The aim of our study was to evaluate nonspecific defence mechanisms (phagocytosis and acute phase response) in dogs during an experimental infection caused by subcutaneous application of Staphylococcus aureus \((1 \times 10^8 \text{ CFU/mL})\). The neutrophils phagocytosis (evaluated by fluorescein isothiocyanate labeled Staphylococcus aureus) is activated at 48 h and the phagocytic number remained high at the 72\textsuperscript{nd} h of infection. During the early stage of the infection process (between the 2\textsuperscript{nd} and 24\textsuperscript{th} h) the absolute segmented neutrophil count, the absolute band count, band-neutrophil ratio, tend to have high sensitivity, whereas an abnormal leukocyte count is registered at 48 h.

Fibrinogen increase is registered the earliest (at 24\textsuperscript{th} h). Its concentrations remain high till the end of the study. The changes in the blood protein profiles show that the \(\alpha_2\)-globulin fraction rises at the 48\textsuperscript{th} and 72\textsuperscript{nd} h. Albumin was significantly lower at 72 h compared to the baseline values. There are no statistically significant changes in the total protein, A/G ratio and sialic acid, which we determined.

The obtained data about the red blood cell parameters shows that Staphylococcus aureus induces a moderate anaemia when applied in dogs. Deviations are also registered in the mean corpuscular hemoglobin, while the other red blood cell indices – mean corpuscular hemoglobin concentration and mean corpuscular volume, are within normal reference ranges.

We conclude that it is always better to combine immunological, hematological and biochemical indices. The key is to choose tests that
are frequently used and to ensure more rapid information about the infectious process.

_key words:_ nonspecific defense mechanisms, dogs, infection, _Staphylococcus aureus_

**Introduction / Uvod**

The frequency of Gram-positive infections in dogs and cats has increased during the last two decades (Dow et al., 1989; Martin et al., 2003). The diseases that _Staphylococcus aureus_ causes, vary from small furuncles to sepsis (Scott, 2001). This facultative bacteria can provoke the host defence by different mechanisms. It has been shown to release exotoxins that act as superantigens (Alouf and Muller-Alouf, 2003). The key elements of bacteria are peptidoglycan (PGN), lipoteichoic acid (LTA) (De Kimpe et al., 1995; Ruhland and Fiedler, 1990). The wide spectrum of structural and secretory components, by which the staphylococi comes into complex relationships with the macroorganism (Allaker et al., 1991; Jarraud et al., 2001; Moulding et al., 1999), their high variability and the rapidly developing antibiotic resistance (Bergoge-Berezin, 2000; Toshkova, 1994; Urumova, 2004) are only part of the reasons troubling the struggle against these agents.

Monshouwer (1996) indicates, that acute–phase response of the organism can exert a significant influence over the absorption, distribution, metabolism and the excretion of the drugs applied at this early stage of infection, as well as increase or diminish their therapeutic effect. The acute-phase reactants are a separate group of proteins that increase in serum rapidly (within 12-48 h) (Bauermann and Gauldie, 1994; Gabay and Kushner, 1990; Petersen et al., 2004). Raised serum levels are the result of increased hepatic synthesis mediated by cytokines – mainly tumor necrosis factor - \(\alpha\) (TNF-\(\alpha\)), interleukin-1 (IL-1), IL-6 (Medzhitov and Janeway, 2000; Pannen and Robotham, 1995; Van Miert, 1991; Van Miert, 1995).

As a result of the total changes in blood protein profiles, associated with the infection, the plasma viscosity and erythrocyte sedimentation rate (ESR) increase (Gruys et al., 1994; Suffredini, 1999). TNF-\(\alpha\), IL- are released by the activated cell elements in the blood and tissues, the most important of which are the professional phagocytes – polymorphonuclear neutrophilic, eosinophilic granulocytes (microphages) and mononuclear phagocytes (macrophages) (Celada and Nathan, 1994; Krause, 2000). The total microphage cell count is approximately 2.5 \(\times\) \(10^{12}\). Only 5% of these cells are located in the blood (Kayser et al., 2005). Through the blood, however, is realized the transportation and recirculation of the cell elements and their products. It contains the humoral factors of innate immunity – lysozyme, complement (alternative activation pathway), serum proteins (Leitch and Willcox, 1990). Due to this, the analysis of the cell and humoral can
give valuable data for the status of the mobilization of the early mechanisms of defence (Paape et al., 1985; Pagana and Pagana, 1988) that ensure the control of the infection until the induction of adaptive immunity. The use of multiple markers, in particular, combining early sensitive markers with late specific tests will further enhance the diagnostic accuracy in identifying infected cases (Philip and Hewitt, 1980; Rodwell et al., 1993) and may be used for early termination of antibiotic treatment. It is expedient when choosing a given drug for the treatment of a staphylococcal infection to have in mind the occurring changes in the non specific defense mechanisms during the early stage of infection.

The present study had the objectives to examine in dynamics (till the seventy second hour) the neutrophil phagocytosis (by fluorescein isothiocyanate – FITC labeled Staphylococcus aureus), hematological indices, electrophoretic profiles associated with the experimental staphylococcal infection in dogs.

Material and methods / Materijal i metode rada

Experimental animals / Eksperimentalne životinje
The animals used were 9 clinically healthy mixed-breed male dogs at the age of 2-5 years, weighing 14.3±1.8 kg b.w. The animals were bred at a controlled temperature of 21°C, 60 % humidity, and a 12/12 light regimen in the Hospital for Small Animals, Faculty of Veterinary Medicine, Trakia University. Twice daily, the animals were allowed a 30 minute walk. The dogs were fed twice a day with a granulated food (“Jambo dog”, Gallisman S.A., Bulgaria), and the water supply was ad libitum.

Thirty days prior to their inclusion in the experiment the dogs were de-wormed with the combination of praziquantel and abamectin (Prazimec-D, Biovet JSC, Peshtera, Bulgaria) at a dose of 1 tablet for 10 kg b.w. Ectoparasites were treated with a combination of permetrin and carbaril (Tapilan-B, Dorvet, Israel).

Experimental design / Plan eksperimenta
The experimental infection was provoked by a subcutaneous injection (s. c.) in the right hind limb of 5 mL, 24-hour broth culture of Staphylococcus aureus – field strain, with a concentration of 1x10⁸ CFU/mL. The identification of the strain was made with a semi-automatic system for identification BD BBL Crystal Gram Positive ID System (Becton Dickinson Diagnostic).

The strain is catalase positive, oxidase negative, plasmocoagulase and desoxyribonuclease producing.

The following was registered for every dog: behavior, appetite, color of conjunctivas, skin integrity, presence of effusions. The possible presence of edema, warmness and painfulness were registered visually and by palpation.
Collection of blood / Sakupljanje krvi

Blood samples were collected immediately prior to the experimental infection with *Staphylococcus aureus* (baseline = 0 h) and at 2, 4, 24, 48 and 72 h after infection, from v. cephalica anterior, between 7.30-8.00 am for the elimination of circadian influence. Blood was collected into vacutainers.

A heparinized (20 IU) sample (1 mL) was used in the phagocytosis assay. It was stored in ice and used within an hour from collection time.

To obtain serum, the blood samples were kept at room temperature at 1 h to allow to clot. They were then centrifuged (1000 x g, 10 min, 20°C).

Red blood cell parameters / Parametri crvenih krvnih zrnaca

Red Blood Cells (RBC) were determined in a Bürker chamber ([Transmedimpex], Vienna, Austria). Hemoglobin (Hb) content was determined with the use of an acid-base analyser ([ABL-3, Radiometer], Denmark). Packed cell volume (PCV) was determined by the microhaematocrit method. The following red blood cell indices were registered: mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV). The erythrocyte sedimentation rate (ESR) was determined by the micromethod of Panchenko (mm/h).

Cellular factors and mechanisms of innate immunity / Celjski faktori i mehanizmi prirodnog imuniteta

White Blood Cell (WBC) count was determined in a Bürker chamber ([Transmedimpex], Vienna, Austria). Differential white blood cell counts were performed on blood smears stained with May-Grünwald and Giemsa solutions by counting 200 leukocytes. As indicators of early infection the following haematological indices were applied: absolute neutrophil count (ANC); absolute band count (ABC); band neutrophil ratio (BNR – was defined as the fraction of immature to total neutrophil forms); immature to mature neutrophil ratio (I/M) were determined. All bands and cell forms less mature than bands were classified together as immature neutrophils. Segmented (mature) neutrophils were defined as having a filamentous bridge between nuclear lobes.

Neutrophil phagocytosis was determined in peripheral blood (Samnaliev et al., 1995) via fluorescein-isothiocyanate (FITC, Sigma, St. Louis, MO) conjugated staphylococci (strain 209) (1x10⁷ CFU/mL), fresh autologous serum.

Fluorescent labeling of bacteria / Fluorescentno obeležavanje bakterija

The culture of *Staphylococcus aureus* (strain 209) was washed once in saline, resuspended in PBS and adjusted to a final concentration of 10⁹ CFU/mL. A volume of 1 mL of the bacterial suspension in 0.1 M Na₂CO₃ buffer - pH 9.6 and 0.06 % FITC solution were mixed by rotation (100 r.p.m.) for 60 min at room temperature, protected from light. After staining, FITC-labeled bacteria were washed twice in PBS and the concentration of live bacteria was adjusted to 3 x 10⁷/mL.
In the phagocytosis assays the neutrophils were stained with ethidium bromide (EB-Sigma – 0.5 μg/mL working solution). Quenching solution was used to extinguish any extracellular fluorescence. The phagocytic neutrophils were expressed from 100 neutrophils as the proportion of cell able to phagocytize more than three bacteria. The phagocytic number was calculated as the ratio of the number of phagocytized staphylococci and the number of phagocytizing cells.

**Humoral factors of innate defence – proteins**

Fibrinogen was determined with a commercial kit (HemoStat Fibrinogen Test Set, Human GmbH, Germany). Biuret's reaction was used for the total protein. Gel electrophoreses was done using a standard method for the determination of albumin, alpha-1 globulin (α₁), alpha-2 globulin (α₂), beta-globulin (β) and gamma (γ) globulin. Protein fractions were registered by densitometer.

The level of serum sialic acid was determined by spectrophotometry (Sydow, 1985).

**Statistical analysis**

The obtained data was processed statistically with a computer program (StatMost, v. 2.5, DataMost Co., USA). The t-test by Student was used, the ANOVA-test was also applied, followed by a post-hoc Tukey’s HSD test. The data were presented as mean ± SD. Differences were considered statistically significant at the p<0.05 level.

**Results**

The experimental *Staphylococcus aureus* infection causes changes in one of the early non-specific defense mechanisms – phagocytosis. The neutrophil phagocytic indices (% neutrophil phagocytosis, phagocytic number) at the 48-th h from the application of the pathogen reach their maximal values – respectively: 30.33±5.02 % and 2.70±1.01 compared to the initial ones – 26.22±3.11 % and 1.70±0.49 (Figure 2). The registered raise of the phagocyte number during this period is statistically significant compared to the initial value (p<0.001), and compared to the value at the 24-th h, when it is 1.93±0.31. This parameter at the 72-nd h of the staphylococcal infection remains at its high level – 2.26±0.36.

The used haematological indices for the evaluation of the changes in the white blood cells of the dogs with experimental staphylococcal infection are shown in Table 1. The WBC is one of the fast reacting and widely used laboratory parameters. Its values decreased as early as the 2-nd hour after the staphylococcal infection to 6.63±1.44.10⁹/L vs. 9.14±2.10.10⁹/L. Although it is statistically significant (p<0.05) this decrease is within the standard for this biological species. Leukocytes subsequently increased up to 15.42±2.73.10⁹/L by hour 48 (p<0.001) of the experimental infection. The value remained high at the 72-nd h as
The changes in the absolute neutrophil count (ANC) are similar to those in WBC. The registered decrease at the second hour is not statistically significant. It is followed by a rapid increase in the values of the index, which at the 48-th h reaches its maximum $10.0 \pm 2.38 \times 10^9/L$ ($P<0.001$) vs. at baseline $5.33 \pm 1.26 \times 10^9/L$, vs. 2 h - $4.26 \pm 1.38 \times 10^9/L$ ($p<0.001$). Neutrophil activation is reflected in the peripheral blood in a rise of the absolute neutrophil count and left shift of neutrophils (increased immature – band to total neutrophil ratio). The statistically significant changes in ABC and the absolute count of the segmented neutrophils occurs already at the 2-4-th h from the infection (Table 1), whereas I/M reaches high levels in the period between the 4-th and – 48-th h.

Figure 1. Dog with erosions on tissues and hair loss in region of inoculation of broth culture of Staphylococcus aureus
Slika 1. Pas sa erozijama na tkivu i gubitkom dlake u predelu mesta inokulacije bujon kulturom Staphylococcus aureus

Figure 2. Phagocytic indices in dogs with experimental infection induced by Staphylococcus aureus
Slika 2. Indeksi fagocita kod pasa sa eksperimentalnom infekcijom izazvanom bakterijom Staphylococcus aureus
Table 1. Haematological tests in dogs with experimental infection induced by Staphylococcus aureus - field strain (1x10^8 CFU/mL), (mean ± SD) / Tabela 1. Hematolo{ki testovi na psima sa eksperimentalnom infekcijom izarvanom terenskim sojom Staphylococcus aureus (1x10^8CFU/mL) (srednja±SD)

<table>
<thead>
<tr>
<th>Dynamics (hour) / Tempo (sati)</th>
<th>n</th>
<th>WBC (10^9/L)</th>
<th>ANC (10^9/L)</th>
<th>ABC (10^9/L)</th>
<th>BNR</th>
<th>Neu-segment (10^9/L)</th>
<th>I/M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>9.14 (2.10)</td>
<td>5.33 (1.26)</td>
<td>0.31 (0.25)</td>
<td>0.05</td>
<td>2.90 (0.73)</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>6.63 (1.44)</td>
<td>4.26 (1.38)</td>
<td>0.30 (0.17)</td>
<td>0.07</td>
<td>1.94 (0.54)</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>8.83 (2.55)</td>
<td>7.12 (2.36)</td>
<td>1.20 (0.79)</td>
<td>0.17</td>
<td>1.26 (0.68)</td>
<td>0.95</td>
</tr>
<tr>
<td>24</td>
<td>9</td>
<td>11.60 (2.96)</td>
<td>9.36 (2.90)</td>
<td>1.50 (0.64)</td>
<td>0.16</td>
<td>1.85 (0.45)</td>
<td>0.81</td>
</tr>
<tr>
<td>48</td>
<td>9</td>
<td>15.42 (2.73)</td>
<td>10.0 (2.38)</td>
<td>1.88 (0.97)</td>
<td>0.19</td>
<td>2.53 (0.89)</td>
<td>0.74</td>
</tr>
<tr>
<td>72</td>
<td>9</td>
<td>11.77 (2.21)</td>
<td>8.05 (2.14)</td>
<td>1.21 (0.69)</td>
<td>0.15</td>
<td>2.52 (0.83)</td>
<td>0.48</td>
</tr>
<tr>
<td>Reference ranges / Referentni rasponi</td>
<td>#; *</td>
<td>3.5-12</td>
<td>#; *</td>
<td>0-0.30</td>
<td>#; *</td>
<td>3-11.5</td>
<td>0.12-0.30</td>
</tr>
</tbody>
</table>

WBC – white blood cell count; ANC – absolute neutrophil count; ABC – absolute band count; BNR – band-neutrophil ratio; Neu-segment – mature neutrophil; I/M – immature to mature (segment) neutrophil ratio / WBC – bela krvna zrnca; ANC – apsolutni broj neutrofila; ABC – apsolutni broj raspona; BNR – odnos raspon-neutrofil; Neu-segment – zreli neutrofil; I/M – odnos nezreli do zreli (segment) neutrofila

# – Comazzi, S. et al., 2004; * – Jain, M.C., 1986

a (p<0.05); a1 (p<0.01); a2 (p<0.001) Significantly different from values at 0 h; b (p<0.05); b1 (p<0.01); b2 (p<0.001) Significantly different from values at 2 h; c (p<0.05); c1 (p<0.01); c2 (p<0.001) Significantly different from values at 4 h; d (p<0.05); d1 (p<0.01); d2 (p<0.001) Significantly different from values at 24 h; e (p<0.05); e1 (p<0.01); e2 (p<0.001) Significantly different from values at 48 h; f (p<0.05); f1 (p<0.01); f2 (p<0.001) Significantly different from values at 72 h / a (p<0.05); a1 (p<0.01); a2 (p<0.001) Značajno različite od vrednosti kod 0h; b (p<0.05); b1 (p<0.01); b2 (p<0.001) Značajno različite od vrednosti kod 2h; c (p<0.05); c1 (p<0.01); c2 (p<0.001) Značajno različite od vrednosti kod 4h; d (p<0.05); d1 (p<0.01); d2 (p<0.001) Značajno različite od vrednosti kod 24h; e (p<0.05); e1 (p<0.01); e2 (p<0.001) Značajno različite od vrednosti kod 48h; f (p<0.05); f1 (p<0.01); f2 (p<0.001) Značajno različite od vrednosti kod 72h

In Table 2 are presented the changes registered till the 72nd h in hemoglobin, packed cell volume, erythrocytes and ed blood cell indices, in dogs with experimental staphylococcal infection. Hemoglobin and erythrocytes during the whole monitored period vary in the characteristic for this biological species,
bordering on the reference (statistical norm) (120-180 g/L for Hb and 5.5-8.5 \(10^{12}/L\) for RBC). The PCV, however, at the seventy second hour is 0.28±0.05 L/L, which is statistically significantly lower than the baseline value 0.33±0.02 L/L \((p<0.05)\); also from the value at the second hour – 0.35±0.07 L/L \((p<0.01)\) and from that typical for the dog species variations of this parameter (Comazzi et al., 2004; Jain, 1986). From the red blood cell indices only MCH at the 72\(^{nd}\) h from the development of the experimental infection shows 31.3±8.67 pg, which is statistically significantly higher than the baseline – 23.4±4.99 pg \((p<0.05)\). The ESR tended to be enhanced at the 48\(^{th}\) and the 72\(^{nd}\) h from the experimental infection when it runs up to 13 and 15 mm/h compared to 4±2.2 mm/h at the baseline \((p<0.05)\).

### Table 2. Hemoglobin, packed cell volume, red blood cell and red blood cell indices in dogs with experimental infection induced by Staphylococcus aureus - field strain \((1\times10^8 \text{CFU/mL})\) \((\text{mean} \pm \text{SD})\)

<table>
<thead>
<tr>
<th>Dynamics (hour) / Tempo (sati)</th>
<th>Hb (G/L)</th>
<th>PCV (L/L)</th>
<th>RBC ((10^{12}/L))</th>
<th>Redblood cell indices / Indeksi crvenih krvnih zrnaca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MCH (pg)</td>
</tr>
<tr>
<td>0</td>
<td>132 (23)</td>
<td>0.33 (0.02)</td>
<td>5.73 (0.38)</td>
<td>23.4 (4.99)</td>
</tr>
<tr>
<td>2</td>
<td>134 (18)</td>
<td>0.35 (0.07)</td>
<td>5.63 (0.55)</td>
<td>27.6 (5.30)</td>
</tr>
<tr>
<td>4</td>
<td>132 (24)</td>
<td>0.30 (0.04)</td>
<td>5.69 (0.56)</td>
<td>26.2 (7.12)</td>
</tr>
<tr>
<td>24</td>
<td>133 (26)</td>
<td>0.32 (0.01)</td>
<td>5.90 (0.19)</td>
<td>25.1 (4.97)</td>
</tr>
<tr>
<td>48</td>
<td>126 (18)</td>
<td>0.29 (0.05)</td>
<td>5.60 (0.50)</td>
<td>22.7 (3.40)</td>
</tr>
<tr>
<td>72</td>
<td>122 (15)</td>
<td>0.28 (0.05)</td>
<td>5.61 (0.80)</td>
<td>31.3 (8.67)</td>
</tr>
</tbody>
</table>

**Reference ranges / Referentni rasponi**

| Hb – hemoglobin; PCV – packed cell volume; RBC – red blood cell; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; MCV – mean corpuscular volume; ESR – erythrocyte sedimentation rate / Hb – hemoglobin; PCV – hematokrit, odnosno volumen čelija pune krvi; RBC – crvena krvna zrnca; MCH – srednji korpuskularni hemoglobin; MCHC – srednja koncentracija korpuskularnog hemoglobina; MCV – srednji volumen korpuskula; ESR – stepen sedimentacije eritrocita |

\# – Comazzi, S. et al., 2004; * – Jain, M.C., 1986; \* – Pannen, B. & J. Robothan, 1995

32
The possible role of some acute phase proteins (APPs) in pathogenesis and diagnosis of staphylococcal infection has been investigated. The electrophoretic profiles, concentration of fibrinogen, sialic acid and A/G ratio associated with the experimental infection are presented in Table 3. In our experimental
model, fibrinogen is the parameter for which is registered the earliest (at the 24th h) statistically significant rise (p<0.001). The fibrinogen concentration remains high till the end of the study. It is followed by a statistically significant rise of the $\alpha_2$-globulins at the 48th and the 72nd h (Table 3). At the 72nd h from the application of Staphylococcus aureus, the dogs show a fall in albumin (p<0.05) compared to the baseline values. There are no statistically significant changes in the sialic acid and the A/G ratio, which we determined.

The experimental staphylococcal infection in dogs is accompanied by painfulness, edematisation and high temperature of the tissues at the site of injection, together with disturbed motor activity followed by an increase of the inguinal lymphatic nodes of the injected limb at the 24th h. Subsequently, hair loss was observed in the region of inoculation of the broth culture, in an area with a diameter of 10-20 cm, and the appearance of crater-like erosions of the tissues 3-5 cm in diameter (Figure 1). The motor activity of the animals was disturbed. Increased thirst and a lack of appetite were also observed.

Discussion / Diskusija

Staphylococcus aureus is a part of the resident microflora of the skin in the dog, but in some cases it multiplies rapidly, colonizes large areas of the epidermis and shows pathogen properties (Scott, 2001). The staphylococcal pyoderma is the most commonly diagnosed skin disease for this biological species. Almost 80% of dogs with allergic and skin symptoms develop a secondary bacterial infection. The afore mentioned facts motivated us to apply this experimental model, involved with a reproduction of bacterial infection in dogs by a subcutaneous application of Staphylococcus aureus. Its possesses a wide spectrum of structural and secretory components, by which it provokes the defense mechanisms of the organism (Onogawa, 2000; Somerville et al., 2003). The phagocytes, which provide the first defence line, when activated release pro-inflammatory mediators (Heumann et al., 1994; Mattsson et al., 1993; Standiford et al., 1994). The large amount of neutrophils and their high phagocytic activity determines them as a dominant factor for elimination of the pathogens that penetrate into the organism (Edwards, 1994). Shearer and Day (Shearer and Day, 1997) prove that the staphylococcal antigen alone, or in a complex with antibodies, is capable of stimulating an oxidative burst in the neutrophils. Based on these in vitro observations, we tested the in vivo neutrophil phagocytosis in dogs with experimental staphylococcal infection. The high values of the phagocytic number at the 48th h (p<0.001) can be treated as proof that when applied on dogs Staphylococcus aureus intensifies the process of internalization (engulfing) of bacteria from the activated phagocytes. Besides this, the use of peripheral blood and fresh autologous serum in the fluorescent assay by which we prove the neutrophil phagocytosis, al-
lows us to give an account of the effects over this process by the other cell and humoral elements. It is known that phagocytosis is a complicated nonspecific defense mechanism the effectiveness of which depends on the influence of a number of factors – complement, lysozyme (Leitch and Willcox, 1999), immunoglobulins (Mazza et al., 1993) adhesion molecules (Serrander et al., 1999; Verdrengh et al., 2000), metabolism (Lechkowitz et al., 2000; Sartorelli et al., 1999) hormonal profile (DeBowes and Anderson, 1991; Van der Goes et al., 2000).

Our studies show that the changes in the phagocytic indices are preceded by changes that occur in other important humoral factors – the serum proteins. The fibrinogen concentration even at the 24-th h reaches a level that is significantly higher compared not only to the baseline (p<0.001), but also to the one at the second h (p<0.001) and the forth h. The values of the fibrinogen remain high till the end of the study – the 72nd h. Therefore this parameter can be considered as a sensitive indicator for a developing infection. The fibrinogen is “positive” acute phase proteins (APPs) and is closely connected to the neutrophil activation, migration and adhesion. These effects are a direct consequence of its possibility to connect specifically with α-subunits of CD11b/CD18 over the surface of the neutrophils (Herrick et al, 1999; Kuhns et al., 2001; Smiley et al., 2001). It is interesting that in the present study the peak concentration of fibrinogen was attained around the registered rise of the α2-globulins at the 48-th h and the 72nd h (Table 3). This electrophoretic change is a result of a rise in the levels of APPs, because many of them migrate to the α1- and α2-globulin zone. The structure of the latter includes a number of acute phase proteins – ceruloplasmin, heptaglobulin α2-macro-globulin, and some components of the complement system – C3, C4. The increased synthesis of “positive” APPs is associated with diminished synthesis of “negative” APPs, such as albumin (Table 3). At the 72nd h from the application of Staphylococcus aureus, the dogs showed a lowering of this parameter, compared to the baseline values (p<0.05). The liver acute phase reactant proteins together with (γ) globulin fraction, including various immunoglobulin classes are best visualised by electrophoresis.

APPs and phagocytosis assays are nonspecific, but they are actually more stable and are strong homeostatic regulatory mechanisms. They can be more closely related to disease progression or recovery.

In experimental dogs, the raised ESR (48 h, 72 h – Table 2) indicate a presence of infection. It could be interpreted with regard to the changes in fibrinogen, which is a humoral factor influencing a function of groups of the sialic acid in the cell membrane of the erythrocytes.

During the early stage (<72 h) of the development of staphylococcal infection WBC is the more sensitively changing hematological parameter compared to RBC. From the used indices the absolute segmented neutrophil count shows a decrease at the 2nd h (p<0.01) (Table 1), followed by changes in ABC – registered at the 4th h, and by similar ones in WBC and ANC, but registered in the period between the 24-th and the 72nd h. The total neutrophil count and ratio of
band to total neutrophil is useful in more than 80% of the cases in the diagnosis (Kalayci et al., 2000; Kupperman et al., 1999). In general, the absolute segmented neutrophil count, and ABC, tends to have high sensitivity, whereas abnormal leucocyte count – the leukocytosis is at the 48-th h. The ratio of band to total neutrophil has been shown to be a good hematological marker in the early diagnosis of neonatal sepsis (Manroe et al., 1979; Rodwell et al., 1993).

The determination of PCV is a fast and comparatively precise and accessible method, which applied in our experimental model, shows a lowering at the seventy second hour from the injection of Staphylococcus aureus, that runs without changes in the erythrocyte and hemoglobin (Table 2). The decrease of the inspected parameter below the values of the referent interval is an indication for the presence of anemia. Proof of this were the changes in another red blood cell index – MCH. However, further testing is needed to confirm a diagnosis. This is so because there is a change only in MCH, and not in MCHC. Pagana and Pagana (1988) state a reference for these red blood cell indices in dogs – MCV = 60-77 fl; MCHC=31-37g/dL; MCH=26-34 pg. The mechanism(s), that provoked the development of anaemia during a staphylococcal infection needs more investigation. The origin of the latter could be from one side a result of direct influence over the erythropoesis from a wide spectrum of proteins which the bacterial cell wall of Staphylococcus aureus possesses or the bacterial cell itself synthesizes (Bohash et al., 1990; De Kimpe et al., 1995; Jarraud et al., 2001). On the other hand, this could be due to an indirect effect achieved from the biologically active agents – cytokines released from the activated cell elements (Cleveland et al., 1996; Danforth et al., 1995), or from acute phase proteins synthesized under their influence such as hepsidin (Nancy, 2004; Thomas, 2006). The hematology results of Comazzi et al. (2004) indicate that anaemia is moderately frequent in dogs. These results confirm a higher sensitivity of PCV than hemoglobin in detecting anaemia. The different percentages of anaemic dogs identified using PCV rather than hemoglobin has already been reported (Tvedten, 1994).

In our experiment the changes could be considered as milder compared to those of the same parameters in severe sepsis and with connection to the developing multiple organ dysfunction syndrome (Aird, 2003; Chapazov, 2006).

**Conclusion / Zaključak**

The results of this study indicated that Staphylococcus aureus applied subcutaneously to dogs strengthens the process of neutrophil phagocytosis. During the early stage (second – twenty fourth hour) of the development of staphylococcal infection WBC is the more sensitively changing hematological parameter compared to RBC. In general, the absolute segmented neutrophil count, ABC, tend to have high sensitivity, contrary to abnormal leukocyte counts.

The red blood cell indices (MCV; MCHC; MCH) and PCV are the more sensitively changing indicators for a staphylococcal infection compared to the...
erythrocyte and the hemoglobin. “Blood tests” should never be viewed as a substitute for a thorough clinical examination and case history-gathering; the results should be carefully assessed in the light of the patient status and taking into account all other information. In general, it is always better to combine immunological, hematological and biochemical indices. The key is to choose tests that are frequently needed and to ensure more rapid information on the infectious process.

### References / Literatura


NESPECFICHESKIE OBORONITEL'NYE MЕKHANIZMЫ U SOBAK V TECHENIE ÈXPERIMENITAL'NOY INFЭKCIИ СТАФИЛОКОККАМИ

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Цель нашего исследования была, совершен нами оценку неспецифических оборонительных механизмов (фагоцитоз и ответ в острой фазе) у собак в течение экспериментальной инфекции субкutanной аппликацией Staphylococcus aureus (1×10^8 CFU/mL). Неутрофильный фагоцитоз (путём Staphylococcus aureus маркированным флуоресцентным изоцианатом) активируется у 48 ч и число фагоцитов осталось высокое у 72 ч инфекции. В течение ранней фазы процесса инфекции (между 2 ч и 24 ч) абсолютное число сегментированных неутрофилов, абсолютное число лент, отношение лента-неутрофил имеют тенденцию к высокой чувствительности, пока ненормальное число лейкоцитов зарегистрировано на 48 ч.

Рост фибриногена зарегистрирован раньше всего (у 24 ч). Его концентрация остаётся высокой до самого конца исследования. Изменения в профилях кровных протеинов показывают, что фракция ñ2-глобулина увеличивается на 48 ч и 72 ч. Альбумин был значительно ниже на 72 ч в сравнении с базисными стоимостями. Нами не установлены статистически значительные изменения в совокупных протеинах, отношению А/Г и слюнной кислоты.

Полученные данные о параметрах красных кровяных телец показывают, что Staphylococcus aureus применён у собак вызывает умеренную анемию. У среднего корпукулярного гемоглобина зарегистрированы девиации, пока другие индексы красных кровяных телец - средня концентрация корпукулярного гемоглобина и срений объём корпукул - находятся внутри нормальных референтных расстояний.

Нами сделан вывод, что всегда лучше комбинировать иммунологические и биохимические индексы. Ключевое отобрать тесты, часто полезные и обеспечить более быстрые информации о процессе инфекции.

Ключевые слова: неспецифические оборонительные механизмы, собаки, инфекция Staphylococcus aureus