Histopathological and Hematological Changes Caused by Head Abscess in Rat

Muhamed Katica[1*], Alma Šeho-Alić[2], Mirza Čelebičić[3], Senad Prašović[2], Nejra Hadžimusić[1], Amer Alić[2]

Abstract

A rat with the mass located on the right lateral aspect of the head in the *regio infraorbitalis, maxillaris, lateralis nasi dextra, et regio dorsalis nasi* was presented for necropsy. Clinically, a severe form of dyspnea and occasional intermittent stridor were observed. Gross examination and histopathology revealed an abscess on the rat's head. Hematological examination showed macrocytic regenerative hypochromic anemia. In addition, in blood smears stained with May Grunwald Giemsa, numerous spherocytes and anulocytes as well as reticulocytes indicating anemia were observed. Morphological changes found in the lymphocyte (1,1%) and neutrophil cytoplasm (20%) were most probably the result of adverse, toxic effects of various products of purulent inflammation in the head abscess.

Key words: abscess, anemia, reticulocytes, spherocytes, anulocytes

[1] Department of Pathological Physiology of Domestic Animals, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina
[2] Department of Pathology, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina
[3] Faculty of Science, University of Sarajevo, Bosnia and Herzegovina
*** Corresponding author:** muhamed.katica@vfs.unsa.ba

Introduction

Unlike neoplastic lesions such as hemangiosarcoma (Cohen et al., 2009), abscesses occur very frequently and are most commonly localized in the internal parenchymatous organs (Barthold et al., 2016), while the reports of abscesses in the rat skin are very scarce. They represent limited areas of necrosis caused by piogenic bacteria (Ackermann, 2011) or gram-negative bacteria, but the anaerobes as a possible etiologic factor (Erdevički et al., 2011) must also be considered. They often occur during injury in fights between rats within the area where they live.

Abscesses are characterized by the pus appearing in bacterial infections when proteolytic enzymes from neutrophils convert dead leukocytes and cellular detritus into a single amorphous fluid mass composed of leukocyte exudate, tissue detritus and microorganisms (Ackermann, 2011).

Generally, the head abscesses can be caused by inflammatory processes from adjacent tissue, middle ear inflammation, often dental abscesses, or by hematogenic spread from distant focal sites. They also arise as a result of complicated craniotrauma or complications of some other neurosurgical procedures, meningitis, etc. (Erdevički et al., 2011; Jamjoom et al., 2009).

Depending on the region where the head abscess is localized, it will cause greater or lesser health problems, or cause a life-threatening respiratory infection. On the other hand, an abscess may be a difficult therapeutic problem because its interior is not vascularized, which prevents the effectiveness of treatment.

There are no available reports describing the subcutaneous form of abscesses on rats' heads with the concomittant respiratory problems, and their repercussions on hematologic parameters.

The aim of the paper was to describe the pathohistological aspect of an abscess localized in and near the upper respiratory tract of a rat, and to determine the possible effect of the abscess and its products on the hematological profile, including morphological changes to the blood corpuscles.

Case history

A 2.5 months old male Wistar rat was clinically diagnosed with subcutaneous, hemorrhagic inflammatory edema, which was progressively spreading into the right side of the head in: *regio infraorbitalis, maxillaris, lacrimalis, lateralis nasi dextra, et regio dorsalis nasi* (NAV, 2017), as shown in Figure 1. The general health condition of the rat deteriorated sharply, and the clinical symptoms identified were: general weakness, exhaustion, a severe form of dyspnea, anorexia and disorientation of the animal. Tempering and pain were found during palpation of the inflammatory edema. Inspiratory stridor was occasionally observed. Inflammatory edema was reminiscent, differentially and diagnostically, of hemangiosarcoma, as shown in Figure 1.

Figure 1. Clinical examination of the rat with the mass on the right lateral aspect of the head

From the moment a change on the head had been observed to the time of the rat's death, three days passed. Serious episodes of dyspnea occurred caused by the partial obstruction of the upper respiratory pathways. Prognosis of any successsful treatment was completely unfavorable, and indicated very rapidly cardiorespiratory arrest due to very difficult inspiration. Hence, it was decided the animal should be euthanized. The rat was put under general anesthesia using 5 mg/kg 2% xylazine hydrochloride and 50 mg/kg ketamine hydrochloride, intramuscularly. After that, to euthanize the animal, pentobarbital sodium was applied - 140 mg / kg, intraperitoneally.

Before the euthanasia, the blood was taken intracardially from the animal for the complete blood count. The "Idexx Laser Cyte" laser flow cytometry analyser was used for this purpose.

Microscopic analysis of the poikilocytotic blood corpuscules implied a blood smear previously taken and colored using Giemsa's method. Poikilocytosis was classified semiquantitatively based on the similar research, following the criteria: non-existing (0%), rare (0.05%–0.5%), mild (>0.5%–3%), modest (>3%–10%), or expressed (>10%). The number and type of poikilocytes were recorded as percentages of the red blood cells (Katica and Gradaščević, 2017; Christopher et al., 2014). According to the semi-quantitative assessment of degenerative changes, leukocytes were classified per percentages as follows: few (5% - 10%), moderate (11% - 30%),many (> 30%) (8,9). For each original stained smear, 2000 erythrocytes and 1000 leukocytes were counted and analyzed using a Motic Type 102 M binocular light microscope with magnification of 900 times (Katica and Gradaščević, 2017; Christopher et al., 2014; Katica et al., 2017; Harvey, 2001).

At necropsy, the whole body and internal organs were examined grossly. Samples of the observed lesions and internal organs collected for histopathology were fixed in 10% neutral buffered formalin for 48 hours. The samples were routinely processed, embedded in parafin blocks, cut at 4 to 6 micrometers and stained with hematoxilin and eosin.

Results and discussion

As shown in Figure 2, at necropsy, a 3 cm grey-white mass was observed in the subcutis of the head in the *regio infra orbitalis, maxillaris, lacrimalis, lateralis nasi dextra, et regio dorsalis nasi.* The mass distended the subcutis, and was surrounded with the extravasated erythrocytes (hematoma) and edematous fluid.

Figure 2. Cross section of the mass on the head (image made post-formalin fixation of the specimen). Note the dark red demarcation (hematoma) on the outer side of the mass (white arrow), and the grey-white abscess (asterisks)

Histopathology of the subcutis from the upper jaw area revealed infiltration of many, predominantly degenerated neutrophils bordered with proliferation of up to 10 layers of fibroblasts, deposited collagen, and scatered leukocytes. Multifocally, between the neutrophils, irregular radial basophilic bacterial colonies were observed, as shown in Figures 3 and 4.

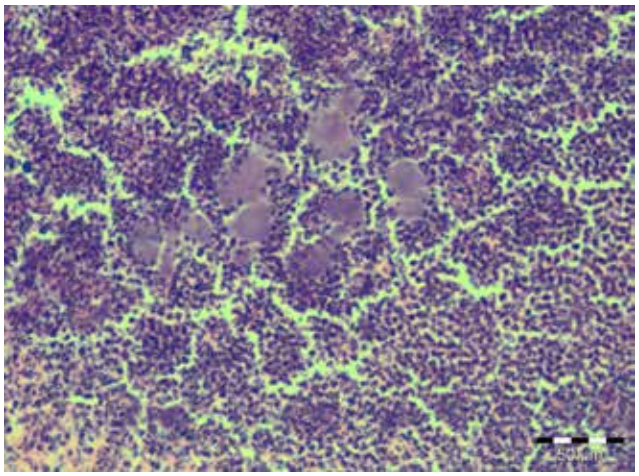

Figure 3. Subcutis, head. Aggregates of numerous, mostly degenerated neutrophils (abscess) in the subcutaneous tissue of the head, 20 X HE

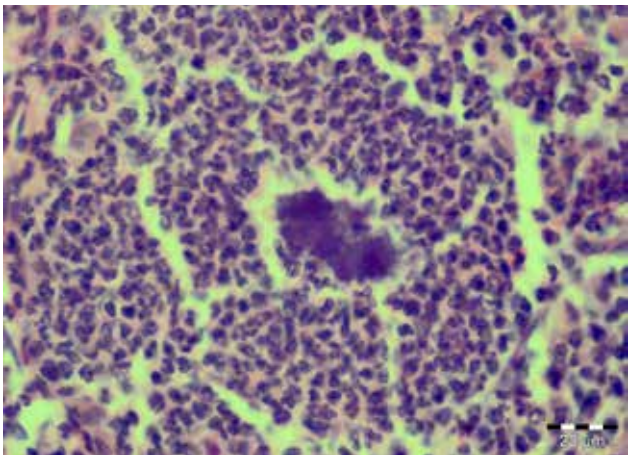

Figure 4. Focal radiating basophilic colonies of bacteria surrounded by numerous neutrophils, 40 X HE

Necropsy did not reveal any foreign body in the lower parts of the respiratory tract that was not affected by the suppurative inflammation. Edema of the larynx and epiglottis was recorded, and the lungs showed multifocal to coalescing areas of alveoli infiltrated by numerous neutrophils and a smaller number of lymphocytes and macrophages. The rest of the pulmonary parenchyma was filled with a clear fluid (edema).

Hematological results indicated a reduced erythrocyte count below the lower physiological limit, and hypochromia, that is, the lower values of other erythrocyte indices (HGB, MCH and MCHC). Hematocrit value and erythrocyte volume were also decreased. Thrombocytopenia was reported alongside erythropenia, as shown in Table 1.

Table 1. Complete blood count of the rat

Hematological parameters	Obtained values	Reference intervals
Erythrocytes (10^{12}/L)	6.1	7.34-8.85#
HGB (g/L)	9.9	14.7-17.3 #
MCV (fl)	59.8	46-57 •
MCH (pg)	18.0	18.6-20.7 #
MCHC (%)	28.4	31.3–34.4 #
PCV (%)	39.3	44.9-51.7 #
Leukocytes (10^9/L)	10.5	6.63-20.35#
Platelets (10^9/L)	696	804-1282 •
Neutrophils (%)	25.5	3.5 - 18.7 %#
Basophils (%)	0.5	0.2 – 0.6 %#
Acidophilus (%)	0.5	0.0 – 0.6 %#
Lymphocytes (%)	67.0	75.8 – 92.9 %#
Monocytes (%)	3.5	0.5 – 3.4 %#

Car et al., 2006
• Kampfmann et al., 2012

The presence of certain erythrocytic poikilocytic forms was also noted in the blood smear. The presence of spherocytes and anulocytes was expressed, while dacryocytes, schizocytes and stomatocytes were mildly represented, as shown in Table 2.

Table 2. Poikilocytotic forms of erythrocytes

Type of erythrocytic poikilocytotic form	Obtained values (%)	Semi-quantitative assessment of poikilocytotic changes
Schistocytes	1.5	Mild (> 0.5–3%)*
Spherocytes	25.0	Expressed (> 10%)*
Stomatocytes	1.5	Mild (> 0.5–3%)*
Dacryocytes	0.9	Mild (> 0.5–3%)*
Anulocytes	13.6	Expressed (> 10%)*
Reticulocytes	5.8	(2.3% -4.5%) •

* Cristopher et al., 2014
• Kampfmann et al., 2012

Moderate neutrophilia with moderate lymphopenia was observed in the differential blood sample analysis, indicating the subacute inflammatory process in the subcutis of the rat's head. Monocytes gravitated towards the upper physiological limit, as shown in Tables 1 and 2.

There were evident morphological changes in the neutrophil cytoplasm and symbolic percentage values in lymphocytes, in the form of azurophilic granules as shown in Table 3, and Figures 7 and 8.

The possible initial cause of the abscess observed here is mechanical trauma. Hematomas were found in the subcutis, as shown in Figures 1 and 2. The exact starting point of the abscess could not be determined, however, the rat's decreased immunity probably exaggerated the condition.

Tissue decay and leukocyte deprivation appeared during the defense of the organism, which became, through the action of proteolytic enzymes, of the fluid consistency, and pus-like creations, with a clearly limited pyogenic membrane with collateral edema, which actually represents an abscess (Piščević, 1995; Mašić, 2011).

The possible etiological factor that caused the suppurative inflammation, i.e. the abscess, is *Staphylococcus aureus*. It causes skin infections, sometimes pneumonia, osteomyelitis, and often produces abscesses (Jamjoom et al., 2009; Kobayashi et al., 2015).

Erythrocytes are highly specialized cells whose basic function is the transport of gases. In physical conditions, specific regulatory mechanisms maintain accurate homeostasis between the saturation of hemoglobins with oxygen, and erythrocyte production. Erythropoietin is a physiological regulator of erythrocyte production that regulates erythrocyte production by stimulating the proliferation and differentiation of erythrocyte progenitor cells, by binding to specific receptors on these cells (Amoroso and Fanelli, 2014).

Table 3. Morphological changes in lymphocytes and neutrophils (%)

Neutrophils	%	Degenerative changes*	Lymphocytes	%	Degenerative changes*
Normal	28	-	Normal	73.3	-
Immature	36	-	Reactive	20	Moderate (11%-30%)*
Hyper-segmented	16	-	Wavy edges	4.4	A few (5%- 10%)*
Acid.granulation	20	Moderate (11%-30%)*	Acid.granulation	1,1	Rare (>0.0–5%)*
Bas.granulation	0	-	Bas.granulation	0	-
Blast form	0	-	Lymphoblasts	1.1	-

* Harvey, 2001

Clinical examination of the rat revealed very difficult inspiration with stridor, while the necropsy revealed the alterations to the tissues of the larynx and epiglottis, indicating inflammation and edema.

Severe dyspnea resulted in tissue hypoxia, which in turn increased erythropoietin production, which ultimately led to accelerated erythropoiesis. The observed reticulocytosis clearly indicates regenerative anemia, as shown in Figure 5 and Table 2.

Appearance of the abscess and its development inversely proportionally caused the rat to lose its appetite eating not enough food. Deficit of essential nutrients, proteins, microelements (Cu, Zn, Fe) and certain vitamins, among other things, significantly contributes to the development of hypochromic anemia (Katica and Gradaščević, 2017). Microscopic blood smear analysis revealed the presence of spherocytes and anulocytes, as shown in Figures 5 and 6, which confirm the evident anemia (Katica and Gradaščević, 2017; Amoroso and Fanelli, 2014). The weak presence of dacryocytes, stomatocytes and schistocytes may be associated with artifacts, which were most likely caused by mechanical trauma during blood transfusion.

By analyzing leukocytic cells, moderate left shift neutrophilia with significant presence of immature neutrophils was observed, indicating accelerated myelopathy in the bone marrow and a subacute inflammatory process as shown in Tables 1 and 3. On the other hand, moderate lymphopenia with altered lymphocyte morphology, reactive lymphocytes and limbs with wavy edges indicate defensive lymphocyte activity within the inflammatory process.

The presence of neutrophils with acidophilic granules in the cytoplasm was moderate, indicating the possible toxic effects of the products of the abscess. The occurrence of neutrophils with such morphological changes in the cytoplasm has been attributed to heavy metal poisoning, drugs (Harvey, 2001) and the adverse impacts of biomaterials implanted in the individual animal tissues (Katica et al., 2017).

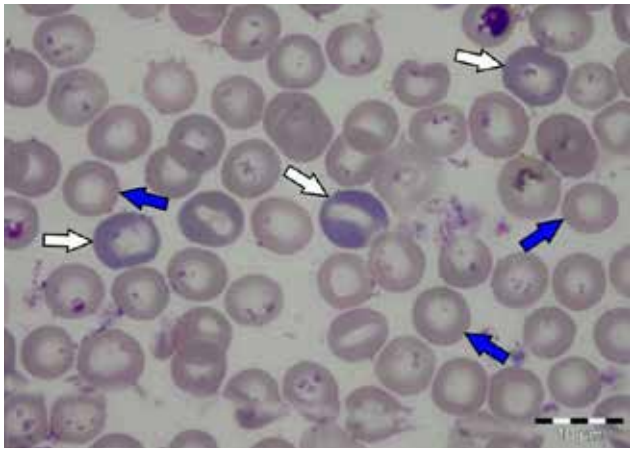

Figure 5. Reticulocytes (white arrow) and hypochromic erythrocytes (anulocytes) (blue arrow), peripheral blood smear 1000 X

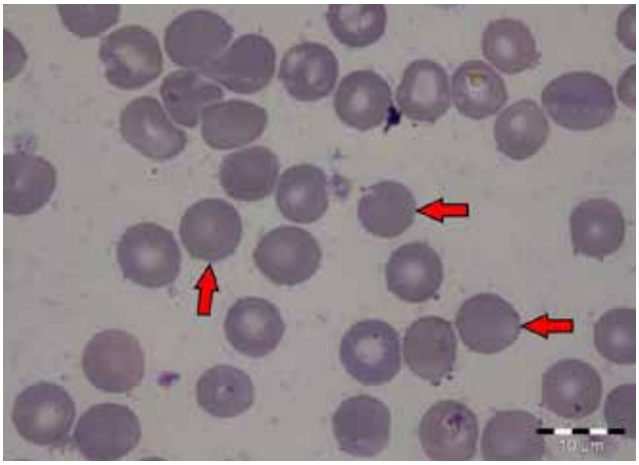

Figure 6. Spherocytes (red arrow), peripheral blood smear, 1000 X

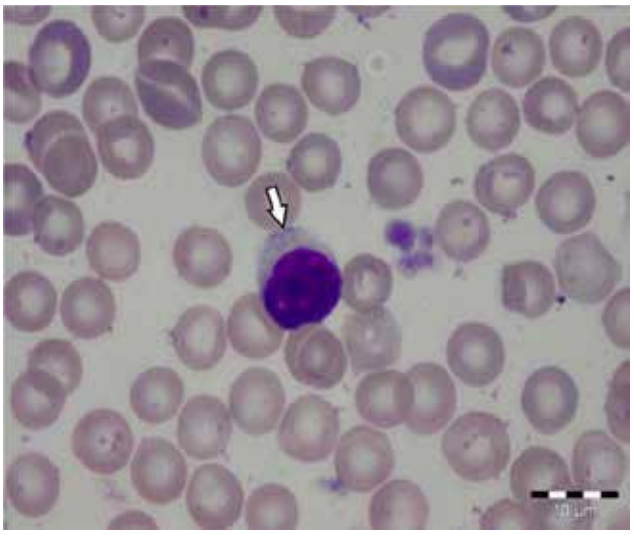

Figure 7. Lymphocyte with azurophilic granules, peripheral blood smear, 1000 X

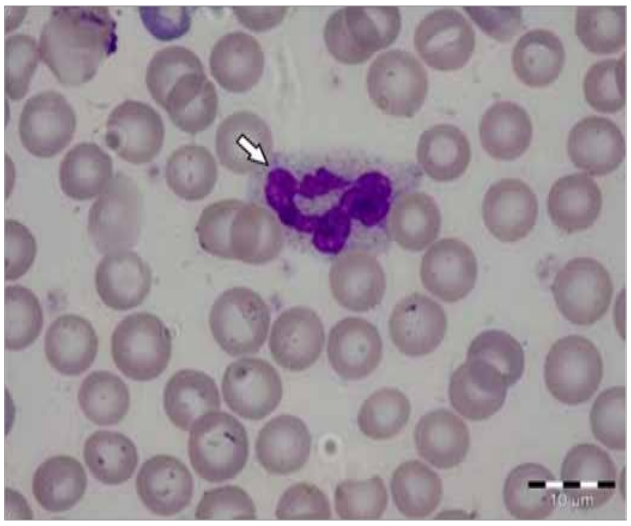

Figure 8. Neutrophil with azurophilic granules, peripheral blood smear, 1000 X

A possible cause of the respiratory disturbances in the rat was the inflammation of the supraglottic structures with edema and inflammation of the epiglottis and surrounding structures, which most likely led to serious obstruction of the respiratory pathways, inspiratory stridor and, ultimately, to cardiorespiratory arrest.

The evident macrocytic regenerative hypochormic anemia was diagnosed by analysis of haematological parameters as well as microscopic examination of morphological changes to the blood corpuscles. In addition to the presence of spherocytes and anulocytes as well as reticulocytes indicating anemia, morphological changes in the lymphocyte and neutrophil cytoplasm were found, indicating the adverse, probably toxic effects of respiratory infection caused by the abscess and its products.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Patohistološke i hematološke promjene uzrokovane abscesom na glavi kod stahora

Sažetak

Predstavljen je obdukcioni nalaz stahora sa izraslinom na desnoj strani glave u regiji infraorbitalis, maxillaris, lateralis nazi dextra i regiji dorsalis nazi. Upalni edem podsjećao je, diferencirano i dijagnostički, na hemangiosarkom. Klinički je uočen teški oblik dispneje i povremeni isprekidani stridor. Obdukcijom nije otkriveno strano tijelo u donjim dijelovima respiratornog trakta koji nije zahvaćen gnojnom upalom. Mogući uzrok otežanog disanja kod stahora je subakutna upala supraglotskih struktura sa edemom i upalom epiglotisa i okolnih struktura. Kompletnim pregledom i histopatologijom otkriven je apsces na glavi stahora. Hematološki pregled pokazao je regenerativnu hipohromnu anemiju makrocitnog tipa. Pored prisustva sferocita i anulocita, kao i retikulocita, što ukazuje na anemiju, uočene su morfološke promjene limfocita (1,1%) i neutrofilne citoplazme (20%), što ukazuje na štetne, vjerovatno toksične, efekte respiratorne infekcije uzrokovane apscesom i njegovim proizvodima.

Ključne riječi: absces, anemija, retikulociti, sferociti, anulociti