Original article

HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS CHARACTERISING THE PROGRESSION OF EXPERIMENTAL PSEUDOMONAS AERUGINOSA SKIN INFECTION IN DOGS

M. ANDONOVA1, V. URUMOVA2, B. PETKOVA3, E. SLAVOV1, P. DZHELEBOV1, TS. CHAPRAZOV4, R. ROYDEV & I. BORISSOV4

1Department of General and Clinical Pathology, 2Department of Veterinary Microbiology, Infectious and Parasitic Diseases; 3Student, FVM, 4Department of Veterinary Surgery, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria

Summary


The aim of the study was to investigate the changes in some rapid, indicative clinical laboratory parameters – white blood cells (WBC), leukogram, erythrocyte sedimentation rate (ESR), total protein (TP), albumin (A), globulins (G), albumin/globulin ratio (A/G) during experimental Pseudomonas aeruginosa skin infection in dogs and to determine their ability to provide information for evaluating such type of infection. Suspension of P. aeruginosa (1×10^8 cfu/mL) was inoculated at a dose 0.3 mL/kg body weight, in five clinically healthy, dogs, 2–5 years old, weighing 24.3 ± 1.8 kg. The control group (n=5) was injected with the same dose of normal saline. The blood samples were taken in the following dynamics: before infection (0 h) and on 4th, 24th, 48th, 72nd hour and on 7th, 10th and 14th day after infection. The data presented clearly suggest that ESR was the most sensitive haematological parameter increasing significantly 4 hours after infection (P<0.01), with values remaining high (P<0.001) till the end of experimental period (day 14). WBC increased significantly on hour 72 (P<0.001). The changes in the leukogram demonstrated increase in band neutrophils on hour 48 and 72 (P<0.01), which is indicative for left shift. Eosinopenia was found on hour 24 and 72 (P<0.01).

Analysis of biochemical parameters demonstrated that the period within 24th to 72nd hour was crucial in progress of P. aeruginosa skin infection. This statement is supported by the significant decrease of albumin concentration (P<0.001), decrease in A/G ratio and hyperglobulinaemia (P<0.001) within this period. These alterations in protein profile did not affect total protein concentration, which remained unchanged during the whole experimental period. The matched analysis of both haematological and biochemical parameters is more accurate and indicative for the progression of bacterial skin infections in dogs. Albumin and globulins concentrations and A/G ratio are sensitive, consistent and reliable parameters, which can be useful for evaluating skin P. aeruginosa infection in dogs.

Key words: albumin, dog, globulin, haematological parameters, P. aeruginosa, skin infection
INTRODUCTION

About half of the canine skin infections are linked to resident flora (Paterson, 1998; Silvestre & Betlloch, 1999). *Pseudomonas aeruginosa* is a Gram-negative bacteria, ubiquitously distributed in soil, water and hospital environment (Botzenhart & Ruden, 1987). The genome of the bacteria consisting of many genes, which control nutrient transport, metabolism, biofilm forming and antibiotic resistance (van Delden & Iglewski, 1998; Lambert, 2002; Williams & Camara, 2009), gives it a phenomenal capability for adapting to changes it temperature, humidity, pH and resistance to disinfectants. The lack of sweat glands, coat, hydration and pH of the canine skin – 7.4 (8.62-6.84) (Meyer & Neurand, 1991), account for the presence of pseudomonads. Impairment of skin structures by ectoparasites, scarification, wounds, irritation, inflammation etc., opens a “gate” for bacterial invasion and colonisation of *P. aeruginosa*. It has a remarkable arsenal of virulent factors, some of which are acquired in the course of infection (Gallagher & Manoil, 2001). Effective function of innate defense mechanisms (surface and chemical barriers; cell and humoral factors; non-specific defense reactions – phagocytosis, inflammation, acute phase response) is a key factor for preventing *Pseudomonas* infection. These defense mechanisms provide control over infection until induction of adaptive immune response (Meglio et al., 2011). Limiting the infection is difficult for the host, as *P. aeruginosa* is able to induce collapse in defense mechanisms (Andonova & Urumova, 2013). Antibiotic therapy is problematic, because of the high bacterial variability, biofilm forming and swift building of drug resistance. This requires a rapid and precise evaluation of host health status during the infectious process.

Routine health status evaluation includes blood testing – erythrocyte count, leukocyte total and differential count, platelet count and ESR (Allen et al., 2002; Weyrich & Zimmerman, 2004, Yonekawa & Harlan, 2005). These parameters are not always a reliable diagnostic source, as their values change not only during infections, but are also influenced by endocrine disorders, dietary and environmental factors. In some cases ESR alterations may last for a longer period of time in spite of the applied effective therapy. Recently some haematological indices have been intensively analysed as possible markers associated with the progression of infection – neutrophil/lymphocyte ratio, mean neutrophil volume, neutrophil volume distribution width, mean platelet volume (Aird, 2003). Studies are also focused on proteins, which may be indicative for some preclinical disorders (Lacour et al., 2001; Hatzistilianou, 2010; Miedema et al., 2011). Albumin may be a precise prognostic indicator for some diseases (Infusino & Panteghini, 2013). In the dog, albumin and fibrinogen represent 62% of blood proteins, while the rest 38% include globulins. Albumin is the main serum protein – its function is to regulate the colloidal osmotic pressure of blood and to transport ions, hormones. It is interesting to note that large amounts of albumin are located in the skin, which is the most significant network for storage of extravascular albumin. Skin infections may induce changes in albumin and epithelial albumin in the dog can provoke allergies (Spitzauer et al., 1993). That is why albumin is a preferable parameter for investigation. Hamrahian et al. (2004) state that the decrease in albumin concentrations under 25
g/L influences serum cortisol levels. Peeples et al. (2005) demonstrated that albumin is a significant target for organophosphate compounds and for that reason decrease of albumin correlates with increased concentration of these toxins. Changes in albumin concentrations, total protein, globulins and albumin/globulin ratio give the possibility to assess more precisely bacterial infections in dogs, especially those characterized by hypoalbuminaemia, accompanied by mild (40–50 g/L) to moderate (50–60 g/L) forms of hyperglobulinaemia (Tyler et al., 2004).

The aim of the present research is to study the dynamics of changes in some rapid, indicative clinical laboratory parameters in dogs during experimentally induced Pseudomonas aeruginosa skin infection and to determine their ability to provide information for evaluating such type of infection.

MATERIALS AND METHODS

Experimental animals

Male mongrel dogs at 2–5 years of age, weighing 24.3±1.8 kg, were used. Animals were treated against ectoparasites with antiparasitic shampoo (Friskies, NE.IT S.p.A., Italy) and Tapilan-B (Dorvert, Israel), and against endoparasites with Prazimec – D (Biovet Peshtera, Bulgaria) at a dose of 1 tablet/10 kg body weight. Animals were kept in individual cages (situated indoors and providing constant room temperature – 15–21°C, humidity – 50–60% and regulated light-dark regimen – 12h/12h) and went for walks twice a day – half an hour in the morning and another walk in the evening. Dogs were fed a standard maintenance diet (Canil Social Gouomarc H, Brazil) and had a free access to water.

Experimental design

Two groups, consisting of 5 dogs each, were formed – experimental group (group A) and control group (group B). Animals from experimental group (A) were injected subcutaneously, in the cervical region (depilated skin), with suspension of Pseudomonas aeruginosa (1×10^8 cfu/mL, a field strain) at a dose 0.3 mL/kg body weight. Dogs from control group were injected subcutaneously, at the same location, with normal saline at a dose 0.3 mL/kg body weight.

Experiments comply with the regulations for protection and humane treatment of experimental animals used in scientific research and education. Experiment was approved by the Ethics Committee of Veterinary Faculty of Trakia University.

Dynamics of sampling

The blood samples were drawn from vena cephalica antebrachii, using venflon catheter (20G Vygon GmbH & Co., Germany) in the following dynamics: before infection (0 h), on 4th, 24th, 48th, 72nd hour and on 7th, 10th and 14th day after infection. Blood samples were taken in fasting state (8.00–8.30 AM) to avoid circadian rhythm influences. Serum was separated after blood was allowed to clot for 30 min at room temperature, followed by a 15 minutes storage at 4–8 °C, after which samples were centrifuged for 10 min at 3000×g.

Clinical laboratory parameters

White blood cells (1×10^9/L) were counted in the Büker chamber; the leukogram – on a blood smear (May-Grunwald Gimsa staining); ESR – Panchenko method (mm/h); total protein – by the Biuret method (g/L); albumin – measured by the bromcresol green assay (g/L); globulins – calculated by subtracting albumin from to-
tal protein; A/G ratio – calculated from albumin and globulin concentrations.

Statistical analysis
Results are presented as mean ± SEM. Data was submitted to one-way ANOVA test (Graph Pad InStat3). Differences were considered statistically significant at the P<0.05 level.

RESULTS
Microbiological data
Microbiological identification of Pseudomonas aeruginosa was done by semi-automatic system BBL Crystal (Diamed, Bulgaria) and Gram-negative bacteria kit.

Clinical laboratory parameters
ESR was the most sensitive haematological parameter increasing significantly on 4th hour after infection (P<0.01), with values remaining high (P<0.001) till the end of the experimental period – day 14 (Fig. 1).

Data in Table 1 demonstrate that WBC increased significantly on hour 72 (P<0.001). The changes in the leukogram demonstrated increase in band neutrophils on hour 48 and 72 (P<0.01), which was indicative for left shift. Eosinopenia was found on hours 24 and 72 (P<0.01).

Analysis of biochemical parameters (Table 2) demonstrate that period within 24th to 72nd hour was crucial in the progress of P. aeruginosa skin infection. This statement is supported by the significant (P<0.001) decrease of albumin concentration in this period, mild hyperglobulinaemia (with concentrations 43–45 g/L) and decrease of A/G ratio (0.583±0.02) on hour 48 of infection. These alterations in protein profile did not affect total protein concentration, which remained unchanged during the whole experimental period.

DISCUSSION
The skin plays an important protective role, isolating the body from the surrounding environments. The skin is also invol-

![Fig. 1. Dynamics of changes in erythrocyte sedimentation rate in dogs with experimental skin infection (mean ± SEM; n=5), induced by application of 1×10^8 cfu/mL bacterial suspension of Pseudomonas aeruginosa. ** P<0.01; *** P<0.001 vs hour 0.](image-url)
Table 1. Changes in total and differential white blood cells counts in dogs with experimental skin infection, induced by subcutaneous application of 1×10⁸ CFU/mL bacterial suspension of *Pseudomonas aeruginosa* (group A; n=5), and in control dogs (group B; n=5)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time after inoculation</th>
<th>0 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>7 day</th>
<th>10 day</th>
<th>14 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count (10⁹/L)</td>
<td>A</td>
<td>11.1±1.7</td>
<td>13.8±1.4</td>
<td>15.4±1.0</td>
<td>16.8±0.9</td>
<td>23.1±1.9**</td>
<td>14.8±1.6</td>
<td>14.4±1.4</td>
<td>10.3±1.1</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8.3±0.7</td>
<td>8.2±0.6</td>
<td>8.7±0.9</td>
<td>8.0±1.4</td>
<td>8.6±1.4</td>
<td>9.3±1.8</td>
<td>8.7±1.0</td>
<td>8.5±0.6</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>A</td>
<td>0.8±0.4</td>
<td>2.8±1.4</td>
<td>3.6±1.6</td>
<td>8.6±2.0**</td>
<td>8.4±1.8**</td>
<td>5.6±1.1</td>
<td>4.2±1.2</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
<td>1.0±0.6</td>
<td>0.6±0.2</td>
<td>0.6±0.4</td>
</tr>
<tr>
<td>Segments (%)</td>
<td>A</td>
<td>57.8±6.2</td>
<td>64.0±4.6</td>
<td>69.4±4.3</td>
<td>63.8±5.0</td>
<td>70.0±3.3</td>
<td>64.0±4.6</td>
<td>63.6±4.4</td>
<td>54.0±5.6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>61.2±1.9</td>
<td>66.6±0.9</td>
<td>65.6±2.2</td>
<td>58.8±2.2</td>
<td>64.8±1.7</td>
<td>64.4±2.4</td>
<td>64.0±1.5</td>
<td>65.8±2.3</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>A</td>
<td>10.0±1.3</td>
<td>7.2±2.6</td>
<td>2.0±0.6**</td>
<td>4.0±1.4</td>
<td>2.4±1.5**</td>
<td>4.8±2.2</td>
<td>4.0±1.7</td>
<td>9.4±1.8</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8.2±1.2</td>
<td>7.4±1.4</td>
<td>7.0±0.8</td>
<td>9.0±0.8</td>
<td>7.2±1.3</td>
<td>7.8±1.4</td>
<td>5.6±1.6</td>
<td>6.6±1.5</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>A</td>
<td>29.2±7.0</td>
<td>24.0±6.0</td>
<td>23.8±4.2</td>
<td>22.4±7.0</td>
<td>18.4±3.4</td>
<td>23.8±4.4</td>
<td>27.8±5.4</td>
<td>34.0±5.9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>30.2±1.1</td>
<td>25.8±0.9</td>
<td>27.0±2.1</td>
<td>32.0±1.5</td>
<td>27.8±1.0</td>
<td>26.2±2.7</td>
<td>27.8±0.6</td>
<td>26.2±1.5</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>A</td>
<td>1.2±0.8</td>
<td>2.0±1.0</td>
<td>1.2±0.4</td>
<td>1.2±0.5</td>
<td>0.8±0.4</td>
<td>1.8±0.8</td>
<td>0.4±0.4</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.4±0.2</td>
<td>0.2±0.2</td>
<td>0.4±0.4</td>
<td>1.2±0.8</td>
<td>0.0±0.0</td>
<td>0.6±0.4</td>
<td>2.0±0.8</td>
<td>0.8±0.5</td>
</tr>
</tbody>
</table>

**P<0.01; ***P<0.001 statistically significant differences vs hour 0
Table 2. Changes in biochemical parameters in dogs with experimental skin infection, induced by subcutaneous application of $1 \times 10^8$ CFU/mL bacterial suspension of *Pseudomonas aeruginosa* (group A; n=5), and in control dogs (group B; n=5)

<table>
<thead>
<tr>
<th>Time after inoculation</th>
<th>Group</th>
<th>0 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>7 day</th>
<th>10 day</th>
<th>14 day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein (g/L)</strong></td>
<td>A</td>
<td>71.0±0.48</td>
<td>70.4±1.22</td>
<td>71.0±0.79</td>
<td>71.2±0.65</td>
<td>71.5±0.71</td>
<td>71.4±0.52</td>
<td>71.7±0.87</td>
<td>71.4±0.49</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>70.9±0.38</td>
<td>71.2±0.39</td>
<td>71.8±0.57</td>
<td>71.7±0.54</td>
<td>71.2±0.75</td>
<td>70.5±0.67</td>
<td>70.9±0.40</td>
<td>72.2±0.41</td>
</tr>
<tr>
<td><strong>Albumin (g/L)</strong></td>
<td>A</td>
<td>37.0±0.35</td>
<td>36.4±1.44</td>
<td>27.4±0.87***</td>
<td>26.2±0.84***</td>
<td>28.2±0.83***</td>
<td>34.5±1.80</td>
<td>36.5±0.42</td>
<td>36.2±0.39</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>36.2±0.30</td>
<td>35.6±0.28</td>
<td>36.1±0.19</td>
<td>36.3±0.26</td>
<td>36.6±0.42</td>
<td>35.4±0.35</td>
<td>36.7±0.30</td>
<td>36.9±0.26</td>
</tr>
<tr>
<td><strong>Globulins (g/L)</strong></td>
<td>A</td>
<td>33.9±0.20</td>
<td>33.9±0.64</td>
<td>43.6±1.02***</td>
<td>43.3±0.56***</td>
<td>36.9±1.52</td>
<td>35.2±0.81</td>
<td>35.1±0.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>34.7±0.45</td>
<td>35.5±0.43</td>
<td>35.6±0.48</td>
<td>35.4±0.44</td>
<td>34.6±0.46</td>
<td>35.0±0.40</td>
<td>33.9±0.39</td>
<td>34.4±0.73</td>
</tr>
<tr>
<td><strong>A/G ratio</strong></td>
<td>A</td>
<td>1.089±0.00</td>
<td>1.075±0.05</td>
<td>0.631±0.03</td>
<td>0.583±0.02</td>
<td>0.651±0.02</td>
<td>0.948±0.08</td>
<td>1.039±0.02</td>
<td>1.045±0.02</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.857±0.16</td>
<td>1.003±0.02</td>
<td>1.019±0.01</td>
<td>1.026±0.01</td>
<td>1.056±0.01</td>
<td>1.012±0.01</td>
<td>1.102±0.02</td>
<td>1.065±0.02</td>
</tr>
</tbody>
</table>

*** P<0.001 statistically significant differences vs hour 0
H Hillier 2007) results in infection which provokes impairment of skin structures, of microclimate (humidity, pH) may provoke changes in the dog, but local changes in microclimate (humidity, pH) may provoke impairment of skin structures, which results in infection (Korvik et al., 1991; Hillier et al., 2008).

Prompt, precise and complete evaluation of health status during *Pseudomonas* infection is needed to provide effective control. Experimental models of infection exclude the influence of age, sex, diet, light-dark regimen, humidity and other factors, which may compromise the accurate analysis of changes in haematological and biochemical parameters and overall evaluation of infection progress. Mavrovodi et al. (2013) have analysed the influence of circadian rhythms on immune system, and have demonstrated that they can mediate alterations in erythrocyte count, mononuclear cells count and cytokines. Uzenbaeva et al. (2013) point that melatonin influences neutrophil/lymphocyte ratio in animals. In our study haematological parameters (Table 1) have demonstrated, that in dogs kept under regulated conditions, skin experimental *P. aeruginosa* infection leads to leukocytosis (documented on hour 72) and left shift in the early stage of infection. The left shift is demonstrated by the increase in band neutrophils on hour 48 and 72 (P<0.01). We suggest that the depicted haematological changes are the result of inflammatory response at the site of injection of *P. aeruginosa*. Production of pro-inflammatory cytokines activates leukocytes, key elements of defense against infection (Ping et al., 1995). As compared to other animal species (cattle, horse, cat), the dog has the most pronounced ability to develop neutrophilia during inflammation. Kharazmi et al. (1984) point that *P. aeruginosa* is capable of inhibiting neutrophil chemotaxis. Leidal et al. (2003) state that this Gram-negative bacterium can destruct some chemokines (RANTES, MCP-1), thus inhibiting migration of blood cells.

Our study has demonstrated that the experimental *Pseudomonas* skin infection is characterised by significant decrease in eosinophil count (24th and 72nd hour). Eosinophils are an important component of defense system of organism. Eosinophil granule proteins have antibacterial properties – major basic protein, eosinophil cationic protein and eosinophil peroxidase with antibacterial activity against *P. aeruginosa* (Lehrer et al., 1989; Linch et al., 2009). Bass et al. (1980) have registered eosinopenia during the acute phase of infection, while Abidi et al. (2008) point it as a marker of sepsis. In our study ESR is the most sensitive haematological parameter increasing significantly on 4th hour after infection (P<0.01), with values remaining high (P<0.001) till the end of experimental period. These changes in ESR are the reflection of the inflammatory response to the injected pseudomonads, which cause tissue damage by producing toxins (Pollack, 1980). In the later stages, the increased hepatic production of acute phase proteins also influences ESR, which demonstrates the activation of another non-specific defense reaction – the acute...
phase response. ESR is not a specific indicator, as its values increase not only in inflammation, but also in malignancies, rheumatic disorders, autoimmune diseases and mono- or polyclonal gammopathies (Saadeh, 1998). The thesis for the leading mechanism of inflammation in the progression of the experimental skin infection is supported by the decrease of A/G ratio (Table 2) on 24th, 48th and 72nd hour under the reference range for the dog (0.8–2.0). A/G ratio decreases when albumin concentrations are low or when globulins are high. During acute inflammation production of some acute phase proteins is increased, which is accompanied by dramatic decrease in production of albumin, being a negative acute phase protein (Kaneko et al., 1997). The documented decrease in albumin concentration in the period within 24th to 72nd hour in the dogs with skin P. aeruginosa infection (Table 2), could be the result of increased vascular permeability, which is associated with the release of bacterial toxins. Thus albumin passes into the interstitial space of surrounding tissues, which becomes filled with exudate. This is manifested by the significant local oedema, registered as early as the 4th hour after injection of the bacterial suspension and yet present after the 72nd hour in all experimental dogs.

In conclusion, the simultaneous analysis of both haematological (ESR, leukogram) and biochemical parameters (TP, A, A/G ratio) together with the clinical examination, provide sufficient information about the health status during the progression of skin Pseudomonas aeruginosa infection in dogs.

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Correspondence:
Assoc. Prof. Maria Andonova PhD
Department of General and Clinical Pathology, Functional Pathology and Immunology Unit, Faculty of Veterinary Medicine, Student's Campus, 6000 Stara Zagora, Bulgaria, e-mail: andonova_m@yahoo.com