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Review

Pulmonary Hypertension Secondary to Myxomatous Mitral Valve Disease in Dogs: Current Insights into the Histological Manifestation and Its Determining Factors

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Abstract: Pulmonary venous hypertension (PVH) is caused by deteriorating left ventricular function. The most common cause of PVH in dogs is myxomatous mitral valve degeneration (MMVD). It causes left ventricular volume overload and an increase in left atrial and pulmonary venous pressure (PVH), which leads to pulmonary vascular wall remodeling and contributes to the perpetuation and worsening of PVH. Pulmonary vascular wall remodeling is also characteristic of pulmonary arterial hypertension (PAH). However, the changes in PVH arise secondary to heart failure and vascular remodeling progresses as the disease progresses. On the other hand, PAH is a primary disease that can be triggered, for example, by the use of certain drugs. Similar structural changes may suggest the influence of similar pathophysiological mechanisms or the intermediation of similar mediators. Therefore, this article discusses recent and hitherto uncommented findings elucidating the pathophysiology of the processes and influences on the pattern of histological changes observed in pulmonary hypertension secondary to degenerative mitral valve disease. In particular, we focus on the activity of factors such as endothelin, serotonin, and nitric oxide, which are involved in pulmonary vascular wall remodeling in both PVH and PAH.

Keywords: endothelin; nitric oxide; serotonin; myxomatous mitral valve disease; pulmonary vessels; pulmonary

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1. Introduction

Mitral valve regurgitation is the most common heart disease in dogs. It is suffered primarily by dogs of small and medium-sized breeds. The most common cause of mitral valve regurgitation is myxomatous mitral valve degeneration (MMVD) [1,2]. Analysis of the population of dogs with MMVD indicates that the development of the disease has a genetic basis, and it is particularly conserved in the population of Cavalier King Charles Spaniels [3–6]. The disease leads to non-inflammatory degenerative changes in the mitral valve leaflet margins, resulting in thickening. This leads to the formation of pathological mitral regurgitation (MR), as well as the rupture of the tendinous cords [7]. The backflow of blood into the left atrium causes an increase in the pressure of blood pushing against its walls (volume overload), resulting in the secretion of atrial natriuretic peptide (ANP), which lowers blood pressure inside the atrium [8–10]. ANP also contributes to cardiac remodeling, i.e., atrial dilatation. This process, called eccentric hypertrophy, allows the size

of the left atrium to adjust to the blood volume so that the left atrial pressure value returns to normal values. The remodeling process involves the left ventricle in parallel, followed by the right ventricle and the right atrium. Typical of the course of congestive heart failure (CHF) due to MMVD are multiple recurrences of pulmonary edema of varying degrees of severity [11], and pulmonary hypertension (PH) [12,13]. Right heart enlargement develops in more severe and/or chronic cases of PH [11].

Hypertension is also often accompanied by a chronic cough [14,15]. Other symptoms commonly associated with PH are syncope and restlessness. Exercise intolerance, weakness, weight loss, and depression are considered non-specific but associated with PH [1,12,13,16–19]. In symptomatic heart failure (stage C according to the American College of Veterinary Internal Medicine, ACVIM), patients with a high probability of PH have a significantly worse prognosis, with a survival time about 2 months shorter compared to patients with a lower probability of PH [16,17,20]. Even after surgical intervention, there are cases of PH recurrence [21]. PH in dogs is found when pulmonary artery blood pressure rises above pulmonary artery pressure (PAP) $\geq$ 25 mm Hg during echographic examination [22,23]. Estimation of PH is based on the assessment of systolic PAP. It is calculated by quantifying the peak velocity of tricuspid regurgitation (TRV) and then deriving the pressure gradient (PG) between the RV and right atrium (simplified Bernoulli equations (PG = 4 $\times$ velocity [m/s]^2) [23]. It is also important to assess the pulmonary artery wedge pressure (PAWP) and the size of the left atrium (LA) [23]. The range of PAP's value in dogs is higher than that in humans, in whom PH, according to the current World Symposium on Pulmonary Hypertension guidelines, is found at values exceeding 20 mm Hg [24,25].

In general, increased PAP may be due to six reasons (pulmonary arterial hypertension (PAH), venous pulmonary hypertension (PVH), pulmonary hypertension secondary to respiratory disease/ischemia, pulmonary embolism, due to cardiovascular parasites, and idiopathic pulmonary hypertension) [23]. However, for this review, the first two are the most important: pulmonary arterial hypertension (classified in the group of precapillary PH conditions), which is caused by primary vascular lesions in the pulmonary arteries [26–29], and pulmonary venous hypertension, which is associated with left ventricular heart failure (classified in the group of postcapillary PH conditions) [23]. The first disease is much rarer and the former is a primary, chronic, and progressive disorder leading to endothelial and smooth muscle proliferation and dysfunction, and is one of the most serious vasculopathy, in addition, the exact causes of the disease's development are still unknown [30,31]. It is characterized by mean PAP $\geq$ 25 mm Hg, PAWP $\leq$ 15 mm Hg, and consistently elevated pulmonary vascular resistance (PVR) (no left atrial enlargement is observed) [23]. The second disease is most often seen in dogs with MMVD, dilated cardiomyopathy, or congenital defects such as ductus arteriosus persistence (hypertension associated with left heart disease). In the context of venous pulmonary hypertension, it is also said that there are two phases in its pathomechanism. The first phase is isolated postcapillary PH (Inc-PH); a phase in which mean PAP $\geq$ 25 mm Hg, pulmonary artery wedge pressure PAWP > 15 mm Hg, diastolic pressure gradient (DPG) < 7 mm Hg, and there is no diagnosis of increased PVR (presence of enlarged atrium). This is the result of the transfer of the blood pressure gradient from the left atrium to the pulmonary veins. The second phase is called the combined precapillary and postcapillary PH (Cpc-PH) [32]. In the combined phase, higher values of PVR, and DPG $\geq$ 7 mm Hg are recorded, and this is also the phase of pulmonary vascular remodeling [23,33]. Experimental studies have demonstrated the timing of the transition from the Inc-PH phase to Cpc-PH, and have been able to link the expression of certain genes to the combined phase [34,35]. Differentiating these two phases is proving to be clinically important and provides a better understanding of the mechanism of hypertension secondary to MMVD.

Recently, several studies have been presented that have shed new light on the pathogenesis of venous pulmonary hypertension, so the purpose of this review is to summarize the knowledge on this topic and highlight the processes that lead to the development of the different stages of PVH, which in some places have points in common with PAH.

2. Histopathology in PVH Secondary to MMVD

Lesions caused by abnormal function or structure of the left heart, which leads to venous pulmonary hypertension, primarily involve blood vessels. However, their presence is also noted in the lung tissue and lymphatic vessels [36–38] (Table 1). These changes are the result of disturbed mechanisms related to increased vasoconstriction, suppressed vasodilatory effect, and disruption of cell cycle mechanisms.

2.1. Pulmonary Vessels

The biphasic nature of the pathomechanism of PVH is reflected in the sequence of histological changes. Ipc-PH is the passive phase of venous hypertension, as its features are conditioned by the underlying cardiac disease (e.g., MMVD) [39]. This is manifested by increased filling and congestion of pulmonary veins. Persistent chronic PH stimulates the initiation and progression of vascular remodeling [40]. The presence of changes in the histological structure of the vessels is considered a feature of PVH in the Cpc-PH phase.

Long-term PVH causes an increase in arterial and venous vessel wall thickness, in both humans and animals [22,37,41]. Thickening is seen in all layers of the vascular wall [42]. Analysis of the literature indicates that two processes are responsible for this condition: activation of the processes of hypertrophy and hyperplasia and inhibition of apoptosis of pulmonary vascular wall cells. In dogs, hypertrophy, hyperplasia of smooth muscle cells in the medial layer, and deposition of connective tissue in the appendage have been most commonly demonstrated in PVH [37]. Among them, by far the greatest changes are in the muscular layer of the vasculature, which is known as the thickening of the medial [37]. An increase in external diameter due to MMVD is observed both in PVH conditions secondary to the disease and even in the period preceding PH [43]. However, fully masculinized vessels represent an increasingly high percentage in dogs with PVH [43]. Patients with MMVD and coexisting PVH also showed a higher ratio of medial layer thickness to total vessel diameter compared to patients with MMVD without PVH [37,44–46]. It should also be added that this ratio increases in proportion to increasing PVH [44,47]. Other types of PH, of unknown origin, showed concentric (obstructive) hypertrophy of the vessel wall [48]. One such cause is pulmonary arteriopathy, which leads to PAH [49]. Interestingly, vascular occlusion is not observed in PVH. The difference between PVH and PAH in terms of the degree of endothelial proliferation is puzzling. In the case of PVH, the presence of excessive endothelial proliferation was excluded, which is typical in PAH [37,43].

The increased number of muscle fibers is due to an increase in the number of actively proliferating smooth muscle cells, which is proportional to the increase in pressure, significantly increasing the number of smooth muscle cells in the middle layer [37,45]. These results confirm previous speculations that the processes of hyperplasia and hypertrophy are involved in the pathogenesis of PVH [50,51]. Vascular epithelial growth factor (VEGF) has an active role in this process. The main role of VEGF is to promote vascular remodeling during normal development, but also during disease. VEGF has been linked to increased vascular resistance, vascular remodeling, and lung congestion [52–54]. Studies during experimental damage to cardiac function in dogs showed increased expression of mRNA corresponding to VEGF [55]. The increase in VEGF appeared to be most significant in PVH, in contrast to PAH [56]. In addition, there was a less pronounced, but also significant, increase in VEGF receptor 2 expression (VEGF2). VEGFR2 expression data may have therapeutic relevance to the availability of VEGFR2 inhibitors. Other studies have shown that the increased number of smooth muscle cells may also result from inhibition of pro-apoptotic activity [45]. It has been indicated that the appearance of PVH in dogs with left heart failure leads to a significant decrease in the synthesis of bcl-2-like protein 4 (BAX), caspase-3, and caspase-8 proteins (pro-apoptotic proteins) and an increase in the expression of anti-apoptotic B-cell lymphoma-2 (BCL-2) [45]. Proteomic studies, in dogs with MMVD and PVH, revealed an increase in the synthesis of apoptosis resistance proteins and the progression of medial thickening [57]. The study detected the proteins whose changes were most significant: up-regulation of salt-induced kinase 3 (SIK3), collagen type I alpha

chain 1 (COL1A1), and transforming growth factor alpha (TGF-α) and down-regulation of apoptosis-associated tyrosine kinase (AATYK), hepatocyte growth factor activator (HGFA) and non-receptor protein tyrosine phosphatase type 13 (PTPN13) [57]. The SIK family of proteins is one of the regulators of myocardial inflammation and fibrosis through the activation of apoptosis signal-regulating kinase 1 (ASK1) [58,59]. Intermediates in the signal for apoptosis through the ASK1 pathway are growth factors [59]. Stimulation of growth factor activation also occurs in tumor cells, which are known to have impaired apoptosis processes, which may account for the higher TGF-α activity in vessel wall cells [60]. Interestingly, differences between the pulmonary artery and peripheral pulmonary arteries were indicated in terms of which proteins were synthesized the most [57]. One study showed a difference in the expression of DNA-binding inhibitor (ID) proteins, factors that regulate DNA transcription, at different phases of PVH [35]. Namely, the protein expression of ID2 activity, a factor that controls, for example, VEGF (one of the stimulators of endothelial proliferation, which also controls the proliferation of muscle cells found in the vascular wall), has been linked to the Cpc-PH phase [61]. Arwood et al. (2019) also noted that pathways they called "important for the cell cycle" were significantly involved, thus arguing the hypothesis of reduced apoptosis intensity among vessel wall cells [35]. A similar genetic response was obtained in a mouse model during a validation study in the same paper. Obtaining similar results is a strong argument for the hypothesis that these genes are responsible for the development of Cpc-PH, especially since the effect of their expression is consistent with the phenotype of Cpc-PH. In contrast, the peptide barcode for the different phases of MMVD in dogs is shown, distinguishing the presence or absence of PVH [62].

Collagen deposition in the layers of blood vessels was documented in an experimental model of pulmonary hypertension in other species [63], and it is caused by increased proliferation of myofibroblasts. The process most likely aims to strengthen the basal lamina to stop excessive accumulation of extracellular fluid. Endothelin and VEGF activity may lead to such changes. In one case of primary PAH in dogs, in addition to severe medial thickening, fibrosis limited only to the vessels was indicated, located mainly in the arterial vessel wall [64]. Current work on PVH in dogs lacks direct studies on changes in myofibroblast activity.

The current suggestion is that inflammatory cells are involved in increasing pulmonary vascular diameter [43,65]. In one study, the perivascular number of B lymphocytes, T lymphocytes, and macrophages was significantly increased in the MMVD + PVH group compared to the MMVD without PVH and control group. The accumulation of these cells was observed even before the onset of remodeling in larger vessels, while small arterioles were already undergoing masculinization processes [43]. Inflammatory cell infiltration in PVH can be stimulated by many factors. Serotonin, in addition to the vasoactive properties described earlier, can enhance the immune response [66–69].

Table 1. Histological changes in the muscular layer of arterial vessels in dogs with MMVD and MMVD + PH. SMCs—smooth muscle cells (average); %MT—percentage of medial thickness; %PCNA—the percentage of proliferating cell nuclear antigen.

Source	Tissue		Parameter		Control Group	MMVD	MMVD + PH
[37]	Lung		Diameter	Internal	266.15 ± 20.47	268.56 ± 20.62	255.92 ± 28.97
				External	299.46 ± 23.06	345.51 ± 28.38	376.76 ± 29.06
[43]	Lung	20–100 µm	%MT		13.04 ± 0.82	23.70 ± 1.47	32.44 ± 1.65
		101–200 µm			12.60 ± 1.09	24.54 ± 1.45	30.48 ± 2.09
		201–300 µm			12.77 ± 1.27	23.44 ± 1.28	30.51 ± 1.82
		301–400 µm			11.71 ± 0.86	22.40 ± 1.58	32.52 ± 1.63
[37,44,45]	Lung		%MT		11.04 ± 0.95	22.36 ± 1.44	32.25 ± 5.06

Table 1. *Cont.*

Source	Tissue	Parameter	Control Group	MMVD	MMVD + PH
[45]	Lung	SMCs	4.87 ± 0.24	16.61 ± 1.91	23.44 ± 2.18
[45]	Lung	SMCs	29.04 ± 2.15	51.65 ± 4.11	69.53 ± 8.33
[37]	Lung	%PCNA	1.91 ± 0.21	14.99 ± 1.07	6.06 ± 0.61

2.2. Lymphatic Vessels

Excessive hydrostatic pressure may result in dysregulation of the lymphatic system and the expansion of the lymphatic bed [70,71]. VEGF, which is also a stimulator of lymphogenesis, may also be involved in this process, and an increase in its activity has been noted in PH [55,56,72] (Table 1). VEGF also causes an increase in vascular permeability, which is why it is sometimes called vascular permeability growth factor [73]. VEGF exists in multiple splicing forms that bind to appropriate cellular receptors. The VEGF-C and VEGF-D forms that bind to receptor 3 (VEGFR3) are responsible for the development of lymphatic vessels [74]. The strongest stimulator of VEGF production is hypoxia [75,76], and one of the consequences of PVH development is hypoxia [77].

2.3. Pulmonary Tissue

Pulmonary hypertension leads to congestion, pulmonary edema, and thickened alveolar septae [45,78]. The accumulation of cells in air spaces in dogs is still a poorly studied topic. Red blood cells that enter the alveoli are engulfed by macrophages contributing to the formation of siderophages (cardiac defect cells) (Figure 1). In PVH, VEGF creates favorable conditions that promote the formation of transudates and the passage of blood cells into the alveoli [36,73]. In addition, thickening of the inter-alveolar septa due to collagen deposition is observed [36,45,79]. In particular, areas of atelectasis are predisposed to collagen deposition [78]. It is suggested that this is related to the remodeling of the extracellular matrix (mainly glycosaminoglycans), which becomes a storage site for extravascular fluid created by increased hydrostatic pressure in the vessels [80].

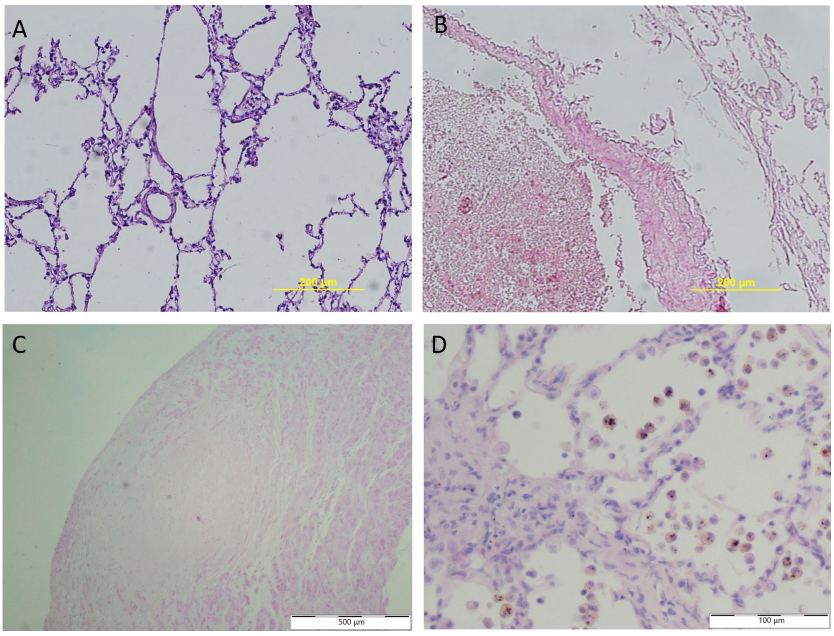

Figure 1. Microscopic changes during venous hypertension in dogs. (**A**) Enhanced emphysematous changes in the lungs, (**B**) Thickening of the pulmonary artery wall, (**C**) Degenerative changes in the pulmonary artery membrane, (**D**) Hemosiderin-laden macrophages in the alveolar lumen. Hematoxylin-eosin staining.

2.4. Morphology of Blood Cells

Assessing the relationship between changes in blood parameters and the occurrence of PH is difficult due to the impact of various disease states on hematological and biochemical values. This is no different in the case of MMVD, where changes have been identified in, among other things, oxidative status, urea levels, creatinine, or Red blood cell distribution width (RDW) [81–84]. Several attempts have been made to correlate RDW with PH and indicate its clinical utility. However, an increase in the anisocytosis rate was obtained only in severe MMVD in dogs [85]. Other papers did not even indicate a difference between postcapillary PH and control dogs [86,87]. It also failed to show a difference in RDW values between dogs with precapillary PH and postcapillary PH [85–87].

3. Molecular Changes in PVH Secondary to MMVD

The changes in vascular structure outlined earlier are mainly the result of disruption of various signaling pathways and in the secretion of cell mediators (Table 2). In the following paragraphs, we attempt to discuss the effects of individual substances on the development of PVH.

3.1. Endothelin 1

Endothelin 1 (ET1) is a major vascular remodeling factor. ET1 is a peptide that plays a key role in vasoconstriction and is secreted by vascular endothelial cells, enabling a significant reduction in vessel lumen within a short time of secretion [88,89]. Its higher levels have been demonstrated in various conditions caused by heart disease or in the experimental induction of cardiac dysfunction in dogs [55], and it is highly active in the lungs in pathological conditions [90]. ET1, acting through its receptor ET-A or B, whose expression can increase up to threefold in hypertensive states, strongly stimulates arterial constriction [55]. In dogs, the ET-B receptor is the main one responsible for removing ET1 from the circulation via the respiratory system. Therefore, the removal of ET1 from the circulation will mainly be influenced by perfusion in the pulmonary vasculature, which is known to be impaired in hypertensive states [91]. The threefold increase in ET-B expression in hypertensive states is most likely a response to increased ET-1 concentrations in the circulation. Accumulation of ET1 in the circulation leads to an increased efficiency of ET1 binding to the ET-A receptor, which is mainly located in the blood vessel [92]. The crucial role of the ET1 and its receptors was demonstrated, in a canine model of chronic embolic pulmonary hypertension [93]. Dogs in which hypertension was induced were divided into a treated and untreated group with bosentan, which is a nonselective ET-1 receptor antagonist. Dogs did not show significant changes in vascular wall structure as a result of treatment, but both treated and untreated dogs showed an increase in ET1 [93]. Hence, ET1 is one of the most important factors leading to increased PVR. High PVR is characteristic of the Cpc-PH phase, making it like PAH. It should be noted that Cpc-PH also has other features of PAH, namely: vascular changes and high levels of ET1 [94]. The fact that PVH at some point takes on the characteristics of PAH is confirmed by studies on dogs and swine [34,56]. In dogs, ET1 levels were compared in patients with PVH, including Ipc-PH and Cpc-PH in patients with PAH. The highest increase in ET1 levels was in the PAH group, but a significant increase was also seen in PVH [56]. In dogs, ET1 levels were compared in patients with PVH, including Ipc-PH and Cpc-PH in patients with PAH. The highest increase in ET1 levels was in the PAH group, but a significant increase was also seen in PVH [34]. Perhaps, if the Ipc-PH phase had been distinguished from the Cpc-PH phase in the dog study, similar changes in endothelin levels would also have been recorded. ET1 accumulation in the circulation can be induced experimentally. The consequences of impaired pulmonary perfusion are illustrated by a study in rats with ETA antagonists and ETB receptor deficiency [65]. Suppressed receptor function leads to the development of severe pulmonary hypertension and the accumulation of reactive oxygen species [65]. The hemodynamic changes occurring during Ipc-PH lead to ET-1 accumulation and increased effects on the vessel wall, resulting in vascular remodeling. However, further research is needed to be certain of this.

Table 2. Values and changes; endothelin, VEGF, nitric oxide AND serotonin in healthy dogs and dogs with MMVD (PH −/+). (ppa—The average percentage of positive areas, rpe—The relative protein expression). Abbreviations: tryptophan hydroxylase 1—TPH-1; 5-hydroxytryptamine (serotonin) receptor 2a—5-HTR2A; serotonin transporter—SERT; extracellular regulated kinase ½—ERK1/2; phosphorylated ERK1/2—pERK1/2; serotonin—SRT; nitric oxide—NO; vascular endothelial growth factor—VEGF; vascular endothelial growth factor receptor—VEGFR; endotheline—ET; endotheline B-receptor—ET, B; not applicable—n/a. Green—significant increase; red—significant decrease.

Source	Tissue		Biomarker [Unit]	Control Group	MMVD	
					MMVD (PH+)	MMVD (PH−)
Endothelin and endothelin's receptors						
[55]	Blood (plasma)		Et [pg/mL]	17.8 (15.0–19.2)	20.6 (17.2–23.1)	n/a
[71]	Lung		ET [−]	n/a	3-fold increase in the concentration	n/a
[71]	Lung		ET, B [−]	n/a	3-fold increase in the concentration	n/a
Vascular endothelial growth factor and VEGF's receptors						
[55]	Blood (plasma)		VEGF [pg/mL]	33.1 (29.7–36.9)	81.2 (73.3–96.2)	n/a
[71]	Lung		VEGF [−]	n/a	3-fold increase in the concentration	n/a
[71]	Lung		VEGFR [−]	n/a	3-fold increase in the concentration	n/a
Oxide nitric						
[95]	Blood (plasma)		NO [µM]	n/a	25.88 (15.08–36.71)	n/a
Serotonin value, expression of the receptors, and associated protein						
[55]	Blood (plasma)		SRT [ng/mL]	26.1 (21.0–30.7)	26.6 (22.4–30.5)	n/a
[96]	Blood	plasma	SRT (ng/mL)	2.92 (1.76–7.50)	1.75 (1.19–2.72)	1.23 (0.27–4.23)
[96]	Blood	Platelet	SRT [ng/109 platelets]	179.73 (102.37–352.24)	135.11 (21.21–312.22)	325.99 (96.84–407.66)
[44]	Lung		TPH1 [ppa]	0.86 ± 0.19	8.07 ± 0.73	5.43 ± 0.34
			SERT [ppa]	0.58 ± 0.05	9.87 ± 0.43	4.56 ± 0.44
			5HT2A [ppa]	1.48 ± 0.11	7.06 ± 0.83	45 ± 0.46
			ERK [ppa]	0.80 ± 0.2	7.78 ± 0.35	1.61 ± 0.47
			pERK [ppa]	0.00 ± 0.0	6.61 ± 0.85	0.00 ± 0.00
[97]	Lung		TPH-1 [rpe]	2.33 (0.58–4.09)	1.56 (0.68–5.08)	1.59 (0.52–5.43)
			SERT [rpe]	1.14 (0.09–3.10)	1.49 (0.24–1.69)	0.46 (0.04–1.00)
			5-HTR2A [rpe]	4.40 (0.97–10.75)	3.15 (2.36–8.88)	2.59 (1.12–9.46)
			ERK1/2 [rpe]	1.74 (0.68–6.37)	2.16 (1.04–4.29)	1.41 (0.72–5.38)
			pERK1/2 [rpe]	0.72 (0.13–2.01)	0.21 (0.07–2.59)	1.02 (0.35–4.09)
	Arteries		TPH-1 [rpe]	0.65 (0.49–1.12)	1.85 (0.51–2.95)	0.81 (0.29–4.09)
			SERT [rpe]	0.23 (0.14–0.39)	0.40 (0.04–0.74)	0.14 (0.04–0.63)
			5-HTR2A [rpe]	0.68 (0.44–1.00)	1.58 (0.50–2.57)	0.99 (0.37–4.67)
			ERK1/2 [rpe]	0.86 (0.73–1.04)	1.04 (0.48–4.15)	0.81 (0.54–4.23)
			pERK1/2 [rpe]	0.61 (0.35–0.89)	0.56 (0.10–2.87)	0.45 (0.11–2.26)

3.2. Nitric Oxide

Nitric oxide (NO) is one of the mediators of the dynamic balance between vasoconstriction and vasodilation of blood vessels [98]. NO leads to the relaxation of the smooth muscle of the middle layer of the vessel wall through the activation of guanosine cyclase [99]. NO synthesis takes place in the endothelium of blood vessels [98]. NO is a radical with an unstable structure, so it reacts easily and has a short half-life, so its level is estimated from the concentration of its metabolites in the blood, which have greater stability [100]. Higher values of nitrate and nitrite have been indicated in dogs with cardiac conditions, but it has not been possible to relate their severity and type [100]. In contrast, in humans, increasing NO production is indicated in PVH states, and its values are higher compared to other PH [42,95,101]. However, the cause–effect relationship between changes in NO activity is still not precisely known. Given that the severity of MMVD in dogs is associated with vascular endothelial dysfunction [102], therefore, a decrease in NO can be seen early in the disease [103]. However, the increase in NO with the progression of the disease is not explained, and an increase in compensatory processes is considered a possible cause [95]. Endothelial dysfunction is a possible cause of the lower levels of cyclic guanosine monophosphate (cGMP) in the middle layer of the blood vessel wall [104] and may be the reason for the low levels of cGMP noted in PH, thus the weaker dilation effect [105,106]. Abnormalities of the phosphatidylinositol 3-kinase-protein kinase B pathway of endothelial NO synthesis (PIK-3k/AKT/eNOS) are an important cause of reduced NO production and persistence of high cellular calcium concentrations [107,108]. Disruption of this mechanism may result, among other things, from the activity of reactive oxygen species (ROS), elevated concentrations of which are observed in MMVD states [65,109,110]. An important cofactor in the synthesis of nitric oxide (eNOS), whose activity is impaired by ROSs, is tetrahydrobiopterin (BH4) [111]. ROSs, as compounds with high redox potential, readily react with BH4. The oxidation reaction results in the formation of BH2, which cannot integrate into the NO metabolism pathway, so it is inhibited [111]. In dogs, the concentration of BH4 increases as the disease progresses, due to a compensatory increase in BH4 production [102]. BH4 accumulation is also potentiated by competing with BH2, for a binding site with eNOS [102]. Increased BH2 levels, associated with MMVD severity, are correlated with progressive vascular endothelial damage [102]. The effects of NO on PVH pathophysiology are complex and still poorly understood; however, clinicians have successfully stimulated NO production using phosphodiesterase-5 (PDE-5) inhibitors, which act by blocking the degradative action of cGMP-specific phosphodiesterase type 5 (PDE5) on cyclic GMP in smooth muscle cells [98,105,112–114].

3.3. Serotonin

Serotonin (SRT) is a hormone well known for its activity in the central nervous system. Its biosynthesis takes place in the nervous system, it is also accumulated in thrombocytes [115]. SRT, similar to ET-1, has vasoconstrictive effects in dogs [116–119]. The effect of SRT on blood vessels is demonstrated by the use of serotonin reuptake inhibitors in dogs, as in the case of fenfluramine, which leads to vasoconstriction and PAH [120]. In another study, chronic treatment of dogs with fenfluramine resulted in increased activation of protein kinase C, which is one of the main factors in smooth muscle contractility in blood vessels [121]. The correlation between increased SRT concentrations in the circulation and increased activity of the PLC/IP3 pathway, which activates protein kinase C, signals that SRT and ET-1 may act in a synergistic manner, which is also involved in protein kinase C activation [122]. Elevated serum SRT levels were detected in dogs with MMVD which, depending on the severity of the disease, may suffer from PVH [123]. SRT secreted from platelets has a direct mitogenic effect on vascular smooth muscle cells, which may be one of the factors stimulating hyperplasia and hypertrophy of vessel walls [124,125], and a decrease in the number of SRT granules in platelets is associated with an increase in PVH or an increase in MMVD [96,126–129]. In PVH, there is increased expression of proteins such as the serotonin transporter (SERT), tryptophan hydroxylase 1 (TPH1), extracellular

regulated kinase ½ (ERK1/2), and phosphorylated ERK1/2 (pERK1/2) [44]. In addition, pERK1/2 was only noted in the PH state [44]. These changes were recorded mainly in the middle layer of the canine artery, which is similar to processes in humans [44,130]. A negative correlation between thrombocytic SRT and increasing plasma and serum SRT levels has also been described in humans. Due to the evidenced effects of SRT, most authors consider SRT to be one of the factors causing the development of MMVD [131–134], however, recent studies have not shown a clear role of SRT pathway gene expression in the development of PH in dogs, but have indicated increased protein synthesis of tryptophan hydroxylase 1 (TPH-1) and 5-hydroxytryptamine (serotonin) receptor 2a (5-HTR2A) in the MMVD and MMVD + PH states relative to the control group [97].

In conclusion, the high activity of SRT pathway proteins and increased expression of serotonin receptors suggests that it may be one of the factors enhancing PVH pathogenesis, but not one critical to it. An obstacle to realistically determining the role of SRT in PVH pathogenesis is the interracial differences in its reference level [135].

4. Conclusions

PVH in dogs is most often the result of the progress of degenerative mitral valve disease. Histological and molecular changes in the pulmonary vessels, pulmonary tissue, and lymphatic bed are certainly a compilation of malfunctioning pathways controlling vasoconstriction and vasodilation. However, it is still unclear which mechanism is crucial to the histologic picture we obtain. At the forefront are the consequences of increased endothelin, whose removal is impeded by altered pulmonary vascular perfusion. The second key factor, in our opinion, is damage to the vascular endothelium, by which the balance of vascular tone is severely disturbed, through reduced nitric oxide secretion. In addition, the pressure that is exerted on the vascular wall, combined with increased oxidative activity in the patient, can lead to cell cycle disruption in the cells of the muscle wall. There is also no doubt that the vessels, defending themselves against PVH, take compensatory actions such as remodeling of perivascular connective tissue, including thickening of interalveolar septae, enrichment of blood vessel walls with deposits of collagen, or increasing the lymphatic bed (e.g., as a consequence of increased hydrostatic pressure in the veins). However, it is not easy to determine whether a particular histopathological/molecular change in response is the result of an imbalance in the cardiovascular system or is already a compensatory mechanism (as in the case of nitric oxide, which had lower levels in patients with early MMVD than in healthy controls and had higher levels in patients with advanced MMVD than in controls). In conclusion, the topic requires further histological and molecular studies for the best understanding of these mechanisms. It also appears that the changes seen in PVH are like those seen in PAH; however, they are less expressed (as in the correlation between endothelin increase and the onset of vascular lesions). However, some aspects significantly differentiate the two entities, such as, among others, increased endothelial proliferation in PAH, which has not yet been demonstrated in PVH dogs. Primary vascular lesions may become a source of inspiration for further research on PVH, and research should therefore be promoted on PAH as well.

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