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HISTOPATHOLOGICAL ASPECTS OF AGONAL THROMBUS AND ITS ROLE IN AGONAL DEATH DIAGNOSIS - PRELIMINARY STUDY

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Abstract

The mechanisms of blood coagulation in cases of agonal death are rarely studied in veterinary and human medicine. Agonal thrombi are considered as being formed antemortem. Nevertheless, those are misdiagnosed as cruors because of some gross common features. This preliminary study aims to highlight histopathological differences between agonal thrombi and post-mortem clots. Thirteen cases were included: ten domestic carnivores and three chickens. Agonal death diagnosis was established based on the clinical history and followed by standard necropsy and histopathological special staining techniques (Mallory and Masson Trichrome Stain) to highlight the fibrin deposition and pattern. Histological findings of intraventricular and intra-atrial agonal thrombi were similar in all cases and consist of: presence of Zahn's lines, layered display of fibrin deposition, intact or altered erythrocytes and mononuclear infiltration. One case displayed similar features in subepicardial veins. Histopathological examination of clots did not reveal the presence of fibrin deposition, nor Zahn's lines. Considering that in all cases of agonal thrombi the histopathological findings were different from the clots, the agonal thrombus can be associated with agonal death, ruling out the sudden death.

Key words*: agonal thrombus, agonal death, sudden death, cruors.*

INTRODUCTION

The mechanisms of blood coagulation are well studied nowadays, but the lack of research regarding agonal coagulation keeps an open door to this topic. One of the most encountered types of death is the agonal one. Most of the researchers discuss about thrombi (which are formed intra-vitae) or cruors (post-mortem coagulation), but few references about a third category: agonal thrombus, with no true separation between them (Hansma et al., 2015; Kondou et al., 2020; Malone et al., 2008).

It is well known that thrombosis is a pathology that takes place during the animal lifetime, usually associated with impaired mechanisms of coagulation, while cruors are referring to post-mortem clots (Di Fazio et al., 2021; Jackowski et al., 2006; Jackowski et al., 2011). Their gross examination has important differences: thrombi are pale-coloured (because of their high content of fibrin) usually found within the blood vessels with strong adherence to the vascular wall, while post-mortem clots are red-dark coloured, with soft consistency and non-adherent qualities (Kalubert et al., 1988; Kappler et al., 2017; Van Winkle & Bruce, 1993).

The gross examination of agonal thrombi reveals a yellow to red colour, soft to mild elasticity and can be found in the blood vessels, but mainly in the heart chambers with a mild adhesion to the endothelium (Ciobotaru, 2013; Hansma et al,. 2015; Michaud et al., 2013; Roberts et al., 2012).

Considering the previously published studies, there are no references regarding agonal thrombosis in animals. Consequently, the present study aims to establish the histopathological differences between agonal thrombi and cruors in animals and also to have the diagnostic standard in order to rule out sudden death.

MATERIALS AND METHODS

In this preliminary study were included thirteen cases: ten domestic carnivores and three

chickens who all suffered of agonal death. All the cases that took part of this study were animals which was well known that died with a period of agonal suffering. In order to take samples of the agonal thrombus, standard necropsy was performed in a period between one to twelve hours post-mortem. The gross examination of the agonal thrombus was followed by samplings for histopathological investigation, 10% formaldehyde fixation for 24 hours, routine triming, routine hematoxilin-eosine staining and special staining techniques: Mallory and Masson trichrome (Mansueto et al., 2019; Nagasaky et al., 2008; Van Winkle & Bruce, 1993).

Mallory and Masson trichrome are special staining techniques used for the visualization of fibrin (red stained). The aforementioned special stainings were used in order to confirm the presence of fibrin deposition in the agonal thrombus.

RESULTS AND DISCUSSIONS

The recent publications on agonal thrombus tend to describe separately this category with the main objective to associate it with the cases that had an extended agonal period. Usually, the agonal thrombus in humans is found in the right chambers of the heart, but some studies also involve the pulmonary vessels (Hansma et al., 2015).

The necropsy findings in domestic carnivores and chickens were similar to previously published studies in humans: all the agonal thrombus was found in the chambers of the heart, pale-reddish in colour with e soft-mild consistency and fine adherences to the endothelium (Figure 1). Another characteristic found in all cases is the intertwining of the agonal mass to the pectinate muscles and trabecula of the heart, proving that agonal thrombi are formed during the lifetime of the animal when the contractility of the heart is still present.

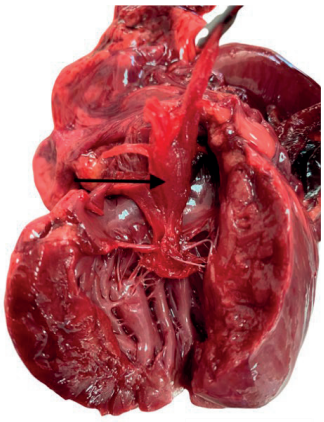

Figure 1. Agonal thrombus in the left heart chambers (dog)

One of the most important element of the intra-vitae thrombus is the presence of fibrin deposition arranged in a layered pattern (Zahn's lines) which are not found in the post-mortem clots (Mansueto et al., 2019). Also, the intra-vitae thrombus formation is based on the Virchow's Triad: impaired blood flow (turbulence or stasis), hypercoagulability and endothelial injury (Mansueto et al., 2019; Zachary, 2017,). These are the two main characteristics of a thrombus that are not associated with cruors. Cruors have no adherences to the endothelium, do not content fibrin depositions, being mainly formed out of erythrocytes and mononuclear infiltration (Fineschi et al., 2008).

Microscopical examination of agonal thrombi revealed the same elements for all cases, the most important being the presence of fibrin depositions, identified especially with Masson Trichrome staining. Alongside fibrin identification, a specific layering of it starts to form the pattern of Zahn's line (Figure 2 A, B)

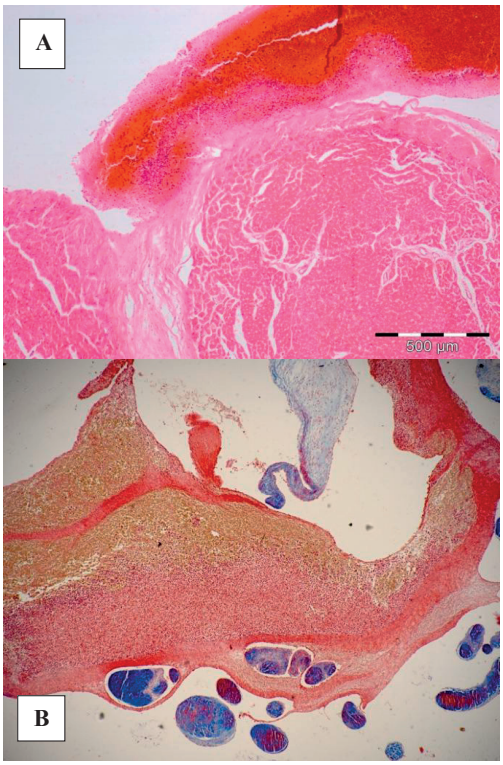

Figure 2**.** **A**-Agonal thrombus (dog), adhesion to the endothelium, HE stain, x50; **B**- Lines of Zahn (arrow) (dog), Masson Trichrome stain, x50

Fine, interrupted adherence between oxyphil fibrin network of agonal thrombus and endothelial cells were identified in histopathological investigation, as well. The cellular component of the agonal thrombus was represented by erythrocytes and clustered leukocytes. (Figure 3 A, B)

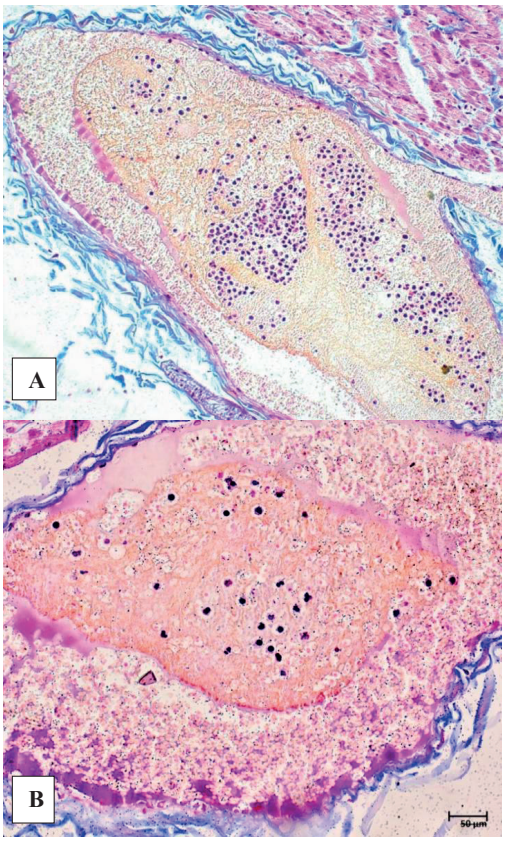

Figure 3. **A -** Agonal thrombus (dog), fibrin layering in the subepicardial veins, Mallory stain, x200; **B -** Clustered mononuclear infiltration in the agonal thrombus (dog), Mallory stain, x400

The microscopic view of wave-like fibrin layering was seen in the mass of the agonal thrombus in all cases presented, with random distribution (Figure 4).

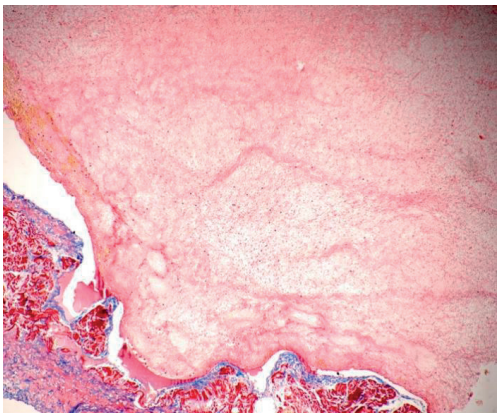

Figure 4. Agonal thrombus (dog), moderate fibrin deposition Masson Trichrome stain, x200

One particular aspect of agonal thrombosis was found in a carnivore where agonal thrombus was found also in the subepicardial veins. Agonal thrombus found in the small veins proves that agonal suffering probably alters the blood flow in all of the circulatory system (Figure 3A).

Even though the literature divides the coagulation of blood in ante-mortem or post-mortem, the agonal blood coagulation, that is to be associated with agonal death, needs to be studied for its importance to the forensic pathology field. All the gross and histopathologic differences of the agonal thrombi versus cruors tend to give us the explanation that this category exists and, even more, is the way to rule out sudden death scenarios in forensic pathology. Although they do not have a clinical significance, because of forming in agonal death (meaning the animal already suffered from other pathology that determine the agonal period), their importance to the pathology field is bigger. Knowing that agonal thrombi are structures that form in the *articulo-mortis* (the point of death), those are not to be misdiagnosed with thrombus vera nor with cruors as expression of the post-mortem coagulation of blood (Barkhausen et al., 2002; Hansma et al., 2015)

CONCLUSIONS

The differences between ante-mortem, post-mortem and agonal thrombi described previously lay the path for finding the ways to distinguish them at necropsy, those should be categorized differently giving the fact that do not form in the same way nor do they have the same characteristics. The agonal thrombus seems to have more common elements with the thrombus which is formed ante mortem then with the cruors, proving that they form strictly during the agonal period of death while the heart of the animal is still active.

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