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## Pre- and postcapillary pulmonary hypertension in dogs: Circulating biomarkers

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### Abstract

**Background:** Pulmonary hypertension (PH) in dogs is a syndrome that could be primary or secondary due to pulmonary disease, pulmonary thromboembolism, heartworm disease, and heart failure. Due to the inability of right heart catheterization in veterinary patients, there is a lack of differential criteria between PH forms. In some acute cases, it is impossible to provide a full EchoCG or catheterization study. In this situation, circulating markers may be useful to discover the possible mechanism of PH form and provide specific therapy.

**Aim:** Following all previous data in human and veterinary studies, we assumed that plasma concentration of serotonin, endothelin-1 (ET-1), and vascular endothelial growth factor D (VEGF-D) would show a predominance in affected part of pulmonary circulation.

**Methods:** We studied 59 small-breed dogs of different sexes and ages. Groups were formed according to a primary pathology: healthy dogs ( $n = 8$ ); dogs with myxomatous mitral valve disease (MMVD) and postcapillary PH (PostPH,  $n = 23$ ); dogs with MMVD and precapillary PH (PrePH,  $n = 28$ ). Animals in the study were diagnosed with the primary disease by standard echocardiographic methods and algorithms. Blood samples were collected at the moment of presentation and frozen in a  $-80^{\circ}\text{C}$  fridge. For biochemistry analysis, we used species-specific ELISA kits, provided by Cloud-Clone Corp. (USA). The tests were provided by the means of Almazov National Medical Research Center, IEM laboratory.

**Results:** Dogs with EchoCG-proved PostPH had a higher concentration of VEGF-D in comparison to control and PrePH ( $p < 0.001$ , for both). There was no difference between the control and PrePH groups ( $p > 0.05$ ). ET-1 was higher in PrePH in comparison to PostPH and control dogs ( $p < 0.001$ , for both). In addition, there was no difference between the control and PostPH groups ( $p > 0.05$ ). Serotonin concentration did not have a difference between controls and PostPH. However, it was higher in PrePH than in control ( $p < 0.033$ ) and PostPH group ( $p < 0.006$ ). Receiver operating curve analysis showed that plasma concentrations of ET-1 (0.99) and VEGF-D (0.92) had high effectiveness in the differentiation of PostPH and PrePH.

**Conclusion:** This study showed a correlation between circulating biomarkers (serotonin, ET-1, and VEGF-D). We found a connection between ET-1 and right-sided heart failure as well as VEGF-D and left heart failure in the PH context.

**Keywords:** Biomarkers, Endothelin-1, Pulmonary hypertension, Serotonin, VEGF-D.

### Introduction

Pulmonary hypertension (PH) in dogs is a syndrome that could be primary or secondary due to pulmonary disease, pulmonary thromboembolism, heartworm disease, and heart failure (Reinero *et al.*, 2020). There is a growing body of data for echocardiography assessment in PH diagnostics. But there is still a lack of “gold standard” in right heart catheterization studies, therefore some of the EchoCG-based parameters may be inaccurate (Johnson *et al.*, 1999; Kelliham and Stepien, 2010; Visser, 2017; Jaffey *et al.*, 2019; Menciotti *et al.*, 2021). Even so, in acute cases, sometimes it is impossible to provide full EchoCG to identify PH and its possible cause.

In dogs, acute respiratory distress could be associated with PH-associated states: progression of left-heart disease, pulmonary edema (a reactive form of PH), lung disease, dirofilariasis, and thromboembolism of the pulmonary artery (Reinero *et al.*, 2020). In this acute context, circulating markers that show the prevalence of the damaged part of lung circulation is in demand. Human studies show defying circulating markers: ANP, brain natriuretic peptide (BNP), NTproBNP, ADMA, PDGF, vascular endothelial growth factor (VEGF), PIGF, etc. (Hewes *et al.*, 2020). Nowadays, the most valuable circulating biomarkers in human medicine are natriuretic peptides. In human research, BNP was found to be the most reliable factor of right chambers overload, due to its production predominantly by

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overstretched right ventricle muscle (Såleby *et al.*, 2019). In studies, BNP showed prognostic value in chronic pulmonary artery thromboembolism (PAT) but was not suspected as an early marker of PH (Hewes *et al.*, 2020). In veterinary medicine, there are several studies of natriuretic peptides, most of them associated with NT-proBNP clinic relevance to pulmonary edema or left-sided heart failure (LSHF) (Nagaya *et al.*, 1998; Heishima *et al.*, 2018; Hezzell *et al.*, 2018). Moreover, there are studies on endothelin-1 (ET-1) concentration in PH patients and its correlation with pulmonary artery pressure (Nootens *et al.*, 1995; Dupuis and Hoepfer, 2008; Warwick *et al.*, 2008). Also, there is a lot of information about VEGF's role in PH, but the data is not homogenous, possibly because of different VEGF ligands (A, B, C, D) and variable effects with different receptors (Kanazawa *et al.*, 2007; Voelkel and Gomez-Arroyo, 2014; Ahmed *et al.*, 2020).

By contrast, in veterinary medicine, several studies are elucidating the role of serotonin in lung remodeling and LSHF due to myxomatous mitral valve disease (MMVD) (Mangklabruks and Surachetpong, 2014; Höglund *et al.*, 2018; Sakarin *et al.*, 2020).

Following all previous data in human and veterinary studies, we supposed that plasm concentration of serotonin, ET-1, and VEGF-D would show a predominance of affected part of pulmonary circulation.

#### Materials and Methods

In this pilot study, we included dogs of different sexes, predominantly of age above 10 years old, with a history of pre-existent MMVD at stage C of ACVIM classification. The dogs were treated with standard therapy (Pimobendan, Furosemide, Spironolactone +/- ACEi).

All the dogs were divided into three groups: a control group with MMVD but without signs of PH ( $n = 8$ ); dogs with MMVD and signs of precapillary PH (PrePH) ( $n = 28$ ); dogs with signs of postcapillary PH (PostPH) and MMVD ( $n = 23$ ).

All the included dogs were taken to the intensive care unit with signs of acute respiratory distress while being on standard MMVD stage C medication. At the moment of examination, all of them had endocardial murmurs, harsh lung sounds, and crackles.

The control group included dogs with diagnoses of MMVD at stage C. They were treated with standard protocol and did not have signs of respiratory insufficiency, previous pulmonary disease, and PH. In addition, most of them underwent a routine oral cavity sanitation. In parallel, echocardiography and radiology (radiology study of the thoracic cavity in two projections) studies were performed to exclude subclinical forms of diseases.

The dogs that appeared in the intensive care unit had a radiology examination to characterize the insensitivity of pulmonary edema. Then, they were placed in the oxygen camera and were treated in the

standard way until clinical stabilization. After that, they underwent blood sampling for biomarkers study and an echocardiographic study to estimate their heat performance.

To differentiate PrePH and PostPH, we used phenotypic markers. We assumed that all presented dogs previously had the phenotype of LSHF: left chambers dilation, pulmonary veins distention, preserved systolic function, diastolic dysfunction above class 2, absence of right chamber's dilatation, and low velocity of tricuspid regurgitation. The cases, with preserved phenotypic markers of LSHF and addition of new-onset PH features (tricuspid regurgitation above 2.7 m/s, presence of pulmonary artery regurgitation, decreased right pulmonary artery distensibility index, right ventricle wall hypertrophy, and right chambers dilation), were assumed as patients with PostPH according to ACVIM consensus in PH. Meanwhile, dogs with signs of PH onset (as mentioned above), but with loss of LSHF signs were marked as PrePH. This subjective method is mentioned to find out some features closely connected with different forms of PH from the blank.

We excluded dogs with pre-existing PH and its therapy, congenital defects, arrhythmia, lung disease, and systemic diseases affecting blood flow (arterial hypertension, cushing's disease, PAT, dirofilariasis, sepsis, electrolyte abnormalities, etc.) and dogs without previous history of MMVD.

We performed echocardiography in standard methods, with an estimation of chamber diameter, and systolic and diastolic function of the left ventricle. Left atria and right atria diameter were studied in long parasternal 4 chambers-view axis. Right ventricle diameter and right ventricle free wall thickness were estimated in the long parasternal 4 chambers-view axis. Additional criteria included subjective characterization of dilated/non-dilated chambers and right wall hypertrophy presence. The longitudinal systolic function of the right heart was studied by TPASE. Systolic and diastolic diameter of the left ventricle, wall thickness, and shortening fraction was studied in M-mode by the standard methods.

The velocity measurements were performed in standard methods with PW-and CW-Doppler. The pulmonary flow was studied on the right short axis: we analyzed anterograde flow, its phenotype, the ratio between acceleration time and effusion time, and regurgitation velocity. In this projection, the ratio between PA and aorta was subjectively estimated. In the context of associated changes of the right ventricle, the velocity of tricuspid regurgitation above 2.7 m/s was assumed as a PH marker. Velocity less than 2.2 m/s was declared as non-significant. The transmitral flow was studied in the context of mitral regurgitation, a diastolic function of the left ventricle (velocity and ratio between E-wave and A-wave calculation). Additionally, tissue Doppler measurements were performed: S'-wave on the tricuspid valve, e'-wave on the interventricular septum, and its ratio with transmitral E-wave. In

parallel, we performed subjective characterization of right pulmonary artery distensibility. The PH diagnosis was verified by echocardiography means, using conventional criteria (echocardiography data could be found: Oleynikov and Yi, 2022)

Blood samples were collected at the moment of presentation and frozen in a  $-80^{\circ}\text{C}$  fridge. For biochemistry analysis, we used species-specific ELISA kits, provided by Cloud-Clone Corp. (USA) The tests were provided by the means of Almazov National Medical Research Center, IEM laboratory.

Statistical analysis was performed by SPSS version 23.0 software (IBM Corporation, Armonk, NY) and GraphPad Prism 8.00 (GraphPad Software Inc., La Jolla, CA). The comparison of group characteristics, and echocardiographic indices was performed by the nonparametric Mann–Whitney test (Appendix Table 1) significance of the differences in categorical variables was calculated by Fisher’s exact test. All  $p$  values were 2-sided, and  $p < 0.05$  was considered to be statistically significant. The correlation between variables was evaluated by Spearman’s rank correlation coefficient analysis.

**Ethical approval**

The study was approved at the internal clinical conference.

**Results**

This study included 59 dogs of a senior (9–11 years old) age, different sexes, and breeds, predominantly of toy and small breeds. Statistically, a significant weight difference was not observed ( $p > 0.05$ , for all groups). Average heart rhythm was more rapid in the group with PostPH, but did not statistically differ from other groups ( $p > 0.05$ , for all groups, Table 1).

The PH diagnosis was verified by echocardiography means, using conventional criteria (echocardiography data could be found: Oleynikov and Yi, 2022).

In the PostPH (Table 2, Fig. 1) group plasma, VEGF-D concentration was significantly elevated in comparison to the control dogs and PrePH group ( $p < 0.001$ , for both). There was no difference between the control and PrePH groups ( $p > 0.05$ ).

The plasma concentration of ET-1 in the PrePH (Table 2, Fig. 2) group was statistically higher than in the control and PostPH groups. There was no difference between control and PostPH groups ( $p > 0.05$ ).

There was no significant difference between serotonin (Table 2, Fig. 3) concentration in control and PostPH groups. Albeit, plasma serotonin was increased in the PrePH group in comparison to the control dogs ( $p = 0.033$ ) and the PostPH group ( $p = 0.006$ ).

Receiver operating curve (ROC) analysis of the VEGF D, ET-1, serotonin showed that VEGF D AUC is 0,92 (95%; CI = 0.85–1.0;  $p < 0.001$ ); for the ET-1 is 0.99 (95%; CI = 0.97–1.0;  $p < 0.001$ ); for the serotonin is 0.73 (95% CI = 0.59–0.86;  $p = 0.0057$ ) (Fig. 4). However, AUC showed predictive value: ET-1 > VEGF

Table 1. Weight and heart rate analyze.

| Indices         | Control (N = 8)     | PrePH (N = 28)      | PostPH (N = 23)     | Differences between control and PrePH | Differences between control and PostPH | Differences between PrePH and PostPH |
|-----------------|---------------------|---------------------|---------------------|---------------------------------------|--|--------------------------------------|
| Weight (kg)     | 4.3 (3.1–5.9)       | 3.9 (3.2–5.7)       | 4.5 (2.9–7.8)       | >0.05                                 | >0.05                                  | >0.05                                |
| Heart rate, bmp | 133.0 (118.5–140.8) | 130.0 (110.0–159.0) | 148.0 (140.0–156.0) | >0.05                                 | 0.030                                  | >0.05                                |

Table 2. Serum biomarkers.

| Indices            | Control (N = 8)  | PrePH (N = 28)   | PostPH (N = 23)  | Differences between control and PrePH | Differences between control and PostPH | Differences between PrePH and PostPH |
|--------------------|------------------|------------------|------------------|---------------------------------------|--|--------------------------------------|
| VEGF D (pg/ml)     | 33.1 (29.7–36.9) | 36.0 (26.1–59.8) | 81.2 (73.3–96.2) | >0.05                                 | <0.001                                 | <0.001                               |
| Endothelin (pg/ml) | 17.8 (15.0–19.2) | 36.6 (33.1–39.0) | 20.6 (17.2–23.1) | <0.001                                | >0.05                                  | <0.001                               |
| Serotonin (ng/ml)  | 26.1 (21.0–30.7) | 30.2 (26.1–67.9) | 26.6 (22.4–30.5) | 0.033                                 | >0.05                                  | 0.006                                |

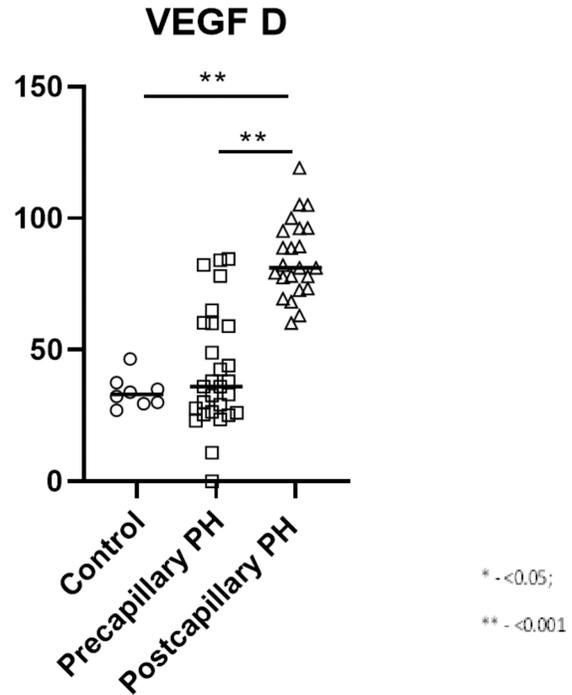


Fig. 1. Graphic representation of the VEGF-D plasma concentration in PrePH, PostPH, and control groups.

D> serotonin, which means that ET-1 and VEGF-D has high diagnostic value for differentiation between Pre- and PostPH in dogs.

Plasma markers showed correlation to EchoCG findings, analyzed by Spearman Heatmap. We observed reverse correlation between VEGF-D and LVFW for PrePH dogs ( $r = -0.53, p = 0.004$ ), ET-1 and PA effusion time ( $r = -0.50, p = 0.006$ ); positive correlation for serotonin and presence of PA regurgitation ( $r = 0.60, p = 0.035$ ). In Post PH group, we found reverse correlation of these serotonin ( $r = -0.59, p = 0.003$ ) and VEGF-D ( $r = -0.62, p = 0.002$ ) to the IVS width; reverse correlation of ET-1 and MR ( $r = -0.51, p = 0.012$ ); positive correlation of the serotonin concentration and PA acceleration time ( $r = 0.58, p = 0.004$ ).

### Discussion

In this study, we tried to find a correlation between EchoCG parameters and serum biomarkers in PH forms differentiation. We suspect that our findings in biomarkers could help with PH diagnosis in cases where EchoCG should be avoided or is unavailable.

There is a tendency in human medicine to replace or find alternative circulating biomarkers to avoid invasive methods. In the context of this study, which includes dogs with previously diagnosed LSHF, we could not use natriuretic peptides due to possible difficulties in the interpretation.

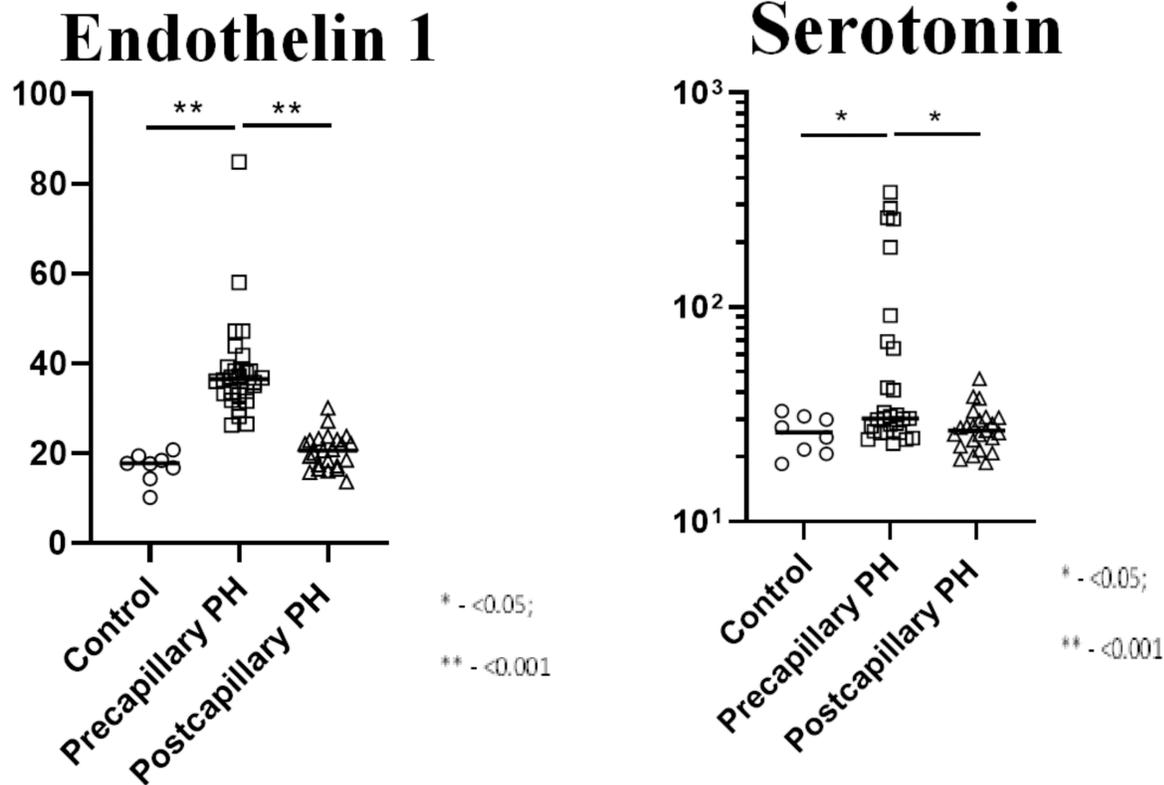


Fig. 2. Graphic representation of the ET-1 plasma concentration in PrePH, PostPH, and control groups.

The pathologies of chronic pulmonary congestion predispose to compensatory increased volume drainage, which is done by the lymphatic system. Physiological dog studies discovered that lymph drainage could be enhanced above 300% in case of chronic volume overload (Uhley *et al.*, 1962). VEGF-D is a predominantly lymphatic vessels growth factor, taking part in lymphatic pool remodeling both in chronic interstitial lung diseases and LSHF (Achen *et al.*, 1998). Human clinical studies found that patients with dyspnea and later diagnosed heart failure had elevated plasma VEGF-D concentration. Authors suspected that this condition/symptom developed as a consequence of pulmonary circulation congestion and compensatory lymphatic system remodeling. This could also be associated with PostPH development due to pulmonary arterial wedge pressure (PAWP) increase and alteration in the microcirculatory drainage (Borné *et al.*, 2018). This statement was validated by the study which showed a correlation between PAWP and VEGF-D (Houston *et al.*, 2019). Additionally, VEGF-D rise was admitted in cases of chronic PAT, which was interpreted as a remodeling due to local congestion in the context of the redistributive edema (Säleby *et al.*, 2019). In both cases, VEGF-D was associated with increased pulmonary vascular resistance, vessels remodeling

Fig. 3. Graphic representation of the serotonin plasma concentration in PrePH, PostPH, and control groups.

due to chronic congestion. Moreover, VEGF-D is a mortality factor for patients with PostPH (Miller *et al.*, 2013). One more interesting study in terminal heart failure and cardiac transplantation showed lowering of VEGF-D levels due to a decrease in signs of pulmonary congestion and pulmonary vascular resistance (Ahmed *et al.*, 2020).

Another rather specific biomarker is ET-1. This factor is more connected with the rise of pulmonary vascular resistance. ET-1 could be increased both by hyperproduction and by altered clearance (Cody *et al.*, 1992; Staniloae *et al.*, 2004). Human clinical studies showed that ET-1 clearance decreased in patients with PrePH and there was a correlation between ET-1 plasma level and pulmonary vascular resistance determined by right heart catheterization (Meoli *et al.*, 2018). In pathophysiological dog studies, ET-1 rise was found in experimental chronic PAT and decreased ET-1 clearance, showing utilization alteration in pulmonary circulation (Kim *et al.*, 2000). Additionally, we found data about successful bosentan therapy in PrePH and increased circulating ET-1 level (Kim *et al.*, 2000).

There is an enhancing factor for ET-1 associated PH—serotonin, which could be a synergist for ET-1 sensitivity in the context of its decreased clearance (Channick *et al.*, 2001; Rubin *et al.*, 2002; Eickelberg *et al.*, 2003; Campbell, 2007). Additionally, serotonin is

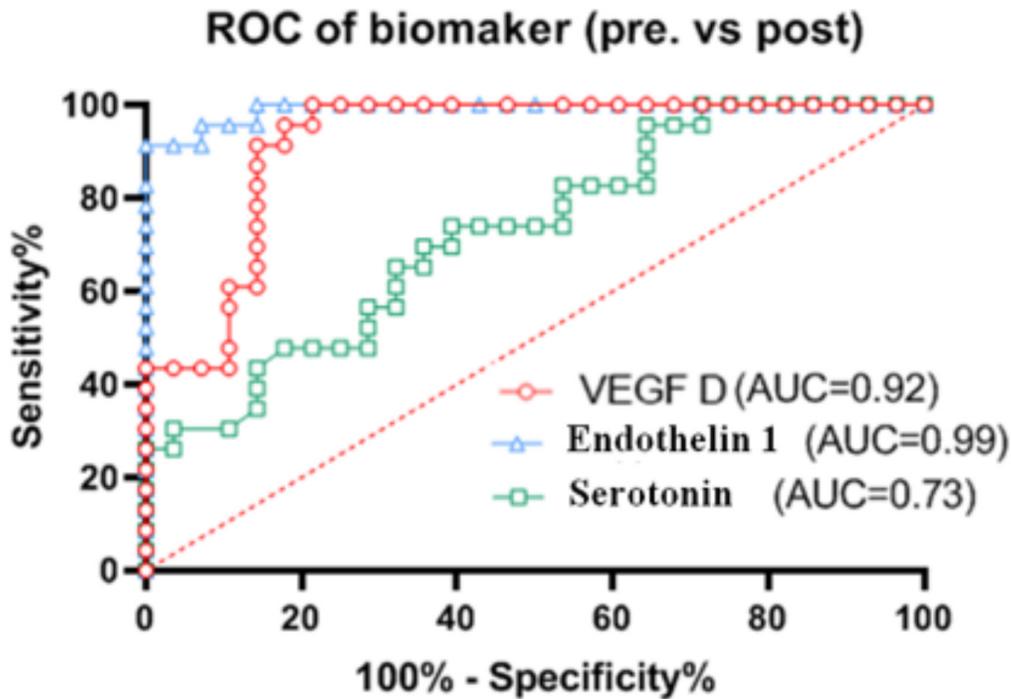


Fig. 4. Grafical ROC with diagnostic ability of the blood markers.

a proliferating factor for pulmonary vessels media and muscles (Eddahibi *et al.*, 2000; Marcos *et al.*, 2004). In veterinary studies, dogs with myxomatous valvular disease showed a significant role of serotonin pathways in PrePH and PostPH development (Hashimoto *et al.*, 2000; Oyama and Levy, 2010; Ljungvall *et al.*, 2013; Roels *et al.*, 2015). In a recent study, serotonin pathway proteins were studied in dogs with PostPH (Sakarín *et al.*, 2020). Increased serotonin-associated proteins were found in pulmonary vessels, which was interpreted as a consequence of chronic hypoxia and vascular muscular cells remodeling (Eddahibi *et al.*, 2000; Marcos *et al.*, 2004). However, the study did not show a correlation between plasma concentration and pulmonary tissue findings. In our data, we did not find a clear difference in serotonin plasma between groups. This data should be assumed in the context of pulmonary circulation changes associated with two paths. The first one is associated with enhancing effect on ET-1-induced vasoconstriction, as mentioned above, in this variant serotonin plasma level could differ in an unrecognizable limit, but its action on the receptors and increasing to ET-1 sensibility would be enough to provoke severe pulmonary vessel remodeling. The second path is associated with increased serotonin pulmonary circulation and an increased window of acting; this could be sustained by VEGF and serotonin interactions and prolonged pulmonary transition time,

observed in dogs with myxomatous valvular disease (Lord *et al.*, 2003).

Our VEGF-D observations in this study showed that in dogs with EchoCG diagnosed PostPH, VEGF-D concentration almost doubled. This result is similar to a prospective study in human medicine, which elucidates the correlation between PAWP and VEGF-D (Houston *et al.*, 2019). Based on the physiological features of lymphatic drainage in cases of chronic volume overload described in early physiological studies, we suspected these changes are mostly attributed to lymphatics proliferation to prevent pulmonary congestion in dogs with myxomatous valvular disease (Uhley *et al.*, 1962). Logically we can predict a correlation between LA pressure, PAWP and blood VEGF-D concentration.

As for ET-1, it was significantly higher in dogs with PrePH, than in the PostPH and control groups. This factor could be explained by microvascular bed remodeling both in the late stages of LSHF and secondary pulmonary arterial remodeling due to lung interstitial disease or pneumonitis. In this context, we can suspect a correlation between ET-1 and right-sided heart failure, because in human studies we can find data about the dependence between ET-1 level and right atrial pressure, and pulmonary artery oxygen saturation (Nootens *et al.*, 1995). Moreover, a recent veterinary study found that dogs with PrePH have a median lifespan of about 276 days, while PostPH dogs—576

days, which shows us possible benefits in diagnosis, monitoring and treatment of the found PH (Borgarelli *et al.*, 2015; Jaffey *et al.*, 2019).

In conclusion, we can say, that the most valuable circulating marker in this study is ET-1. It rose in both Post- and PrePH, which reflects its own diagnostic power of the PH differentiation. But in combination with VEGF-D, it could show the difference between the forms. Subjectively we can recommend these markers as diagnostic criteria of PH in absence of echocardiography, and prediction of the right heart or the left heart failure phenotype.

In this study, several limitations are present. We lack the opportunity to differentiate some specific diseases such as hemangiomas or veno-occlusive disease, which could be presented with similar symptoms, observed in this study's dogs, and could show EcoCG signs of both Pre- and PostPH or shifting between phenotypes (Reinero *et al.*, 2019). But our findings are pointing at the leading wing of the pulmonary microcirculation bed damage. Additionally, we studied acute manifestations, predisposing to speculations in chronic states interpretations or comorbidities. Of course, this study should be powered by more wide prospective study, including more dogs and re-analyzing time and treatment interactions. And we have to find out the specific circulatory and respiratory systems threshold.

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#### Author's contribution

Oleynikov D.: collected data, provided diagnostics and treatment, coordinated the data-analysis and contributed to the writing of the manuscript. Ma Yi: coordinated the data analysis and contributed to the writing of the manuscript.

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**Appendix Table 1.** Echocardiographic-derived measurements and indices. Complex analyze and statistics.

| Indices                           | Quality markers | Control (N = 8)    | PrePH (N = 28)   | PostPH (N = 23)     | Differences between control and PrePH | Differences between control and PostPH | Differences between PrePH and PostPH |
|-----------------------------------|-----------------|--------------------|------------------|---------------------|---------------------------------------|--|--------------------------------------|
| TAPSE (mm)                        |                 | 10.0 (9.6–10.6)    | 10.1 (9.2–12.7)  | 11.0 (10.0–12.8)    | >0.05                                 | 0.040                                  | >0.05                                |
| LA (mm)                           |                 | 25.0 (22.3–35.5)   | 21.5 (19.6–29.9) | 37.0 (33.0–44.8)    | >0.05                                 | 0.004                                  | <0.001                               |
| Ao (mm)                           |                 | 13.8 (11.4–18.8)   | 13.8 (12.1–14.6) | 13.3 (12.0–15.5)    | >0.05                                 | >0.05                                  | >0.05                                |
| LA/Ao                             |                 | 1.9 (1.8–2.0)      | 1.6 (1.4–2.1)    | 2.7 (2.3–3.2)       | >0.05                                 | <0.001                                 | <0.001                               |
| IVS (mm)                          |                 | 6.4 (5.5–8.2)      | 7.4 (6.6–8.2)    | 6.8 (6.0–7.4)       | >0.05                                 | >0.05                                  | 0.026                                |
| LVFW (mm)                         |                 | 6.1 (5.4–7.0)      | 6.3 (5.9–7.1)    | 5.5 (5.0–6.3)       | >0.05                                 | >0.05                                  | 0.005                                |
| LVIDd norm to mass                |                 | 1.9 (1.8–2.0)      | 1.5 (1.4–1.9)    | 2.4 (2.2–2.5)       | 0.024                                 | <0.001                                 | <0.001                               |
| LVIDs norm to mass                |                 | 1.0 (1.0–1.1)      | 0.8 (0.7–0.9)    | 1.2 (1.1–1.3)       | 0.005                                 | >0.05                                  | <0.001                               |
| MR velocity (m/s)                 |                 | 5.5 (5.0–5.8)      | 5.0 (4.5–5.5)    | 5.0 (4.4–5.6)       | >0.05                                 | >0.05                                  | >0.05                                |
| MR degree N (%)                   | 0.              | 0 (0)              | 2 (7.1)          | 0 (0)               | >0.05 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
|                                   | 1.              | 1 (12.5%)          | 5 (17.9%)        | 0 (0%)              | >0.05 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
|                                   | 2.              | 6 (75.0%)          | 16 (57.1%)       | 6 (26.1%)           | >0.05 <sup>a</sup>                    | 0.032 <sup>a</sup>                     | 0.046 <sup>a</sup>                   |
|                                   | 3.              | 1 (12.5%)          | 5 (17.9%)        | 15 (65.2%)          | >0.05 <sup>a</sup>                    | 0.016 <sup>a</sup>                     | 0.001 <sup>a</sup>                   |
|                                   | 4.              | 0 (0%)             | 0 (0%)           | 2 (8.7%)            | >0.05 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
| E (sm/s)                          |                 | 114.5 (98.0–123.8) | 65.0 (48.0–88.8) | 138.0 (120.0–164.0) | <0.001                                | 0.002                                  | <0.001                               |
| A (sm/s)                          |                 | 87.5 (79.8–95.8)   | 53.5 (46.0–83.0) | 79.0 (60.0–100.0)   | 0.018                                 | >0.05                                  | 0.018                                |
| E/A                               |                 | 1.3 (1.1–1.4)      | 0.9 (0.8–1.5)    | 1.8 (1.6–2.2)       | >0.05                                 | <0.001                                 | <0.001                               |
| Diastolic dysfunction class N (%) | 0 cl.           | 1 (12.5)           | 2 (7.1)          | 0 (0)               | >0.05 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
|                                   | 1 cl.           | 0 (0)              | 15 (53.6)        | 0 (0)               | 0.011 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | <0.001 <sup>a</sup>                  |
|                                   | 2 cl.           | 7 (87.5)           | 9 (32.1)         | 12 (52.2)           | 0.012 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
|                                   | 3 cl.           | 0 (0)              | 2 (7.1)          | 11 (47.8)           | >0.05 <sup>a</sup>                    | 0.028 <sup>a</sup>                     | 0.001 <sup>a</sup>                   |
| PV (mm)                           |                 | 10.9 (9.7–13.0)    | 6.6 (4.3–11.0)   | 13.3 (12.5–16.1)    | 0.021                                 | 0.013                                  | <0.001                               |

Continued

| Indices  | Quality markers | Control (N = 8)     | PrePH (N = 28)      | PostPH (N = 23)    | Differences between control and PrePH | Differences between control and PostPH | Differences between PrePH and PostPH |
|--|-----------------|---------------------|---------------------|--------------------|---------------------------------------|--|--------------------------------------|
| RPA (mm)   |                 | 5.8 (5.0–6.9)       | 8.2 (7.0–9.2)       | 6.7 (5.5–7.6)      | 0.001                                 | >0.05                                  | 0.005                                |
| PV/RPA   |                 | 1.9 (1.8–2.1)       | 0.8 (0.6–1.3)       | 2.1 (1.8–2.4)      | <0.001                                | >0.05                                  | <0.001                               |
| Right pulmonary artery dispensability N (%)                  | 0               | 0 (0)               | 16 (57.1)           | 4 (17.4)           | 0.005 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | 0.005 <sup>a</sup>                   |
| (absent-0, weak dispensability -1, normal dispensability -2) | 1               | 0 (0%)              | 11 (39.3%)          | 9 (39.1%)          | >0.05 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
|  | 2               | 8 (100%)            | 1 (3.6%)            | 10 (43.5%)         | <0.001 <sup>a</sup>                   | 0.010 <sup>a</sup>                     | 0.001 <sup>a</sup>                   |
| RV (mm)  |                 | 6.9 (5.6–9.3)       | 11.0 (7.6–13.1)     | 10.5 (8.0–12.0)    | 0.021                                 | 0.009                                  | >0.05                                |
| RV dilated N, (%)  |                 | 0 (0)               | 16 (57.1)           | 10 (43.5)          | 0.005 <sup>a</sup>                    | 0.032 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
| RA (mm)  |                 | 12.3 (11.9–13.2)    | 16.9 (14.9–25.1)    | 17.7 (15.1–19.0)   | <0.001                                | <0.001                                 | >0.05                                |
| RA dilated N, (%)  |                 | 0 (0)               | 26 (92.9)           | 18 (78.3)          | <0.001 <sup>a</sup>                   | <0.001 <sup>a</sup>                    | >0.05 <sup>a</sup>                   |
| RV wall (mm)   |                 | 4 (3.6–4.4)         | 6.0 (5.4–6.8)       | 5.0 (4.2–6.2)      | <0.001                                | 0.005                                  | 0.012                                |
| RV wall hypertrophied N, (%)                                 |                 | 0 (0)               | 21 (75.0)           | 8 (34.8)           | <0.001 <sup>a</sup>                   | >0.05 <sup>a</sup>                     | 0.005 <sup>a</sup>                   |
| PA (mm)  |                 | 10.8 (10.0–11.0)    | 12.0 (10.7–14.3)    | 11.5 (10.0–13.4)   | 0.036                                 | >0.05                                  | >0.05                                |
| PA dilated N, (%)  |                 | 0 (0)               | 28 (100)            | 16 (69.6)          | <0.001 <sup>a</sup>                   | <0.001 <sup>a</sup>                    | 0.002 <sup>a</sup>                   |
| AT (ms)  |                 | 73.5 (66.8–85.8)    | 47.5 (33.5–53.0)    | 50.0 (42.0–63.0)   | 0.005                                 | 0.001                                  | >0.05                                |
| ET (ms)  |                 | 140.5 (123.3–153.0) | 131.0 (109.3–157.5) | 102.0 (92.0–121.0) | >0.05                                 | 0.005                                  | 0.014                                |
| AT/ET  |                 | 0.6 (0.5–0.6)       | 0.4 (0.3–0.5)       | 0.5 (0.4–0.5)      | 0.003                                 | 0.043                                  | 0.005                                |
| LA regurgitation (m/s)                                       |                 | /                   | 2.3 (1.5–3.5)       | 1.7 (1.6–2.4)      | /                                     | /                                      | >0.05                                |

Continued

| Indices                   | Quality markers | Control (N = 8)  | PrePH (N = 28)   | PostPH (N = 23)  | Differences between control and PrePH | Differences between control and PostPH | Differences between PrePH and PostPH |
|---------------------------|-----------------|------------------|------------------|------------------|---------------------------------------|--|--------------------------------------|
| PH by the waveform (N, %) |                 | 0 (0)            | 25 (89.3)        | 14 (60.9)        | <0.001 <sup>a</sup>                   | 0.004 <sup>a</sup>                     | 0.023 <sup>a</sup>                   |
| TR degree (N, %)          | 0               | 4 (50)           | 0 (0)            | 0 (0)            | 0.001 <sup>a</sup>                    | 0.002 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
|                           | 1               | 4 (50)           | 14 (50)          | 12 (52.2)        | >0.05 <sup>a</sup>                    | >0.05 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
|                           | 2               | 0 (0)            | 14 (50)          | 11 (47.8)        | 0.013 <sup>a</sup>                    | 0.028 <sup>a</sup>                     | >0.05 <sup>a</sup>                   |
| TR velocity (m/c)         |                 | 2.5 (1.1–3.0)    | 3.7 (2.9–4.2)    | 3.0 (2.7–4.0)    | 0.001                                 | 0.001                                  | >0.05                                |
| E', sm/s                  |                 | 10.0 (9.0–10.75) | 8.0 (7.0–9.0)    | 8.0 (8.0–9.0)    | 0.017                                 | 0.006                                  | >0.05                                |
| ePLAR                     |                 | 0.08 (0.0–0.24)  | 0.42 (0.30–0.57) | 0.20 (0.16–0.23) | <0.001                                | >0.05                                  | <0.001                               |