A/G ratios were strongly and negatively associated to the globulin and fibrinogen concentrations and positively to the albuminaemia.

The present results demonstrate similar patterns in variations of the plasma globulin and fibrinogen concentrations in *S. aureus* inoculated dogs with maximal values on day 3 whereas albuminaemia and the A/G ratios fluctuated simultaneously in an opposite way and reached minimal values on day 7, corroborating that albumin may be considered as a negative APP and fibrinogen as a positive APP. Furthermore, variations in fibrinogen concentrations appeared early in the post-inoculation period (since the 6th hour), persisted until the 21st day and were intense, leading to the conclusion that fibrinogen concentration is a more reliable marker of bacterial infection than changes in albuminaemia or in globulin concentrations.

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