



## COMPLETE BLOOD COUNTS IN DOGS WITH HAEMANGIOSARCOMA OF THE SPLEEN

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### Summary

Hristov, Ts. T., 2022. Complete blood counts in dogs with haemangiosarcoma of the spleen. *Bulg. J. Vet. Med.*, **25**, No 2, 255–261.

Haemangiosarcoma is the commonest splenic tumour in dogs. In this study, haematological studies were carried out in 21 dogs with splenic haemangiosarcoma. The findings comprised microcytic, hypochromic anaemia (MCV  $60.05 \pm 2.24$  fL, MCH  $21.33 \pm 0.87$  pg, Hb  $103.47 \pm 8.85$  g/L), erythropania ( $4.72 \pm 0.43$  T/L), thrombocytopaenia ( $222.67 \pm 26.36$  G/L), neutrophilic leukocytosis ( $16.41 \pm 1.78$  G/L), lymphopaenia ( $16.47 \pm 1.67\%$ ), and eosinopaenia ( $1.28 \pm 0.38\%$ ).

**Key words:** complete blood counts, dogs, splenic haemangiosarcoma

### INTRODUCTION

Haemangiosarcoma (malignant haemangioendothelioma or angiosarcoma) is an exceptionally aggressive malignancy originating from vascular endothelium. This neoplasm is encountered markedly more frequently in dogs than in other animal species (Lana *et al.*, 2007). A survey among 655 Golden Retriever dogs dead from neoplasms in the USA has found out that haemangiosarcomas (22.64% of all tumours) and lymphomas (about 18.4%) were the more important neoplasms in this breed (Kent *et al.*, 2018). Haemangiosarcoma is the commonest splenic tumour in dogs. Its prevalence was estimated to more than 50% of all splenic tumours and about 70% of all splenic malignancies (Thamm, 2007). In visceral forms, clinical

signs are variable, depending on the location of the primary tumour.

Secondary changes in blood parameters (haematological paraneoplastic syndrome) in cancer patients are the commonest observed paraclinical abnormalities. The changes may vary in different tumour types with varying tendencies, but the most specific findings for haemangiosarcomas are anaemia, haemolysis, thrombocytopaenia, hypofibrinogenaemia (Karabağlı *et al.*, 2011). In some cases, abnormal blood picture may be the earliest sign of a current neoplastic process.

The present study is part of a large-scale research aimed at determination of occurring haematological changes (red

and white blood pictures) in dogs affected with some malignant tumours.

#### MATERIALS AND METHODS

The studies were performed in 21 canine patients of Small Animal Clinic of the Faculty of Veterinary Medicine – Stara Zagora, from 2007 to 2015. Out of them, 17 were from large and giant breeds. The average age of patients was  $9.6 \pm 0.63$  years. All were with histopathologically confirmed splenic haemangiosarcoma.

Blood samples were collected from v. cephalica antebrachii, in EDTA-anticoagulated tubes. Samples were assayed immediately. Red and white blood parameters: haemoglobin (g/L), haematocrit (%), red blood cell counts (T/L), mean corpuscular volume (MCV, fL), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g/L), red cell distribution width (RDW, %), total white blood cell counts (G/L), platelet counts (G/L) were determined on an automated haematological analyser Mindray BC-2800VET (China). Differential white blood cell counts and abnormal erythrocyte morphology were evaluated on blood smears stained with Hemacolor® rapid staining kit (Merck, Germany).

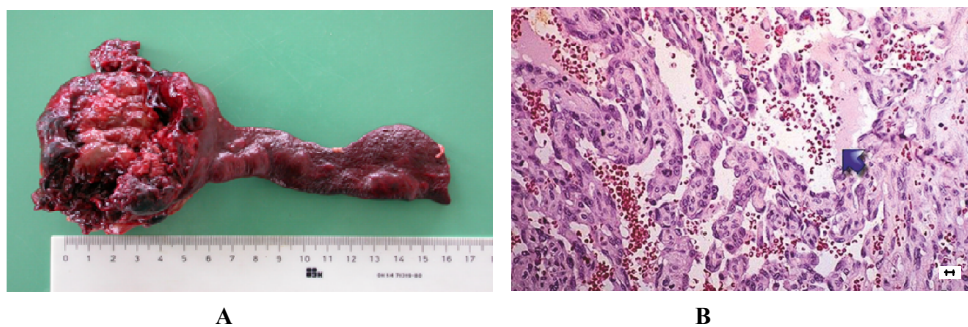
The control group of dogs consisted of ten clinically healthy dogs of both sexes, weighing 8–24 kg, 4–11 years of age.

Samples from various areas of the neoplastic growths were collected for histopathological study. Specimens were fixed in 10% neutral formalin, routinely processed (Dzhurov *et al.*, 1989), embedded in paraffin, cut on a microtome (thickness 5  $\mu$ m) and stained with haematoxylin-eosin (H/E).

Data were analysed by means of statistical software (Statistica v. 6.1, StatSoft Inc., 2002). Descriptive statistics was used to calculate mean values and standard errors of means. Differences between groups were evaluated with the Student's t-test at level of significance  $P < 0.05$ .

#### RESULTS

Macroscopic and histological examinations confirmed categorically the diagnosis splenic haemangiosarcoma (Fig. 1). Microscopic findings depended on the grade of tumour differentiation. Differentiated forms exhibited ovoid fusiform cells with various grade of differentiation, forming irregular lumens of blood vessels, filled with blood.



**Fig. 1.** Spleen haemangiosarcoma in a dog. A. Gross finding. B. Histological findings – pleiomorphic cells, irregular blood vessels filled with blood (H & E, bar=5  $\mu$ m).

**Table 1.** Changes in red blood picture parameters in dogs with haemangiosarcoma (n=21) and healthy control dogs (n=10). Data are presented as mean±SEM

Parameters	Dogs with haemangiosarcoma	Control dogs
Haemoglobin (g/L)	103.47±8.85**	174.2±7.45
Erythrocytes (T/L)	4.72±0.43**	7.44±0.35
Haematocrit (%)	29.85±2.29**	52.62±2.96
Mean corpuscular volume (fL)	60.05±2.24**	69.17±1.28
Mean corpuscular haemoglobin (pg)	21.33±0.87**	23.06±0.80
Mean corpuscular haemoglobin concentration (g/L)	326.33±11.89**	351.7±3.50
Red cell distribution width (%)	17.70±0.96**	14.46±1.02
Platelets (G/L)	222.67±26.36**	302.5±23.96

\*P<0.05, \*\*P<0.01 vs control dogs.

**Table 2.** Changes in white blood picture parameters in dogs with haemangiosarcoma (n=21) and healthy control dogs (n=10). Data are presented as mean±SEM

Parameters	Dogs with haemangiosarcoma	Control dogs
Total leukocyte count, G/L	16.41±1.78**	10.55±0.74
Eosinophils, %	1.28±0.38**	9.90±2.34
Myelocytes, %	–	–
Metamyelocytes, %	0.90±0.41	–
Band neutrophils, %	8.05±1.29**	3.9±0.77
Segmented neutrophils, %	69.0±1.89**	56.5±2.66
Lymphocytes, %	16.47±1.67**	26.3±1.72
Monocytes, %	4.28±0.73**	4.86±0.77

\*P<0.05, \*\*P<0.01 vs control dogs.

Complete blood counts in dogs with splenic haemangiosarcoma and control healthy dogs are shown in Table 1 and 2.

All red blood picture parameters were statistically significantly altered (P<0.01) compared to healthy controls. The reduction of haemoglobin content was marked, with values below 120 g/L in 71.4% of dogs (n=15). The lowest individual measured value was 45 g/L. The same tendency was observed for red blood cells and haematocrit, with strong (P<0.05) positive correlation between haemoglobin and red blood cell counts (Pearson's cor-

relation coefficient  $r=0.969$ ). In 76.16% of dogs (n=16) erythropania was established. A substantial reduction (P<0.01) was detected with respect to red blood cell indices (MCV, MCH and MCHC), accompanied with increased deviation of red cell volume (RDW). Platelet counts in dogs affected with haemangiosarcoma were also lower than those of controls, with 47.6% demonstrating thrombocytopenia.

Determination of the grade of anaemia according to WHO scale (Grotto, 2008) showed low-grade anaemia (grade I and II) in 42.84% of dogs, grade III anaemia

Complete blood counts in dogs with haemangiosarcoma of the spleen

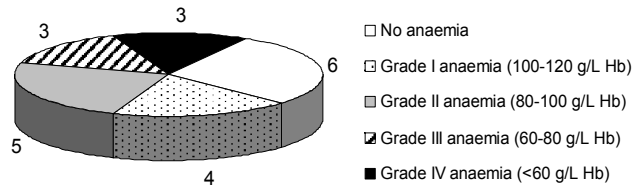


Fig. 2. Distribution of dogs with haemangiosarcoma according to anaemia grade.

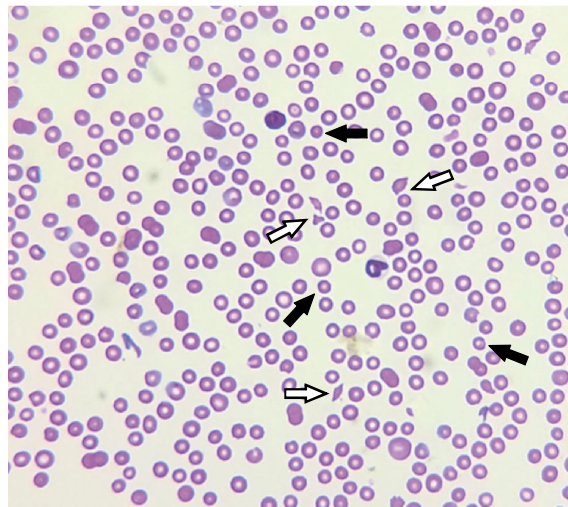


Fig. 3. Blood smear from a dog with spleen haemangiosarcoma demonstrating schistocytes (white arrows) and microcytes (black arrows).

in 14.28% and the same proportion (14.28%) – with the most severe, grade IV anaemia (Fig. 2).

Microscopic examination of blood smears revealed a significant share of schistocytes and microcytes (Fig. 3).

The changes in white blood cell counts are shown in Table 2. Total white blood cell counts in dogs with haemangiosarcoma were statistically significantly higher ( $P < 0.01$ ) vs controls. Leukocytosis was found out in 38.08% of dogs ( $n=8$ ), whereas 5 patients (23.8%) exhibited hyperleukocytosis, up to 34.2 G/L.

Reduced eosinophil percentage was present in 66.64% ( $n=14$ ) of dogs with cancer, and 11 out of them (52.78%) were aneosinophilic. Neutrophilia with regenerative left shift was found out in nine dogs.

Lymphocyte and monocyte percentages tended to decrease considerably. Lymphocytopenia was evident in 33.32% of dogs, with lowest percentage of 3%. On the other hand, two of dogs were with lymphocytosis (up to 32%). Monocytopenia affected 38.08% ( $n=8$ ) of patients.

## DISCUSSION

The observed anaemia and erythropania are a typical expression of haematological paraneoplastic syndrome accompanying the malignancy. Studies in almost all types of cancer have shown that anaemia is an important prognostic marker of shorter survival in cancer patients (Caro *et al.*,

2001). In haemangiosarcomas, anaemia often develops due to inhibited erythropoiesis, decreased life span of red blood cells and blood loss consequently to haemorrhages from the tumour growth (Pintar *et al.*, 2003). Damaged vascular endothelium of haemangiosarcomas often causes microangiopathic haemolytic anaemia (Madewell & Feldman, 1980). In such cases, the presence of schistocytes and spherocytes in blood smears are typical microscopic findings, observed also in the present study. The reduced values of red blood cell indices undoubtedly determine anaemia in patients with haemangiosarcoma as microcytic/hypochromic, of the non-regenerative type (Neiger *et al.*, 2002). These data correspond to our results, demonstrating serious changes in red blood picture in dogs with haemangiosarcoma. The fact that more than 70% of our patients developed hypochromaemia and erythropania indicated severe disturbances of erythropoiesis, bone marrow and erythrocytes.

The reduced haematocrit in cancer dog patients is frequently reported. There was a positive correlation between lower haematocrit values and the malignancy stage (Da Silva *et al.*, 2014). Similar results are anticipated taking into consideration the influence of erythrocytes on haematocrit values. Developing anaemia and erythropania inevitably result in corresponding degree of haematocrit reduction.

Thrombocytopenia in humans and animals with neoplastic diseases was described consequently to chemotherapy. Nevertheless, in more than 36% of cases in dogs with tumours, thrombocytopenia has been observed before the start of chemotherapy (Bergman, 2007). Paraneoplastic thrombocytopenia is particularly specific for vascular splenic tumours (haemangiosarcoma) and lymphoproliferative tumours (lymphoma and leukaemia) (Pintar, 2003; Bergman, 2007). It was proved that the mechanism of thrombocytopenic paraneoplastic syndrome included enhanced destruction of platelets combined with secondary reduction of their production due to direct damage of bone marrow and its lower production potential (Bergman, 2007). In addition, some researchers described also an autoimmune-mediated paraneoplastic thrombocytopenia (Rozanski *et al.*, 2002; Finora, 2003).

Neutrophilic leukocytosis is often reported in dogs with various neoplasms. Leukocytosis with neutrophilia develops in the majority of animals as a response to inflammatory-necrotic changes in affected organs and tissue breakdown of the tumour mass (Ahlyum *et al.*, 2011). The mechanisms of paraneoplastic leukocytic response is not completely elucidated, but is probably related to production of haemopoietic growth factors, e.g. granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (G-CSF), which enhance the synthesis and release of neutrophils. In line with these facts, Petteirino *et al.* (2011) reported a case of tumour-secreted G-CSF and GM-CSF in a dog with kidney carcinoma. Tumour-secreted G-CSF was also detected in cats with cutaneous adenocarcinoma and pulmonary squamous cell carcinoma (Dole *et al.*, 2004). Some cytokines – interleukin-1 and tumour necrosis factor alpha could also trigger neutrophilia by promoting growth factors synthesis (Duda *et al.*, 2017). These mechanisms in the development of tumour-associated leukocytosis could provide explanation to two relatively frequent phenomena in oncology: the hyperleukocytosis (23.8% of patients in this study) from one part, and the im-

possibility to control this leukocytic response with antibiotics on the other.

The lymphopaenia observed in this study was also reported by other studies in dogs with splenic haemangiosarcoma (Aoki *et al.*, 2015). Although a definite reason for this state could not be suggested, we agree with the opinion of Kritsepi-Konstantinou & Oikonomidis (2016) that lymphopaenia observed in oncological patients was due to endogenous stress and influence of proinflammatory cytokines, and induced competitive shifting of myelopoiesis to synthesis of granulocytes (neutrophils).

Altered eosinophil counts have been rarely discussed in veterinary oncology. In our patients, eosinopaenia was detected in 38.31% of dogs with splenic haemangiosarcoma. In most research reports on changes occurring in eosinophil counts, paraneoplastic eosinophilia has been observed in mast cell tumours, pericardial leiomyosarcoma, T-cell intestinal lymphoma, anaplastic mammary carcinoma, fibrosarcoma etc. (Musser *et al.*, 2018). Single reports about tumour-dependent eosinopaenia in adrenal tumours are available (Ramsey & Ristic, 2007). As the primary mechanism for development of eosinopaenia is associated with high glucocorticoid concentrations, it may be hypothesised that the stress provoked by the malignancy was at the background of this event (Kritsepi-Konstantinou & Oikonomidis, 2016).

## CONCLUSION

Paraneoplastic haematological changes are often encountered in various types of neoplasms, including splenic haemangiosarcoma. Anaemia (hypochromaemia with erythrocytopenia), thrombocytopaenia, neutrophilic leukocytosis, lymphopaenia and

eosinopaenia appeared to be important haematological changes associated with haemangiosarcoma of the spleen. A distinct feature in these malignancies is the type of anaemia – microcytic/hypochromic and non-regenerative.

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Paper received 18.03.2020; accepted for publication 18.05.2020

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