Study on the hematological and biochemical changes in dogs infected by *Dirofilaria immitis*

**Studii asupra modificărilor hematologice și biochimice la câinii instați cu *Dirofilaria immitis***

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**Abstract**

In this study, a total population of 31 naturally infected with *D. immitis* dogs from the area of Thessaloniki Greece, were screened for hematological and biochemical alterations. The main hematological changes were anemia in 38.71% (n=12), increased numbers of White Blood Cells in 29% (n=19), peripheral eosinophilia in 38.71% (n=12), basophilia in 35.48% (n=11) and decreased hemoglobin (HgB) concentration in, 19.35% (n=6) of the examined dogs.

The most important biochemical findings were increased activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Createnini Kinase (CK), in 54.84% (n=17), 51.61% (n=16), and 54.84% (n=17) of dogs, respectively. Increased total proteins concentration (TP) in 25.81% (n=8), and elevated Urea concentration in 32.26% (n=10) of examined dogs also has recorded.

**Key words:** *Dirofilaria immitis*, hematological, biochemical

**Introduction**

*Dirofilaria immitis* is a nematode parasite. It is a frequently seen parasite in dogs and in other species such as cats, foxes, bears, wolves, and horses and rarely in humans (Quin et al, 1997; Araujo et al, 2003). Environmental factors like temperature and mosquitos play an important role in the prevalence of Dirofilariosis in dogs (Cringoli et al, 2001). The nematode parasite *Dirofilaria immitis* has a worldwide distribution (Bowman et al 2003; Otto et al. 1969). The geographical distribution is associated with the availability of intermediate host (mosquito). Dirofilariosis is a common and important disease of dogs in tropical subtropical and temperate regions of the world (Quinn et al, 1997). At the present time, Dirofilariosis is not endemic in most of the north-western European countries (Greeve at al 1983; Schrey, 1996), but it is an important parasitosis of dogs in Greece (Haralabidis 2003) as well as in other Mediterranean countries and so it is a common disease in routine clinical practice. The mature form of the parasite (the female measures 25-30cm and the male 15-18cm) lives in the right ventricle of the heart or in the pulmonary artery (Quinn et al, 1997; Araujo et al, 2003; Muro et al, 1999; Montoya et al, 1998; Araujo et al, 2003; Fan et al, 2001). The female forms release several thousand larvae into the blood (Quinn et al, 1997; Montoya et al, 1998; Araujo et al, 2003; Bidgood and Collins, 1996). The microfilariae are ingested by mosquito during feeding. Within the mosquito (*Culicidae* genus *culex*) the larvae (L1) migrate to the stomach and then to the mouthparts (L3) during development. When the mosquito feed again, the infective larvae (L3) are deposited on the skin of the animal and enter through the bite wound. A single mosquito can transmit up to 10-12 L3. The L3 stages molt and migrate to the pulmonary arteries nearly in 100 days after infection. The life cycle in dog, is completed in 6-7 moths.

The main pathogenesis is damage to the pulmonary arteries and mechanical obstruction of blood flow depending on the number of parasites presenting in the right ventricle heart. Antigen tests detect specific antigens from adult female heartworms, and are used with success to detect canine heartworm infection. Currently, assays are
available as in-clinic tests, as well as at many veterinary reference laboratories. The severity of the pathology is influenced not only by the number of worms but also by the stress of high blood flow. Hypertrophy of the right ventricle from increased cardiac outputs and increased pulmonary vascular resistance. No clinical signs have been associated with circulating microfilariae. The clinical signs of dicrofilariosis depend on the stage of the life cycle of the worm, the severity of the infection, and the host response to infection. Signs of heart failure are related to the number of adult worms in the heart and great vessels. Antibody-antigen reactions cause kidney disease and arteritis; emboli can cause lesions in lungs, kidneys and brain (Sodicof, 1995).

Materials and methods

In this study 31 cases of naturally infected dogs from the area of Prefecture of Thessaloniki, were investigated for clinicopathological changes, observed during routine hematological and serum biochemical tests. From those 20 dogs belonged to mixed breeds and 11 were pure bred, 8 dogs belonged to small size (under of 10 kilograms of weight), 11 belonged to medium size (between 10 to 20 kilograms of weight) and 12 belonged to large size (over of 20 kg of weight) dogs, 10 dogs were living with an indoor-outdoor status and 21 dogs were living permanently outdoors, 19 were males (2 neutered) and 12 females (4 spayed), and their age ranged from 1.5 year to 13.5 years. Of the above dogs, 14 were shorthaired (shorter than 4 cm in long) and 17 were longhaired (longer than 4 cm in long), 8 dogs were asymptomatic (stage 1) and the rest 23 presented clinical signs.

Diagnosis was based on an in-clinic antigen test (IDEXXX, heartworm, Antigen Test Kit), history, and recognition of the clinical signs, from dogs which had symptomatic disease. Antigen tests detect specific antigens from adult female heartworms. They are very efficient in identifying canine heartworm infection (Steere, 1989). Currently, assays are available as in-clinic tests, and also at many veterinary diagnostic laboratories. In this study, the PetChek kit Snap (IDDEX Laboratories, ELISA, Portland USA) was used, according to the manufacturer’s instructions. This test is an enzyme immunoassay designed to detect the presence of circulating antigens from an adult female in serum or plasma. Sensitivities calculated for samples taken from dogs with very low burdens (≤ 2 adult female worms) were 92.9 % and 79.2% (Steere, 1989). Sensitivities calculated for samples taken from dogs with more than 2 adult female worms were 99.2% and 97.2% (Greene, 1990).

A statistically significant analysis of the variation in the seropositive rate in relation to hematological and biochemical rates was conducted by using the Chi-square, test and the significant difference factor was P < 0.005.

Results and discussion

This study has record that from a total population of 31 naturally infected dogs with *D. immitis* originated from the area of Thessaloniki Greece, the following results, regarding hematological changes: 12 were anemic (38.71%), 19 with increased numbers of White Blood Cells (61.29 %). 12 had peripheral eosinophilia (38.71 %), 11 had basophilia (35.48 %) and 6 had decreased hemoglobin (HgB) concentration (19.35 %).

The most important biochemical findings were increased serum activity of aspartate aminotransferase (AST) in 17 (54.84 %) dogs, increased activity of alanine aminotransferase (ALT) in 16 (51.61 %) dogs, and also increased activity of Createnini Kinase (CK) in 17 (54.84 %) dogs. Increased total proteins concentration (TP) has recorded in 8 (25.81 %), and elevated Urea concentration in 10 (32.26 %) of screened dogs.

Individual serological tests were carried out to identify specific antigens of *D. immitis* in the bloodstream. False negative test results mainly occur in the case of light infections, or in the absence of adult female worms (Hoover, 1996). Antigen tests are currently recommended by the American Heartworm Society for primary screening and confirmation of heartworm infection in dogs (Wei et al, 1985; Anon, 1995).

ELISA antigen tests detect specific circulating proteins released by the reproductive tract of the mature female worm, and a strongly positive antigen test is normally correlated with a heavy heartworm infestation (Shaw and Day, 1995). However, the antigen level can also vary between animals with identical worm burdens (Shaw and Day, 2005). The above-mentioned tests are available either as ‘in-clinic’ tests or laboratory tests, and their sensitivity approaches 98% but decreases to 35% in dogs with low worm burdens (Shaw and Day, 2005).

Specificity approaches 100% for all the available kits (Shaw and Day, 1995). Small worm burdens, the presence of immature females or male-only infections are common causes of low antigen titles and false negative results (Hoover et al 1990). Hoover et al (1990) found that the
Dirofilaria immitis causes anemia, hemoglobinuria, and hemoglobulinemia. Other studies have recorded haematological findings of this disease, those of anemia, hemoglobinemia and hemoglobinuria (Kitagawa et al, 1990 ; Murdoch, 1984 ; Ishihara et al, 1988 ; Kitagawa et al, 1989). Mild regenerative anemia is present in less than 30% of cases and is thought to result from hemolysis. (Nelson and Couto. 2003) Schaer (2003) says that haemolysis and hemoglobinuria may occur due to the fragmentation of RBCs.

This study has recorded that 61.29% of infected dogs had increased numbers of White Blood Cells, with respect to this finding Nelson and Couto. (2003) refer that monocytosis is inconsistent findings in the complete blood count in dogs infected by Dirofilaria immitis.

This study has recorded that 38.71% and 35.48% of infected dogs had eosinophilia and basophilia respectively. Allergic reaction to the parasite can cause peripheral eosinophilia and basophilia. (Bush, 1991 ; Sodicof, 1995 Quinn et al, 1997 ; Nelson and Couto. 2003 ; Schaer 2003).Dirofilaria immitis, peak eosinophil counts arise when adult worms first enter the heart or microfilariae fare first released into the blood stream by the female worms (Bush, 1991)

Regarding biochemical changes this study has recorded increased serum activity of aspartate aminotransferase (AST) occurs in 54.84 %, increased activity of alanine aminotransferase (ALT) occurs in 51.61 %, and increased activity of Createnini Kinase (CK)occurs in 54.84 % of screened infected dogs. This study has record also increased total proteins concentration (TP) in 25.81%, and elevated Urea concentration in 32.26 % of screened dogs.

Sodicof (1995), refers that elevated serum ALT and AST activities indicate liver necrosis from increased portal pressure. Also, Kitagawa et al, (1993) and Schaer (2003) refer that increased hepatic enzyme levels, loss of liver functions, proteinuria and uremia can be seen in dirofilariosis with pulmonary, cardiac and hepatic disorders. Nelson and Couto, (1998) record that a mild to moderate elevation in liver enzyme activity and sometimes azotemia may occur in dirofilariosis.

Decreased renal blood flow increases the urea nitrogen (BUN) concentration (Sodicof, 1995). Renal damage by immune mediated glomerulonephritis (Type III) has been documented in some cases and amyloidosis has been reported rarely. Direct "mechanical" damage to glomeruli by microfilaria is reported to result in glomerulosclerosis. Dirofilaria immitis is a cause of hematuria which originated from the kidneys (Bush, 1991) This study has record increased
Total Proteins (TP) concentration in 25.81% of infected dogs and also Schar (2003) refers that serum globulin concentrations are frequently increased and this may increase the total protein concentration Quin et al, (1997). Refer that there may be an increase in particular enzyme activity, hypergammaglobinaemia and hypoalbuminaemia in dirofilariosis.

The role of microfilaria in this disease is unknown. It is known that in occult disease, microfilaria are destroyed primarily within the lung parenchyma. The occult infections are often associated with evidence of right heart enlargement and significant eosinophilic lung disease (P.I.E. = pulmonary infiltrates with eosinophilia). Microfilaria may also play a role in the development of renal lesions, especially glomerulonephritis.

Human infection is abortive and results in radiographic changes referred as “coin lesions”, which have been often misinterpreted as representing neoplasia and can lead to unnecessary thoracic surgery (Bowman et al, 2003).

Regarding haematological and biochemical changes the statistical analysis of the results, (occurring the screened infected by dirofilariosis dogs), has record a significant increase (P< 0.005) in White Blood Cells count, a significant decrease (P< 0.005) in hematocrit value, significant increase (P<0.005) in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Createnini Kinase (CK) enzyme activities. There was also a non significant increase in eosinophil and basophil counts, total proteins concentration (TP) and Urea concentration.

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