

Hematoxylin-eosin-methylene Blue Staining in a Dog Thromboembolism Case

ADRIAN STANCU^{1*}, MARIUS PENTEA¹, MIRELA AHMADI¹, LILIANA CARPINISAN¹, ALINA GHISE¹, RAMONA AMINA POPOVICI^{2*},
ROMEO CRISTINA¹

¹ Banat's University of Agriculture and Veterinary Medicine "King Michael I of Romania" from Timisoara. Faculty of Veterinary Medicine, 119 Calea Aradului, 300645, Timisoara, Romania

² UMF "Victor Babes" Timisoara, Faculty of Dental Medicine, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

A post-mortem examination of a 5 years old Rottweiler dog was performed in order to find out the death cause. Macroscopically examination of lungs highlighted on the pulmonary trunk thrombi which were spread from the origin of the trunk to each pulmonary artery. The dissection of the heart has revealed vegetative structure at the level of the valvular endocardium. For histopathological examination samples were taken from a thrombus and from the left valvular endocardium. For microscopic examination the samples were stained by chemical method using hematoxylin-eosine-methylene blue. Based on the chemical staining the histopathological changes and morphopathological aspects the diagnosis of pulmonary thromboembolism has been established.

Key words: thromboembolism, Rottweiler dog, hematoxylin-eosine-methylene blue staining

Pulmonary thromboembolic disease occurs as a consequence of hemostasis disorder and consists of the development of a thrombus. *Thrombus*, or colloquially a blood clot, is the final product of the blood coagulation step in hemostasis. [2, 3, 5].

Embolus is a solid mass (platelets, fibrin) or a foreign body (fat cells, tumor) travelling within the blood flow.

When embolus reaches the wall of the small blood vessels determine ischemia or necrosis distally to obstruction area. Any part of the body may be affected, but in animals the most frequently locations are: descendent aorta, pulmonary arteries, coronary arteries, cranial vena cava.

When a thrombus occurs for various reasons, fibrinolysis mechanisms begin immediately its lysis, so hemostatic processes are affected, the clot persists, increase the bloodstream and can migrate. There are cases when a clot may suffer changes and takes the shape of the vessel.

Pulmonary thromboembolic disease is a consequence of hemostasis disorder which inside of the blood vessels led to vascular endothelial destruction, vascular stasis or hypercoagulability [3, 5, 7, 8].

Thrombi enter the blood flow, reaching the pulmonary vascular network, producing significant obstruction which lead to pulmonary hemodynamic disorders, and finally the respiration affected.

In the dog the disease is associated with nephropathies by loss of protein, endocarditis, cardiomyopathy, pancreatic necrosis, infectious congenital hemolytic anemia immune mediated, diabetes mellitus, trauma, major surgery [5, 7, 12].

Some authors consider that the incidence of the pulmonary thromboembolism in dog is low even some cases of venous thrombosis were reported. True incidence of PTE at the dog is not known and may be much higher than a shows data [6, 9, 10].

Experimental part

Materials and methods

Post-mortem examination of 5 years old Rottweiler was made, the dog died following a sudden death. There were taken lung and heart samples for histopathological examination.

The samples preparation were carried out as follows: 24 hours alcohol fixation at room temperature (prevent the tissue alteration due to the enzymes activity; preserve the tissue texture; improves the optical differentiation), alcohol dehydration (five steps: 70; 80; 90, 100 and 100% alcohol, each step for two hours), clearing with benzene, paraffin wax at 56°C, embedding tissues into paraffin blocks, trimming of paraffin blocks (6µm), sections mounting on the glass slides (using Meyer albumin), hematoxylin-eosine-methylene blue staining [1, 4, 11].

Staining was performed as follows: deparaffinization of tissue sections in benzene, rehydration using decreasing concentrations of alcohol, rinsing in distilled water, hematoxylin staining, alcohol, eosin staining and methylene blue staining, water removal using increasing concentrations of alcohol, cover slide mounting [1, 11].

Hematoxylin will stain the nuclei in blue and the mucin in light blue. Eosin will stain the cytoplasm in pink, collagen in pale pink, red blood cells in bright red. Methylene blue improves the blue color of the nuclei, making them more visible [1, 11].

The microscopically examination is useful as differentiating diagnosis method only if chemical preparation of samples is applied [1].

Results and discussions

The results were compared with studies of other authors, who have made a study of 29 dogs with confirmed pulmonary embolism, there was ante mortem suspicion of PTE in 11 of 17 (65%) dogs with compatible clinical signs but PTE was suspected in only 11 of 29 (38%) dogs in

* email: astancu2002@yahoo.com; ramonaamina@yahoo.com

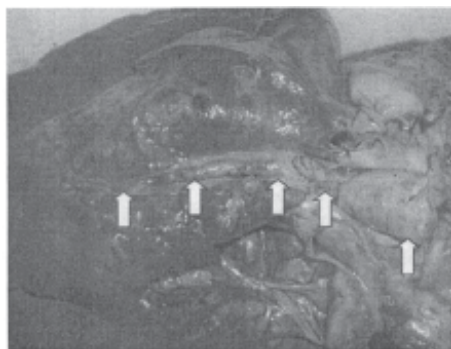


Fig. 1. Pulmonary thromboembolism
Macroscopic appearance

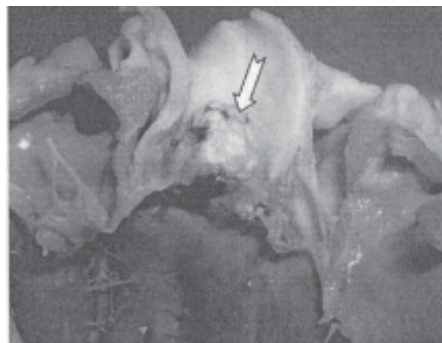


Fig. 2. Ulcerovegetant endocarditis
Macroscopic appearance

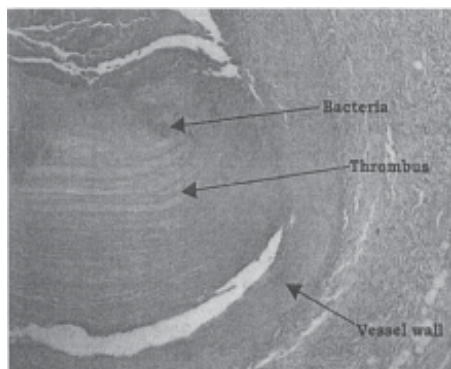


Fig. 3. Pulmonary thromboembolism
Microscopic appearance
Hematoxylin-eosin-methylene blue staining, 20x

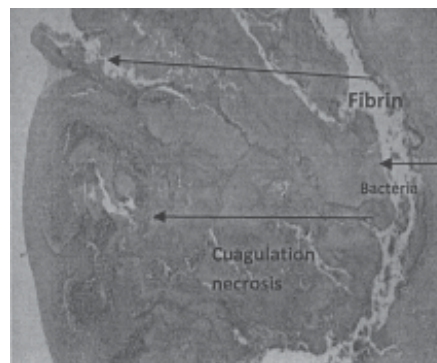


Fig. 4.
Ulcerovegetant endocarditis -
microscopic appearance.
Hematoxylin-eosin-methylene blue staining, 20x

which PTE was subsequently diagnosed at necropsy.³ This study suggested a prevalence of PTE in dogs of 0.9% over a 10-year period. In another study, PTE was suspected before necropsy in 4 of 16 (25%) cats with respiratory signs but PTE was suspected in only 4 of 29 (14%) dogs in which it was subsequently diagnosed postmortem [10].

During the necropsy of heart has been revealed on the pulmonary trunk thrombi which were spread from the origin of the trunk to each pulmonary artery. (fig. 1)

Dissection of the left heart has exposed a vegetative structure in the left valvular endocardium, which is specific for ulcerovegetant endocarditis (fig. 2).

For the histopathological exam the samples were from the site of lesion. The microscopically examination of the thrombi noted also the presence of some bacterial colonies which enlarged the thrombus and obliterated the vessels (fig. 3).

The microscopic examination of the sample taken from valvular endocardium revealed that the vegetant structure contained fibrin, leucocytes, streptococcus spp. located at the base of thrombotic mass. These aspects are characteristic for ulcerovegetant endocarditis (fig. 4).

The obtained results are in line with those of the literature.

Pathogenesis of disease can be explained by affecting morphological integrity of vascular endothelium, which has an important role in the prevention of thromboembolic disease. The lesions of the vascular endothelium firstly produce releasing of endotheline and exposure of sub endothelial collagen, followed by the occurrence of antifibrinolytic factor.

Conclusions

The post mortem gross examination of Rottweiler dog revealed the obstruction of the pulmonary trunk with dense clots of blood, spread from the origin of the latter to the arterial bifurcation into the left and right pulmonary arteries, which macroscopically led to the presumption of thromboembolism.

Macroscopic and microscopic aspects of the lungs, presence of thrombi, bacterial colonies, ulcerovegetant endocarditis caused by the streptococcus infection have confirmed that the sudden death of the dog was produced by pulmonary thromboembolism.

The rare founded pathological aspects, first case encountered in our clinical, shows that PTE has quite low frequency in dogs.

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