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Idiopathic and connective tissue disease-associated pulmonary arterial hypertension (PAH): Similarities, differences and the role of autoimmunity

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ABSTRACT

Pre-capillary pulmonary arterial hypertension (PAH) is hemodynamically characterized by a mean pulmonary arterial pressure (mPAP) ≥ 20 mmHg, pulmonary capillary wedge pressure (PAWP) ≤15 mmHg and pulmonary vascular resistance (PVR) > 2. PAH is classified in six clinical subgroups, including idiopathic PAH (IPAH) and PAH associated to connective tissue diseases (CTD-PAH), that will be the main object of this review. The aim is to compare these two PAH subgroups in terms of epidemiology, histological and pathogenic findings in an attempt to define disease-specific features, including autoimmunity, that may explain the heterogeneity of response to therapy between IPAH and CTD-PAH.

1. Introduction

Pulmonary hypertension (PH) is a hemodynamic condition defined by an increase in mean pulmonary arterial pressure (mPAP) ≥ 20 mmHg at rest, as assessed by right heart catheterization (RHC) [1]. According to the 2022 guidelines of the European Society of Cardiology (ESC) and European Respiratory Society (ERS), PH is classified in five groups based on hemodynamic profile, clinical presentation, pathology, and therapeutic management [1] (Fig. 1). Group 1 PH is characterized by pulmonary capillary wedge pressure (PAWP) ≤15 mmHg and a PVR > 2 Wood units, the opposite of post-capillary PH, which is characterized by a PAWP >15 mmHg and a PVR ≤ 2 [1].

Group 1 PH, that will hereafter be referred to as pre-capillary pulmonary arterial hypertension (PAH), is also histologically characterized by proliferation and remodeling of the pulmonary small arteries, with progressive occlusion and increased pulmonary vascular resistance (PVR), leading to right ventricle hypertrophy/remodeling, hence right heart failure.

A hemodynamic profile similar to PAH is also observed in chronic thromboembolic PH (group IV) (Fig. 1) but, in this case, small vessel occlusion is due to chronic multiple thromboembolisms [2].

As outlined in Fig. 1, PAH is classified in 6 subgroups, namely: 1) idiopathic PAH (IPAH); 2) PAH associated to diseases such as connective tissue diseases (CTD), portal hypertension, HIV and other infections; 3) heritable PAH (HPAH); 4) drugs and toxins-induced PAH, 5) PAH with features of pulmonary venous/capillary involvement (PVOD/PCH), and 6) persistent PH of the newborn (Fig. 1).

Although they share common hemodynamic features, these 6 subgroups differ in terms of epidemiology, prognosis, and response to therapy, indicating different genetic backgrounds and pathogenic

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mechanisms underlying the PAH onset. For instance, IPAH and CTD-associated PAH (CTD-PAH) are the most commons PAH subtype in the Western world [3]. Patients with CTD-PAH have a poorer prognosis than those with IPAH while, among CTD-PAH, systemic lupus erythematosus (SLE)-associated PAH has a better response to therapy than systemic sclerosis (SSc)-associated PAH [4–7].

Aim of this review is to highlight similarities and differences between IPAH and CTD-PAH in terms of epidemiology, histological features, pathogenesis, and role of autoimmunity to gain a better understanding of heterogeneity in the response to therapy.

2. Epidemiology of PAH

Patient registries with PAH have been used as a tool for characterizing the epidemiology, survival and natural history of the disease, and have permitted comparisons between populations in different geographic areas [8].

Even so, the collection of accurate epidemiology data of all forms of PH and its subsets in registries has been a difficult task in this field mainly due to: a) different ethnicity among the groups studied, b) lack of a uniform disease definition, c) differences in the diagnostic approach applied (echocardiography *vs* RHC) and d) community-based heterogeneity, and e) inclusion of both incidental (patients who have just received a diagnosis) or "prevalent" cases (patients who had previously received a diagnosis) preceded by data normalization [8,9].Obviously, an ideal analysis should be based on incident cases only [10], instead of performing statistics on both incidental and prevalent.

For the above reasons, epidemiological data on PAH differ markedly worldwide. In western contemporary registries, the IPAH incidence ranges from 0.9 to 2.6 cases per million inhabitants/ year, and the prevalence from 4.6 to 9 cases per million adults [5,10–18]. In Asian countries only few epidemiological data are available about IPAH, not allowing any substantial comparison with other areas worldwide.

Similarly to CTD-PAH, a female preponderance is commonly observed in IPAH, although a certain variability in the female to male ratio has been reported among registries, ranging from 1.5:1 in Europe [19] to 4:1 in the US [14,20].

Surprisingly, the mean age at diagnosis of IPAH has greatly increased over time, ranging between 45 ± 14 and 65 ± 15 years in current registries, compared to 36 ± 15 years in the first registry created in 1981 (U.S. National Institutes of Health Registry) [19]. The reason for this difference is likely the greater diagnostic accuracy and precision than in the past, in defining PAH clinical settings profiles, thereby reducing cases of wrongly diagnosed IPAH in younger patients.

In Western patients, IPAH is overwhelmingly the most frequent PAH subtype, accounting for 30–48% [11,12,14,17,21], while CTD-PAH is the second most common subtype with an estimated prevalence ranging from 15% to 30% [11,12,14,17,21]. By contrast, the most common subtype in Asia is congenital heart disease (CHD)-PAH (43%), followed by IPAH (35%) and CTD-PAH (19%) [8,22]. Among CTD-PAH, SSc-PAH is the most prevalent in the US (62%) [14] and Europe (61%–76%) [1,15,17], while in Asia high rates of SLE-PAH (51%) have been reported, and SSc-PAH accounts for only 9% [8,22].

3. Pathology of vascular lesions leading to PAH

PAH is characterized by complex arterial lesions involving pre- and intra-acinary pulmonary arteries [23]. Early changes in the endothelial layer are responsible for a specific picture defined as pulmonary vascular disease (PVD) [24]. PVD is characterized by a markedly decreased diffusing lung capacity for carbon monoxide (DLCO), despite an almost normal or only slight decrease of forced vital capacity (FVC), while the FVC/DLCO ratio always remains above 1. At this stage, in most patients PAP is still normal and the patient is usually asymptomatic. Even so, when a FVC/DLCO ratio increase >1.6 occurs, the risk of development of PAH is higher, as suggested by the results of the PHAROS study, showing that a FVC/DLCO ratio > 1.6, along with a DLCO <55%, is highly predictive of PAH development [25,26]. Indeed, in the portion of PVD+ patients that will develop PAH, endothelial changes will evolve dramatically into a continuous anarchic remodeling (vascular remodeling) generating the clinical condition of PAH, one of the major causes of death in CTD-PAH patients.

In patients with PAH, like IPAH and CTD-PAH, vascular remodeling involves all layers of the pulmonary arterial vessels. The histopathological key features of vascular remodeling are listed in Table 1. More specifically, constrictive lesions, such as intimal thickening, media hypertrophy and adventitial thickening, are the result of an imbalance between proliferation and apoptosis of the various cell types forming the vascular walls [23]. Intimal thickening, mainly composed of myofibroblasts and smooth muscle cells (SMC) [27,28] may be eccentric, concentric non-laminar or concentric laminar, the latter being specific to SSc [23]. Media hypertrophy is the result of either expansion of elastic fibers of muscular arteries or SMC hyperplasia and hypertrophy [23]. Adventitial thickening is due to the deposition of collagen by adventitial fibroblasts. Plexogenic lesions consist of slit-like channels of endothelial cells that have undergone proliferation, surrounded by myofibroblasts,

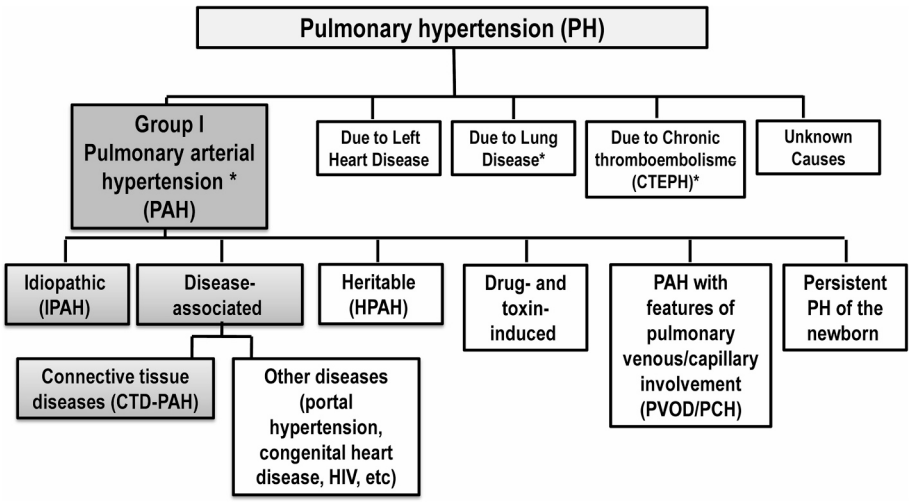

Fig. 1. Clinical classification of pulmonary hypertension according to the 2022 guidelines of the European Society of Cardiology (ESC) and European Respiratory Society (ERS).
PAH subtypes object of this review are in grey boxes. *Pre-capillary PH.

Table 1
Pre-capillary arteries histopathogical changes in idiopathic pulmonary arterial hypertension (IPAH) as compared to connective tissue disease (CTD) -associated PAH (CTD-PAH).

Histology findings	PAH subgroup				
	IPAH	CTD-PAH			
		SSc	MCTD	SLE	RA
Intimal thickening	Yes	Yes	Yes	Yes	Yes
Intimal fibrosis	RO	Yes	Yes	Yes	Yes
Plexogenic lesion	Yes	RO	RO	Yes	NR
Media hypertrophy	Yes	Yes	Yes	Yes	Yes
Adventitial thickening	Yes	NR	NR	NR	NR
Inflammatory infiltrates	Yes	Yes	Yes	Yes	Yes
Fibrinoid vasculitis	Yes	RO	RO	Yes	NR
PVOD-like pattern	No	Yes	SF	SF	SF

MCTD, mixed connective tissue disease; NR, not reported; PAH, precapillary pulmonary arterial hypertension; PVOD, pulmonary veno-occlusive disease; RA, rheumatoid arthritis; RO, rarely observed; SF, severe form; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

SMC and extracellular matrix. Finally, the PVOD-like pattern is characterized by capillary congestion in the alveolar parenchyma, and obliterative fibrosis of venules.

As depicted in Table 1, cellular intimal thickening, media hypertrophy and inflammatory infiltrates [29–31] are all common to both IPAH and CTD-PAH. By contrast, plexogenic lesions and fibrinoid vasculitis are often observed in IPAH and SLE-PAH, but rarely seen in SSc-PAH and MCTD-PAH [29,32,33].

Adventitial thickening occurs more commonly in IPAH than in CTD-PAH [27,34]. On the other hand, arterial intimal fibrosis rarely occurs in IPAH, while it is a common feature of PAH associated to SSc, SLE, MCTD, and rheumatoid arthritis (RA) [29,32].

Similarly, the PVOD-like pattern is rarely observed in IPAH, being more often detected in SSc-PAH and in the most severe forms of CTD-PAH, including SLE, MCTD and RA [29,32] (Table 1).

Overall, the evidence suggests that while PVOD-like pattern and concentric laminar intimal thickening are associated with poor response to therapy, plexogenic lesions and fibrinoid vasculitis, which are more frequently found in IPAH and SLE-PAH, are associated with a more favorable response to therapy, and hence more favorable prognosis.

4. Pathogenesis

4.1. The role of genetics

In the last decades more insight into the pathogenesis of PAH has revealed similarities in genetic background and pathogenic mechanisms between IPAH and CTDs-PAH.

The first evidence for the genetic role in PAH was reported in heritable PAH [35,36], in which a high frequency of bone morphogenic protein (BMP) 2 receptor (BMPR2) gene mutations were found. BMPR2 belongs to the TGF-β superfamily. Upon BMPR2 binding to its ligand BMP, an inhibition of endothelial and SMC proliferation and migration occurs, preventing neointimal formation [37,38]. Some BMPR2 gene mutations are therefore associated to the reduction of BMPR2 expression and of signaling, together with an increased SMC proliferation and migration. These mutations associated to BMPR2 defects of expression (and/or function) have been detected in about 80% of patients with heritable PAH, 10 to 40% of patients with IPAH [39,40], but rarely in *CTD-PAH*, including RA, and Sjögren's syndrome [41,42]. Even so, PAH is observed in only 20% of BMPR2 mutations carriers [43], suggesting that additional, still unknown genetic, epigenetic and/or environmental factors contribute to the development of the disease. In addition, the finding of a BMPR2 protein expression reduction in BMPR2 wild-type patients with IPAH [44] and SSc-PAH [45,46] suggests that additional mechanisms, besides BMPR2 mutations, might decrease BMPR2 expression, thus contributing to the PAH onset. This is the case of the loss of SRY-Box Transcription Factor 17 (SOX17) in endothelial cells shown, by an *in vitro* study, to be associated to the reduction in BMR2 expression, and impairment of BMP signaling [47], predisposing to PAH.

Genome- and exome-sequencing studies have revealed additional genes, other than BMPR2, carrying rare pathogenic mutations, predisposing to both IPAH and CTD-PAH (mainly SSc- and SLE-PAH) (Table 2) [39,42,48–52]. These include other TGF-β/BMP signaling-related genes (GDF2, ACVRL1, ENG, SMAD9), potassium channel genes, namely KCNK3, KCNA5, ABCC8, and transcription factors such as T-Box factor 4 (TBX4) and SOX17, the latter two involved in lung and vascular development, respectively (Table 2). Even so, some of these genes have not yet been validated (Table 2).

4.2. Molecules contributing to endothelial dysfunction

Endothelial dysfunction plays a pivotal role in the pathogenesis of PAH. The imbalance between vasodilator *Vs* vasoconstrictor and/or anticoagulant *Vs* coagulant factors promotes vascular remodeling. More specifically, overproduction of endothelin-1 (ET-1) leading to vasoconstriction, impaired synthesis of the vasodilator nitric oxide (NO) and the prostacyclin (PGI2)/thromboxane A2 (TXA2) imbalance, favoring thrombosis, have been recognized as the main contributors to vascular remodeling in the PAH pathogenesis (Fig. 2).

4.2.1. ET-1

ET-1 is produced by endothelial cells and acts as a potent vasoconstrictor and inducer of smooth muscle cell (SMC) proliferation. These effects occur through ET-1 binding to two endothelin receptor isoforms, namely type A (ETAR) and type B (ETBR). By contrast, the binding of ET-1 to endothelial cell-associated ETBR (the only one to be expressed on these cells) induces the release of NO and PGI2 [53], hence vasodilation and inhibition of platelet aggregation/activation. Increased levels of ET-1 have been reported in the plasma [54] and lungs [53] of patients with IPAH. Elevated plasma ET-1 levels have also been found in both SSc [55] and SSc-PAH patients [56] and are correlated with impaired right ventricular function [55].

4.2.2. NO, and related molecules

NO is a potent pulmonary vasodilator, and acts as an inhibitor of platelet activation and SMC proliferation [57]. It is produced by the vascular endothelium following NO synthase activation. There are three NO synthase isoforms (NOS), namely neuronal (nNOS), endothelial (eNOS) and inducible NOS (iNOS), the latter being activated during inflammation. NO is rapidly converted to its catabolite (NOx), which is the most representative marker of NO production. NOx levels are directly correlated to NO production. In fact, a low NOx plasma level reflects low NO production and this is the reason why NOx is lower in IPAH patients than in controls and inversely correlated with mPAP, PVR and survival [58]. The activity of all three NOS isoforms is negatively regulated by asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor. Plasma levels of ADMA are increased in IPAH patients and associated with an unfavorable pulmonary hemodynamic outcome [59]. Similarly, increased ADMA levels are associated with the presence of PAH, directly correlated with the severity of PAH [60,61] and inversely correlated with 6-min walking test results in CTD-PAH including SSc-PAH [62].

4.2.3. PGI2/TXA2

PGI2 is synthesized and released by endothelial cells. It is the catabolic product of arachidonic acid following the action of two well-known enzymes, namely cyclooxygenase-2 (COX-2) and prostacyclin synthase, the former being up-regulated by inflammation [63] and hypoxia [64] (Fig. 2).

Once released by endothelial cells, PGI2 acts as a powerful inhibitor of platelet aggregation and also promotes vasodilation. Its activity is

Table 2
Gene variants involved in PAH pathogenesis.

Gene	Encoded protein	Protein function	Validation of alleles variants (AV) predicted to be deleterious		References
			Validation	Comments	
GDF2	BMP-9	Ligand of BMPR2 and ALK1, promoting vascular stability	Not validated	NA	[42,49,146]
ACVRL1	ALK1	Endothelial receptor mediating TGFβ/BMP signaling	Validated by clinical and instrumental data	AV are directly associated to high mPaP and PVR and low 6Mwt in FPAH	[39,42,52]
ENG	Endoglin	TGFβ coreceptor, involved in TGFβ/BMP signaling	Not validated	NA	[42,52]
SMAD9	SMAD8	Downstream effectors of BMP signaling	Validated	Assessed by hereditary analyses in HPAH	[42,52]
KCNK3	TASK1	Potassium channel	Validated	Assessed by hereditary analyses in HPAH	[42,52]
KCNK5	KCNA5	Potassium voltage-gated channel	Validated	AV are directly associated to high mPaP and PVR and low 6Mwt	[39,42,50]
ABCC8[a]	SUR1	Subunit of the ATP-sensitive potassium channel	Validated	Loss of function (in IPAH e HPAH)	[42,48,50]
TBX4	TBX4	Regulation of embryonic lung development	Not validated	NA	[42,146]
SOX17	SOX17	Regulation of vascular differentiation	Validated	Assessed by hereditary analyses in HPAH	[42,52]
EIF2AK4	GCN2	Downregulation of protein synthesis	Not validated	NA	[42,146]
ATP13A3	ATPase 13A3	Transport of cations across membranes	Not validated	NA	[42]
CAV1	Caveolin-1	Regulation of eNOS activity	Not validated	NA	[42]
KDR[a]	VEGFR2	Regulation of angiogenesis	Validated	Assessed by hereditary analyses in HPAH	[51]
FBLN2[a]	Fibulin-2	Protection against vascular injury, regulation of blood pressure	Validated	AV are directly associated to high mPaP and PVR and low 6Mwt	[51]
PDGFD[a]	PDGF-D	Regulation of cell proliferation, migration and survival	Validated in PDGF-Knock in mouse overexpressing PDGF in the heart	Vascular smooth muscle cell proliferation and vascular remodeling with wall thickening	[51]

AV, alleles variants; CTD-PAH, connective tissue disease-associated pulmonary arterial hypertension; HPAH, hereditary pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; mPAP, mean pulmonary arterial pressure; NA, not available; PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance; 6MWT, six minutes walking test.
[a] IPAH only.

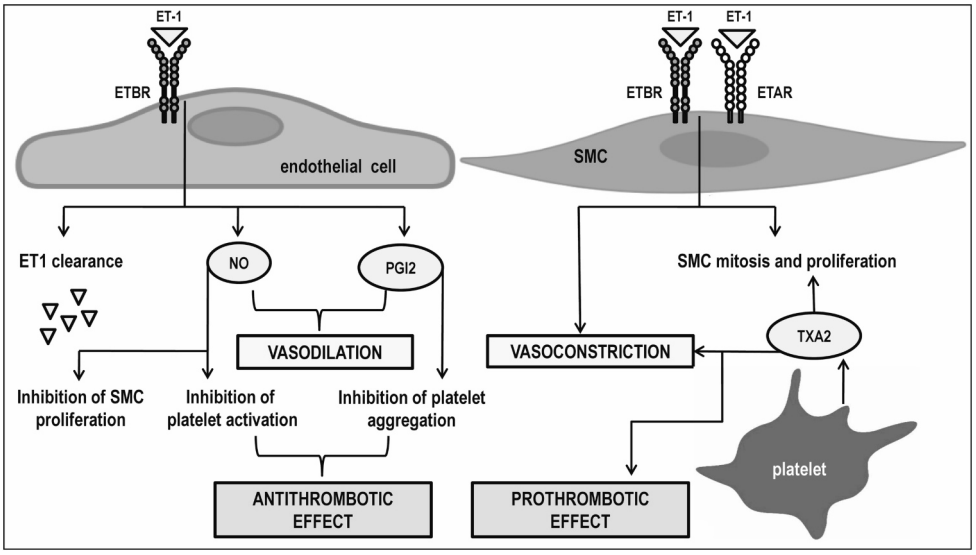

Fig. 2. Vascular tone is maintained by a fine-tuning balance between vasodilator *Vs* vasoconstrictor and/or anticoagulant *Vs* coagulant factors.
The binding of endothelin-1 (ET-1) to the endothelin receptor type B (ETBR) on endothelial cells mediates ET-1 clearance and stimulates the release of nitric oxide (NO) and prostacyclin (PGI2). NO inhibits smooth-muscle cells (SMC) proliferation and, along with PGI2, induces vasodilatation. In addition, both NO and PGI2 produce antithrombotic effects by inhibiting platelet activation and aggregation, respectively. All these protective effects against PAH are counterbalanced by the action of ET-1 itself as well as of the prothrombotic platelet-derived thromboxane A2 (TXA2) on SMC. Indeed, ET-1 binding to its receptors ETBR and ETAR, along with TXA2 signaling, induces vasoconstriction and SMC mitosis and proliferation.

counterbalanced by the prothrombotic and vasoconstrictor effect of TXA2, produced by platelets. An imbalance between PGI2 (decreased production/release) and TXA2 (increased production/release) [65] occurs in IPAH and CTD-PAH, resulting in vasoconstriction and vascular SCM proliferation [65]. In fact, in IPAH patients, a decreased PGI2 expression has been detected in small and medium-sized pulmonary arteries walls, while no expression has been found in plexiform and concentric lesions [66].

4.2.4. *Vascular endothelial growth factor (VEGF)*

In the intricate network of cytokines involved in the pathobiology of PAH, VEGF, a potent angiogenic cytokine which enhances vascular permeability, occupies a pivotal position.

The VEGF family comprises five members, namely VEGF-A, VEGF-B,

VEGF-C, VEGF-D, and Placental Growth Factor. For each member, several VEGF splice-variant isoforms are generated, which signal through specific distinct receptors known as VEGFR1, VEGFR2, and VEGFR3.

VEGF-A is the most powerful angiogenic cytokine with pro-survival and vascular permeability-enhancing effects, exerted primarily by binding to VEGFR2, and to a lesser extent to VEGFR1 [67].

There is considerable evidence supporting the involvement of VEGF-A in PAH, although also some controversy. Elevated plasma levels of VEGF-A have been documented in IPAH [68–71] and SSc-PAH [70,72,73]. In both diseases, plasma [69] or serum [74] VEGF-A levels are directly correlated with PVR. *Adachi* et al. [74] reported higher levels of serum VEGF-A165b, an inhibitory splice variant of VEGF-A, in CTD-PAH as compared with control but not in IPAH. Conversely, another study by *Suzuki S* et al. demonstrated higher plasma levels of VEGF-A165b in IPAH than in controls, but not in CTD-PAH [75]. These discrepancies may be explained by the different biological samples used for VEGF-A165b assay determination, as the former employed serum samples [74] and the latter plasma samples [75].

Placental Growth Factor, a VEGF family member, is a pro-survival and pro-angiogenic factor which exerts these effects through binding to VEGFR-1. Placental Growth Factor is now recognized as a marker for PAH in SSc [76]. In the study by *Adachi* et al. [74], Placental Growth Factor serum levels were higher in CTD-PAH than IPAH or healthy controls and correlated with 6MWD and BNP levels. In the same study, the Placental Growth Factor serum titer was significantly lower in IPAH than in healthy controls [74]. These results are at variance with those reported by *Tiede SL*, who found that Placental Growth Factor levels were significantly elevated in both CTD-PAH and IPAH as compared with the non-PH control group [77].

High levels of soluble VEGFR1 (sVEGFR1) have been detected in CTD-PAH and IPAH [70,78]. In SSc patients, sVEGFR-1 was higher in patients with PH than in those without PH [76], as well as in patients that would later develop PAH than in those who would not [70]. On the other hand, VEGFR-2 has been found to be overexpressed in plexiform lesions from IPAH patients [79,80], while low levels of soluble VEGFR-2 have been detected in IPAH and SSc-PAH as compared to controls [71].

4.2.5. CXCL12

CXCL12, also known as stromal cell-derived factor (SDF-1), is a chemokine belonging to CXC family. It is expressed and secreted by various tissues and cell types and regulates several biological processes, including embryogenesis, organogenesis, angiogenesis and vasculogenesis, and tissue repair. CXCL12 acts through its binding to CXCR4 and CXCR7 receptors. The axis CXCL12/CXCR4 is critical for chemotactic endothelial cell migration [81,82]. There is also evidence that links this axis to enhancement of VEGF signaling. Indeed, *in vitro* studies have suggested that CXCL12 promotes VEGF release [83–86] which, in turn, increases CXCR4 expression on endothelial cells [83], sensitizing these cells to CXCL12 signaling. On the other hand, the axis CXCL12/CXCR7 has been suggested to regulate endothelial proliferation and vascular regeneration and repair [82].

Several evidences have suggested that CXCL12 is involved in the pathogenesis of PAH, including IPAH and CTD-PAH. Indeed, increased plasma levels of CXCL12 have been found in both IPAH and CTD-PAH when compared to controls [82,87], with CXCL12 being associated to a poor prognosis [87]. In addition, a marked increase in the expression of CXCL12 and its receptors CXCR4 and CXCR7 has been detected in lung tissue and pulmonary infiltrates from IPAH patients [82,88]. In SSc, high levels of CXCL12 have been detected in microvascular endothelial cells in early stages of the disease [89], and a CXCL12 polymorphism has been associated with PAH [90].

4.2.6. Caveolin-1

Caveolin-1, the major protein constituent of caveolae plasma membrane, interacts (and forms complexes) with a wide range of proteins, including eNOS, VEGFR [91], and the reactive oxygen species (ROS)-producing enzyme dihydronicotinamide-adenine dinucleotide phosphate (NADPH) oxidases [92] (Fig. 3A). Under physiological conditions, the binding of caveolin-1 to the aforementioned proteins negatively regulates their activity, and maintains ROS and NO in a steady state, favoring vasodilation through Protein Kinase G (PKG) (Fig. 3A).

Defective caveolin-1 expression causes the persistent production of NO and ROS (mainly superoxide) which rapidly inter-react to form peroxynitrite (Fig. 3B). The latter, in turn, induces the nitration of PKG, its functional impairment and hence vasoconstriction [93].

As caveolin-1 is a down regulator of vascular endothelial growth factor (VEGF) receptor, reduced caveolin-1 expression can mediate an up-regulation of VEGF signaling, favoring PAH (Fig. 3B). Low expression of caveolin-1 has been detected in endothelial cells of lung tissues from IPAH patients [93]. Furthermore, caveolin-1 serum levels were significantly lower in IPAH as compared to chronic obstructive pulmonary disease-PAH and non-PAH subjects [94]. Finally, supporting the role of caveolin 1 in PHA, is that its expression in bone marrow mesenchymal stem cells from SSc patients is significantly lower than in cells from healthy subjects [95].

4.2.7. The Warburg effect

Growing evidence has demonstrated that mitochondrial and intracellular metabolism dysfunction are involved in the pathogenesis of PAH [96]. In this context, a major key role is played by the shift from mitochondrial oxidative phosphorylation to glycolysis in the cytosol, known as the Warburg effect, which leads to the inhibition of apoptosis and promotion of proliferation (Fig. 4). The Warburg effect is mediated by the pyruvate dehydrogenase complex (PDC), the gate-keeping enzyme of glucose oxidation. PDC is a multi-enzyme complex composed of pyruvate dehydrogenase (E1 component), dihydrolipoamide acetyltransferase (E2 component), and dihydrolipoamide dehydrogenase (E3 component), whose activity is regulated by the reversible phosphorylation of the E1 component catalyzed by pyruvate dehydrogenase kinase (PDK). There are four PDK isoforms (PDK1–4) that regulate the activity of PDC by modulating its phosphorylation state. PDK-1 and PDK-2 are strongly up-regulated in IPAH-pulmonary SMC and, to a lesser extent, in endothelial cells, fibroblasts, and inflammatory cells [97], while PDK4 expression has been found in pericytes of PAH patients lungs and correlated with pericyte proliferation and survival [98].

Besides PDK, the activity of PDC can be enhanced by Sirtuin-3 (SIRT-3), the main mitochondrial deacetylase. A down-regulation of SIRT-3 has been found in IPAH-pulmonary arterial SMC compared to normal cells [99]. Additionally, a *SIRT-3* loss-of-function polymorphism has been associated with IPAH but not with CTD-PAH [99].

Another molecule which appears to be strictly related to PAH is transcription factor hypoxia inducible factor 1α (HIF-1α), whose activity is regulated by the oxygen state (Fig. 4). Under normoxic conditions, HIF-1α undergoes a proteosomal-dependent degradation while in hypoxic conditions it accumulates, inducing the expression of target genes, including VEGF, CXCR4 and PDK [100,101] (Fig. 4).

HIF-1α is expressed in plexiform lesions [102] and a high HIF-1α expression and transcriptional activity has been found in IPAH-endothelial cells as compared to controls [103].

4.3. The role of autoimmunity

The detection of autoantibodies in IPAH patients sera [104–107] has suggested that autoimmunity might play a role in PAH pathogenesis, similarly to what was observed earlier in CTD-PAH [108–111]. Some autoantibodies (Ab) can be found in both IPAH and CTD–PAH, and some Ab found only in CTD-PAH.

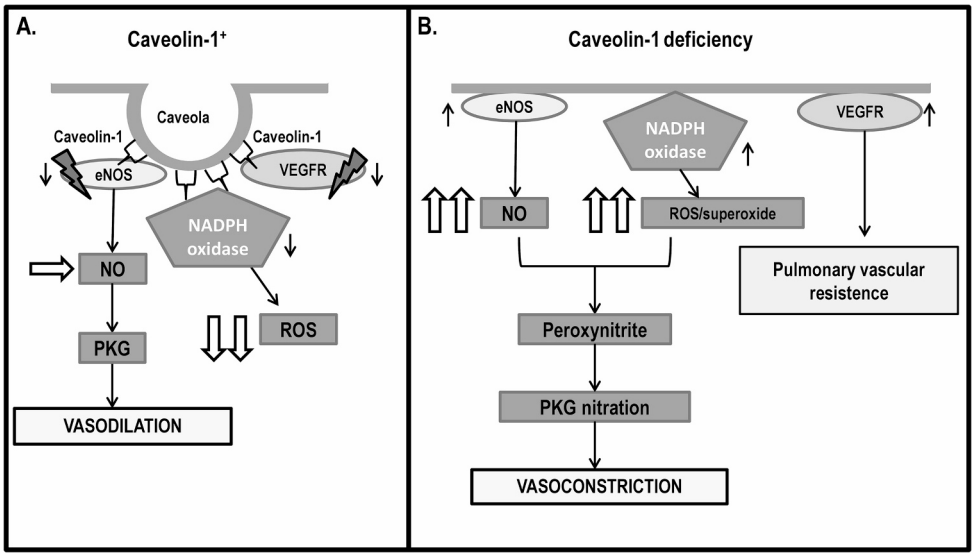

Fig. 3. The role of caveolin-1 in pulmonary arterial hypertension.
eNOS, endothelial nitric oxide synthase; NADPH, dihydronicotinamide-adenine dinucleotide phosphate; NO, nitric oxide; PKG, Protein Kinase G; ROS, reactive oxygen species; VEGFR, vascular endothelial growth factor receptor.

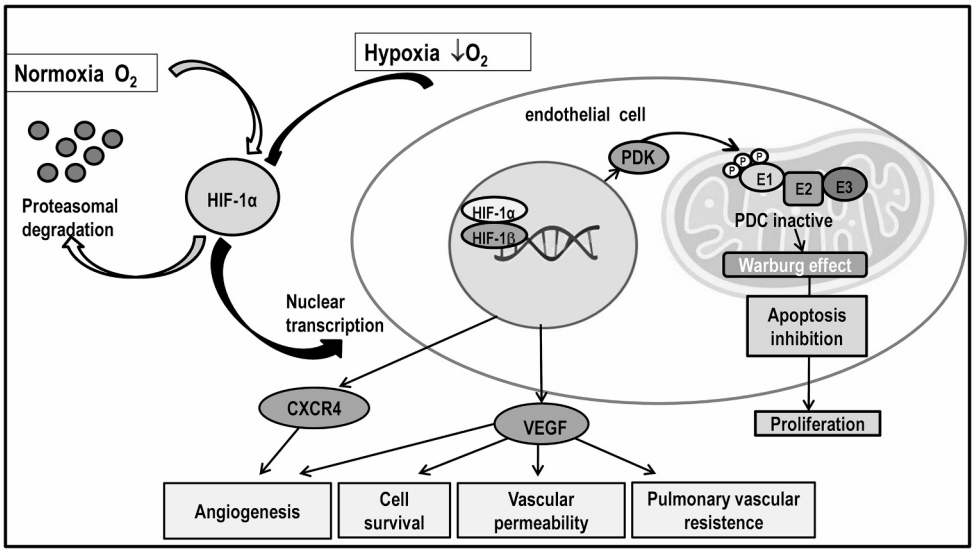

Fig. 4. Hypoxia-inducible factor (HIF) signaling in pulmonary arterial hypertension.
Under normoxia, HIF-1a is degraded by the ubiquitin-proteasome system. Under hypoxic conditions, HIF-1a migrates to the nucleus and activates the transcription of genes, including the pro-angiogenic receptor CXCR4, vascular endothelial growth factor (VEGF) and pyruvate dehydrogenase kinase (PDK). VEGF contributes to pulmonary vascular resistance, besides promoting angiogenesis, cell survival and vascular permeability. On the other hand, PDK inactivates the mitochondrial pyruvate dehydrogenase complex (PDC) through the phosphorylation of the E1 component, and induces the Warburg effect, which results in apoptosis inhibition and vascular cell proliferation.

4.3.1. Autoantibodies common to both IPAH and CTD-PAH

4.3.1.1. Anti-endothelial antibodies (AECA). AECA have been detected in 77.8% of CTD-PAH and in 62.1% of IPAH [105]; their more significant antigenic targets were found to be Laminin A/C and tubulin β-chain [112].

Although expressing distinct reactivity profiles [113], IgG AECA from CTD-PAH and IPAH patients have been demonstrated to have a functional role in enhancing pro-adhesive and pro-inflammatory molecules *in vitro* [114].

In IPAH patients only the AECA IgG/IgM ratio is correlated with the severity of the disease [105]. In SSc and MCTD, the prevalence of AECA is higher in patients with PAH than in those without PAH [105,115,116].

More recently, Ab targeting BMPR2 have been detected in a subset of patients with IPAH but not in CTD-PAH [107]. Ab directed against the type I BPM receptors BMPR1A and Activin Receptor-Like Kinase type 1 (ALK1) have been found in SLE-PAH patients at higher titers than in SLE patients without PAH [117].

Ab to angiotensin receptor type 1 (AT1R) mediate pleiotropic vasoconstrictor and pro-inflammatory effects of angiotensin II. These Ab, together with Ab to endothelin-1 type A receptor (ETAR), have been detected in SSc-PAH at a higher frequency and at higher levels than in IPAH [109]. Moreover, high titers of both Ab have been associated with a worse prognosis in SSc-PAH [109]. In SLE-PAH, anti-ETAR Ab occur more frequently in patients with PAH than in those without PAH, and

their levels appear to be correlated with sPAP, as determined by echocardiograaphy and RHC [110]. Finally, *in vitro* and *in vivo* studies have shown that anti-ETAR and anti-AT1R Ab have also been found to be involved and contribute to the SSc pathogenesis [109,118–120]. More recently, anti-ETBR Ab have been detected in SSc-PAH and IPAH, and higher titers have been found in SSc-PAH patients than in healthy donors [121]. However, the potential pathogenic role of anti-ETBR Ab has not yet been investigated.

4.3.1.2. Anti-fibroblast antibodies (AFA). AFA have been described in SSc [122,123] and have been detected in 30% of patients with SSc-PAH and 40% of those with IPAH [104]. Common AFA target antigens in SSc-PAH and IPAH have been identified by two-dimensional immunoblotting, and include proteins involved in the regulation of cytoskeletal function, cell contraction, oxidative stress, cell energy metabolism, namely vimentin, calumenin, tropomyosin 1, heat shock proteins 27 and 70, glucose-6-phosphate-dehydrogenase [124]. In SSc, a pathogenic role of AFA has been suggested by their ability to promote fibroblast activation and induce a pro-inflammatory and pro-adhesive phenotype [125,126]. Whether AFA can exert similar functions in IPAH remains to be established.

4.3.2. CTD-PAH associated autoantibodies

4.3.2.1. Anti-centromeric proteins (CENP) antibodies. Anti-CENP Ab (ACA), whose main targets are CENP-B and CENP-A, have been detected in 20–30% of patients with SSc and in up to 80% of patients with limited cutaneous involvement [26,123,127,128]. Patients positive for ACA are at higher risk of developing PAH than ACA negative patients [129]. Anti-CENP-B Ab have been suggested to contribute to the pathogenesis of SSc [130–132] by interfering with the normal wound healing co-mediated by CENP-B in pulmonary arterial SMC, and thus promoting vascular remodeling [130,132,133]. To date, there is no evidence of a potential pathogenic role of anti-CENP-A Ab in SSc. Our group has recently identified anti-CENP-A Ab subgroups whose levels are associated with clinical features of PVD in ACA positive patients [26,128].

More recently, we demonstrated that anti-CENP-A Ab subsets can cross-react with the *E2* component of the mitochondrial PDC, the major autoantigen in primary biliary cirrhosis (PBC) [123]. These findings, along with the increased risk of PBC in SSc ACA+ AMA+ patients [134], suggest a pathogenic significance of anti-CENP-A Ab cross-reacting with PDC-E2. Whether these Ab may interfere with the PDC activity, which seems to play a role in PAH, can only be speculated as yet.

4.3.2.2. Anti-U1 ribonucleoprotein (RNP) antibodies. Anti-U1 RNP Ab serve as marker of MCTD but these Ab are detectable in other CTD, such as SSc (6.6% -11.3% of patients) [135,136], and SLE (13–30% of patients) [137]. Several studies have demonstrated a strong association between anti-U1 RNP Ab and PAH in SLE [138–140], as well as in SSc and MCTD [141,142]. Moreover, a recent meta-analysis study has identified anti-U1 RNP Ab as a risk factor for PAH in CTD-patients [143]. *Okawa-Takatsuji* et al. demonstrated that anti-U1 RNP Ab purified from CTD patients are able to induce the expression of adhesion molecules and MHC class II molecules on pulmonary artery endothelial cells [144,145], suggesting their potential role in triggering vasculopathy.

5. Conclusions

The increasing understanding of the PAH pathogenesis has highlighted the central role of endothelial dysfunction in both IPAH and CTD-PAH diseases. Interestingly, growing evidence is accumulating, demonstrating that, like CTD-PAH, IPAH is characterized by an autoimmune signature, with Ab targeting endothelial cells and fibroblasts. These findings may pave the way to novel therapeutic approaches for IPAH. Despite the many pathogenic factors shared by IPAH and SSc-PAH, both diseases are characterized by distinctive features, namely the Warburg effect in the former and the high prevalence of Ab directed against ETAR, AT1R and the presence of ACA in the latter. The evidence that these autoantibodies are pathogenic in SSc might explain, at least in part, the poor response to therapy in this disease as compared to IPAH. Therapies targeting these Ab may offer a valuable strategy to counteract PAH development and/or progress in SSc-PAH.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

[1] Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, et al. 2022 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J 2022 October 11;43(38):3618–731.
[2] Kim NH, Delcroix M, Jais X, Madani MM, Matsubara H, Mayer E, et al. Chronic thromboembolic pulmonary hypertension. Eur Respir J 2019 January;53(1).
[3] Lau EMT, Giannoulatou E, Celermajer DS, Humbert M. Epidemiology and treatment of pulmonary arterial hypertension. Nat Rev Cardiol 2017 October;14 (10):603–14.
[4] Condliffe R, Kiely DG, Peacock AJ, Corris PA, Gibbs JS, Vrapi F, et al. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. Am J Respir Crit Care Med 2009 January 15;179(2):151–7.
[5] Chung L, Liu J, Parsons L, Hassoun PM, McGoon M, Badesch DB, et al. Characterization of connective tissue disease-associated pulmonary arterial hypertension from REVEAL: identifying systemic sclerosis as a unique phenotype. Chest 2010 December;138(6):1383–94.
[6] Prete M, Fatone MC, Vacca A, Racanelli V, Perosa F. Severe pulmonary hypertension as the initial manifestation of systemic lupus erythematosus: a case report and review of the literature. Clin Exp Rheumatol 2014 March;32(2): 267–74.
[7] Rhee RL, Gabler NB, Sangani S, Praestgaard A, Merkel PA, Kawut SM. Comparison of treatment response in idiopathic and connective tissue disease-associated pulmonary arterial hypertension. Am J Respir Crit Care Med 2015 November 1;192(9):1111–7.
[8] McGoon MD, Benza RL, Escribano-Subias P, Jiang X, Miller DP, Peacock AJ, et al. Pulmonary arterial hypertension: epidemiology and registries. J Am Coll Cardiol 2013 December 24;62(25 Suppl):D51–9.
[9] Miller DP, Gomberg-Maitland M, Humbert M. Survivor bias and risk assessment. Eur Respir J 2012 September;40(3):530–2.
[10] Lee WT, Ling Y, Sheares KK, Pepke-Zaba J, Peacock AJ, Johnson MK. Predicting survival in pulmonary arterial hypertension in the UK. Eur Respir J 2012 September;40(3):604–11.
[11] Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Pulmonary arterial hypertension in France: results from a national registry. Am J Respir Crit Care Med 2006 May 1;173(9):1023–30.
[12] Peacock AJ, Murphy NF, McMurray JJ, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. Eur Respir J 2007 July;30(1):104–9.
[13] McGoon MD, Krichman A, Farber HW, Barst RJ, Raskob GE, Liou TG, et al. Design of the REVEAL registry for US patients with pulmonary arterial hypertension. Mayo Clin Proc 2008 August;83(8):923–31.
[14] Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, et al. Pulmonary arterial hypertension: baseline characteristics from the REVEAL registry. Chest 2010 February;137(2):376–87.

[15] Humbert M, Sitbon O, Yaïci A, Montani D, O'Callaghan DS, Jaïs X, et al. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. Eur Respir J 2010 September;36(3):549–55.

[16] Frost AE, Badesch DB, Barst RJ, Benza RL, Elliott CG, Farber HW, et al. The changing picture of patients with pulmonary arterial hypertension in the United States: how REVEAL differs from historic and non-US contemporary registries. Chest 2011 January;139(1):128–37.

[17] Escribano-Subias P, Blanco I, Lopez-Meseguer M, Lopez-Guarch CJ, Roman A, Morales P, et al. Survival in pulmonary hypertension in Spain: insights from the Spanish registry. Eur Respir J 2012 September;40(3):596–603.

[18] Ling Y, Johnson MK, Kiely DG, Condliffe R, Elliot CA, Gibbs JS, et al. Changing demographics, epidemiology, and survival of incident pulmonary arterial hypertension: results from the pulmonary hypertension registry of the United Kingdom and Ireland. Am J Respir Crit Care Med 2012 October 15;186(8):790–6.

[19] Hoeper MM, Huscher D, Ghofrani HA, Delcroix M, Distler O, Schweiger C, et al. Elderly patients diagnosed with idiopathic pulmonary arterial hypertension: results from the COMPERA registry. Int J Cardiol 2013 September 30;168(2):871–80.

[20] Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE, McGoon MD. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL registry. Chest 2012 August;142(2):448–56.

[21] Thenappan T, Shah SJ, Rich S, Gomberg-Maitland M. A USA-based registry for pulmonary arterial hypertension: 1982-2006. Eur Respir J 2007 December;30(6):1103–10.

[22] Zeng WJ, Sun YJ, Gu Q, Xiong CM, Li JJ, He JG. The impact of pulmonary arterial hypertension-targeted therapy on survival in Chinese patients with idiopathic pulmonary arterial hypertension. Pulm Circ 2012 July;2(3):373–8.

[23] Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. J Am Coll Cardiol 2004 June 16;43(12 Suppl S):25S–32S.

[24] Chandra S, Shah SJ, Thenappan T, Archer SL, Rich S, Gomberg-Maitland M. Carbon monoxide diffusing capacity and mortality in pulmonary arterial hypertension. J Heart Lung Transplant 2010 February;29(2):181–7.

[25] Hsu VM, Chung L, Hummers LK, Wigley F, Simms R, Bolster M, et al. Development of pulmonary hypertension in a high-risk population with systemic sclerosis in the pulmonary hypertension assessment and recognition of outcomes in scleroderma (PHAROS) cohort study. Semin Arthritis Rheum 2014 August;44(1):55–62.

[26] Favoino E, Catacchio G, Mininni A, Ruscitti P, Riccieri V, Liakouli V, et al. Novel biomarker for pulmonary vascular disease in systemic sclerosis patients. Clin Exp Rheumatol 2022 October;40(10):1956–63.

[27] Stacher E, Graham BB, Hunt JM, Gandjeva A, Groshong SD, McLaughlin VV, et al. Modern age pathology of pulmonary arterial hypertension. Am J Respir Crit Care Med 2012 August 1;186(3):261–72.

[28] Tuder RM, Stacher E, Robinson J, Kumar R, Graham BB. Pathology of pulmonary hypertension. Clin Chest Med 2013 December;34(4):639–50.

[29] Dorfmüller P, Humbert M, Perros F, Sanchez O, Simonneau G, Müller KM, et al. Fibrous remodeling of the pulmonary venous system in pulmonary arterial hypertension associated with connective tissue diseases. Hum Pathol 2007 June;38(6):893–902.

[30] Liu SF, Nambiar VN, Li Q, Kucherenko MM, Knosalla C, Kuebler WM. Pulmonary hypertension: linking inflammation and pulmonary arterial stiffening. Front Immunol 2022;13:959209.

[31] Parperis K, Velidakis N, Khattab E, Gkougkoudi E, Kadoglou NPE. Systemic lupus erythematosus and pulmonary hypertension. Int J Mol Sci 2023 March 7;24(6).

[32] Overbeek MJ, Vonk MC, Boonstra A, Voskuyl AE, Vonk-Noordegraaf A, Smit EF, et al. Pulmonary arterial hypertension in limited cutaneous systemic sclerosis: a distinctive vasculopathy. Eur Respir J 2009 August;34(2):371–9.

[33] Pogoriler JE, Rich S, Archer SL, Husain AN. Persistence of complex vascular lesions despite prolonged prostacyclin therapy of pulmonary arterial hypertension. Histopathology 2012 October;61(4):597–609.

[34] Chazova I, Loyd JE, Zhdanov VS, Newman JH, Belenkov Y, Meyrick B. Pulmonary artery adventitial changes and venous involvement in primary pulmonary hypertension. Am J Pathol 1995 February;146(2):389–97.

[35] Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. J Med Genet 2000 October;37(10):741–5.

[36] Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips III JA, Loyd JE, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. Nat Genet 2000 September;26(1):81–4.

[37] Valdimarsdottir G, Goumans MJ, Rosendahl A, Brugman M, Itoh S, Lebrin F, et al. Stimulation of Id1 expression by bone morphogenetic protein is sufficient and necessary for bone morphogenetic protein-induced activation of endothelial cells. Circulation 2002 October 22;106(17):2263–70.

[38] Teichert-Kuliszewska K, Kutryk MJ, Kuliszewski MA, Karoubi G, Courtman DW, Zucco L, et al. Bone morphogenetic protein receptor-2 signaling promotes pulmonary arterial endothelial cell survival: implications for loss-of-function mutations in the pathogenesis of pulmonary hypertension. Circ Res 2006 February 3;98(2):209–17.

[39] Pousada G, Baloira A, Vilariño C, Cifrian JM, Valverde D. Novel mutations in BMPR2, ACVRL1 and KCNA5 genes and hemodynamic parameters in patients with pulmonary arterial hypertension. PloS One 2014;9(6):e100261.

[40] Evans JD, Girerd B, Montani D, Wang XJ, Galiè N, Austin ED, et al. BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis. Lancet Respir Med 2016 February;4(2):129–37.

[41] Pousada G, Baloira A, Valverde D. Complex inheritance in pulmonary arterial hypertension patients with several mutations. Sci Rep 2016 September 15;6:33570.

[42] Zhu N, Pauciulo MW, Welch CL, Lutz KA, Coleman AW, Gonzaga-Jauregui C, et al. Novel risk genes and mechanisms implicated by exome sequencing of 2572 individuals with pulmonary arterial hypertension. Genome Med 2019 November 14;11(1):69.

[43] Guignabert C, Bailly S, Humbert M. Restoring BMPRII functions in pulmonary arterial hypertension: opportunities, challenges and limitations. Expert Opin Ther Targets 2017 February;21(2):181–90.

[44] Lavoie JR, Ormiston ML, Perez-Iratxeta C, Courtman DW, Jiang B, Ferrer E, et al. Proteomic analysis implicates translationally controlled tumor protein as a novel mediator of occlusive vascular remodeling in pulmonary arterial hypertension. Circulation 2014 May 27;129(21):2125–35.

[45] Wang Y, Kahaleh B. Epigenetic repression of bone morphogenetic protein receptor II expression in scleroderma. J Cell Mol Med 2013 October;17(10):1291–9.

[46] Gilbane AJ, Rrett-Smith E, Trinder SL, Good RB, Pearce A, Denton CP, et al. Impaired bone morphogenetic protein receptor II signaling in a transforming growth factor-i^2-dependent mouse model of pulmonary hypertension and in systemic sclerosis. Am J Respir Crit Care Med 2015 March 15;191(6):665–77.

[47] Yi D, Liu B, Ding H, Li S, Li R, Pan J, et al. E2F1 mediates SOX17 deficiency-induced pulmonary hypertension. bioRxiv 2023 February 16 [Preprint]. PMID: 36824855.

[48] Bohnen MS, Ma L, Zhu N, Qi H, McClenaghan C, Gonzaga-Jauregui C, et al. Loss-of-function ABCC8 mutations in pulmonary arterial hypertension. Circ Genom Precis Med 2018 October;11(10):e002087.

[49] Wang XJ, Lian TY, Jiang X, Liu SF, Li SQ, Jiang R, et al. Germline BMP9 mutation causes idiopathic pulmonary arterial hypertension. Eur Respir J 2019 March;53(3).

[50] Hernandez-Gonzalez I, Tenorio-Castano J, Ochoa-Parra N, Gallego N, Pérez-Olivares C, Lago-Docampo M, et al. Novel genetic and molecular pathways in pulmonary arterial hypertension associated with connective tissue disease. Cells 2021 June 13;10(6).

[51] Zhu N, Swietlik EM, Welch CL, Pauciulo MW, Hagen JJ, Zhou X, et al. Rare variant analysis of 4241 pulmonary arterial hypertension cases from an international consortium implicates FBLN2, PDGFD, and rare de novo variants in PAH. Genome Med 2021 May 10;13(1):80.

[52] Eichstaedt CA, Sabmannshausen Z, Shaukat M, Cao D, Xanthouli P, Gall H, et al. Gene panel diagnostics reveals new pathogenic variants in pulmonary arterial hypertension. Respir Res 2022 March 27;23(1):74.

[53] Galié N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. Cardiovasc Res 2004 February 1;61(2):227–37.

[54] Jankowich MD, Wu WC, Choudhary G. Association of Elevated Plasma Endothelin-1 levels with pulmonary hypertension, mortality, and heart failure in African American individuals: the Jackson heart study. JAMA Cardiol 2016 July 1;1(4):461–9.

[55] Ciurzynski M, Bienias P, Irzyk K, Kostrubiec M, Bartoszewicz Z, Siwicka M, et al. Serum endothelin-1 and NT-proBNP, but not ADMA, endoglin and TIMP-1 levels, reflect impaired right ventricular function in patients with systemic sclerosis. Clin Rheumatol 2014 January;33(1):83–9.

[56] Coral-Alvarado P, Quintana G, Garces MF, Cepeda LA, Caminos JE, Rondon F, et al. Potential biomarkers for detecting pulmonary arterial hypertension in patients with systemic sclerosis. Rheumatol Int 2009 July;29(9):1017–24.

[57] Chester AH, Yacoub MH, Moncada S. Nitric oxide and pulmonary arterial hypertension. Glob Cardiol Sci Pract 2017 June 30;2017(2):14.

[58] Zhang R, Wang XJ, Zhang HD, Sun XQ, Zhao QH, Wang L, et al. Profiling nitric oxide metabolites in patients with idiopathic pulmonary arterial hypertension. Eur Respir J 2016 November;48(5):1386–95.

[59] Kielstein JT, Bode-Böger SM, Hesse G, Martens-Lobenhoffer J, Takacs A, Fliser D, et al. Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. Arterioscler Thromb Vasc Biol 2005 July;25(7):1414–8.

[60] Thakkar V, Stevens W, Prior D, Rabusa C, Sahhar J, Walker JG, et al. The role of asymmetric dimethylarginine alone and in combination with N-terminal pro-B-type natriuretic peptide as a screening biomarker for systemic sclerosis-related pulmonary arterial hypertension: a case control study. Clin Exp Rheumatol 2016 September;34 Suppl 100(5):129–36.

[61] Liu J, Fu Q, Jiang L, Wang Y. Clinical value of asymmetrical Dimethylarginine detection in patients with connective tissue disease-associated pulmonary arterial hypertension. Cardiol Res Pract 2019;2019:3741909.

[62] Dimitroulas T, Giannakoulas G, Sfetsios T, Karvounis H, Dimitroula H, Koliakos G, et al. Asymmetrical dimethylarginine in systemic sclerosis-related pulmonary arterial hypertension. Rheumatology (Oxford) 2008 November;47(11):1682–5.

[63] Murakami M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. Prog Lipid Res 2004 January;43(1):3–35.

[64] Pidgeon GP, Tamosiuniene R, Chen G, Leonard I, Belton O, Bradford A, et al. Intravascular thrombosis after hypoxia-induced pulmonary hypertension: regulation by cyclooxygenase-2. Circulation 2004 October 26;110(17):2701–7.

[65] Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 1992 July 9;327(2):70–5.

[66] Tuder RM, Cool CD, Geraci MW, Wang J, Abman SH, Wright L, et al. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. Am J Respir Crit Care Med 1999 June;159(6):1925–32.

[67] Peach CJ, Mignone VW, Arruda MA, Alcobia DC, Hill SJ, Kilpatrick LE, et al. Molecular pharmacology of VEGF-A isoforms: binding and Signalling at VEGFR2. Int J Mol Sci 2018 April 23;19(4).

[68] Selimovic N, Bergh CH, Andersson B, Sakiniene E, Carlsten H, Rundqvist B. Growth factors and interleukin-6 across the lung circulation in pulmonary hypertension. Eur Respir J 2009 September;34(3):662–8.

[69] Kümpers P, Nickel N, Lukasz A, Golpon H, Westerkamp V, Olsson KM, et al. Circulating angiopoietins in idiopathic pulmonary arterial hypertension. Eur Heart J 2010 September;31(18):2291–300.

[70] Kylhammar D, Hesselstrand R, Nielsen S, Scheele C, Radegran G. Angiogenic and inflammatory biomarkers for screening and follow-up in patients with pulmonary arterial hypertension. Scand J Rheumatol 2018 July;47(4):319–24.

[71] Säleby J, Bouzina H, Ahmed S, Lundgren J, Radegran G. Plasma receptor tyrosine kinase RET in pulmonary arterial hypertension diagnosis and differentiation. ERJ Open Res 2019 October;5(4).

[72] Papaioannou AI, Zakynthinos E, Kostikas K, Kiropoulos T, Koutsokera A, Ziogas A, et al. Serum VEGF levels are related to the presence of pulmonary arterial hypertension in systemic sclerosis. BMC Pulm Med 2009 May 9;9:18.

[73] Pendergrass SA, Hayes E, Farina G, Lemaire R, Farber HW, Whitfield ML, et al. Limited systemic sclerosis patients with pulmonary arterial hypertension show biomarkers of inflammation and vascular injury. PloS One 2010 August 17;5(8):e12106.

[74] Adachi S, Kikuchi R, Shimokata S, Suzuki A, Yoshida M, Imai R, et al. Endostatin and vascular endothelial growth factor-a(165)b may contribute to classification of pulmonary hypertension. Circ Rep 2021 February 19;3(3):161–9.

[75] Suzuki S, Yoshihisa A, Yokokawa T, Misaka T, Sakamoto N, Sugimoto K, et al. Association between levels of anti-angiogenic isoform of vascular endothelial growth factor a and pulmonary hypertension. Int J Cardiol 2016 November 1;222:416–20.

[76] McMahan Z, Schoenhoff F, Van Eyk JE, Wigley FM, Hummers LK. Biomarkers of pulmonary hypertension in patients with scleroderma: a case-control study. Arthritis Res Ther 2015 August 6;17(1):201.

[77] Tiede SL, Gall H, Dorr O, dos Santos GM, Troidl C, Liebetrau C, et al. New potential diagnostic biomarkers for pulmonary hypertension. Eur Respir J 2015 November;46(5):1390–6.

[78] Malhotra R, Paskin-Flerlage S, Zamanian RT, Zimmerman P, Schmidt JW, Deng DY, et al. Circulating angiogenic modulatory factors predict survival and functional class in pulmonary arterial hypertension. Pulm Circ 2013 April;3(2):369–80.

[79] Hirose S, Hosoda Y, Furuya S, Otsuki T, Ikeda E. Expression of vascular endothelial growth factor and its receptors correlates closely with formation of the plexiform lesion in human pulmonary hypertension. Pathol Int 2000 June;50(6):472–9.

[80] Tuder RM, Chacon M, Alger L, Wang J, Taraseviciene-Stewart L, Kasahara Y, et al. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. J Pathol 2001 October;195(3):367–74.

[81] Liakouli V, Cipriani P, Marrelli A, Alvaro S, Ruscitti P, Giacomelli R. Angiogenic cytokines and growth factors in systemic sclerosis. Autoimmun Rev 2011 August;10(10):590–4.

[82] Costello CM, McCullagh B, Howell K, Sands M, Belperio JA, Keane MP, et al. A role for the CXCL12 receptor, CXCR7, in the pathogenesis of human pulmonary vascular disease. Eur Respir J 2012 June;39(6):1415–24.

[83] Salcedo R, Wasserman K, Young HA, Grimm MC, Howard OM, Anver MR, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: in vivo neovascularization induced by stromal-derived factor-1alpha. Am J Pathol 1999 April;154(4):1125–35.

[84] Mirshahi F, Pourtau J, Li H, Muraine M, Trochon V, Legrand E, et al. SDF-1 activity on microvascular endothelial cells: consequences on angiogenesis in in vitro and in vivo models. Thromb Res 2000 September 15;99(6):587–94.

[85] Liang Z, Brooks J, Willard M, Liang K, Yoon Y, Kang S, et al. CXCR4/CXCL12 axis promotes VEGF-mediated tumor angiogenesis through Akt signaling pathway. Biochem Biophys Res Commun 2007 August 3;359(3):716–22.

[86] Liu X, Duan B, Cheng Z, Jia X, Mao L, Fu H, et al. SDF-1/CXCR4 axis modulates bone marrow mesenchymal stem cell apoptosis, migration and cytokine secretion. Protein Cell 2011 October;2(10):845–54.

[87] McCullagh BN, Costello CM, Li L, O'Connell C, Codd M, Lawrie A, et al. Elevated plasma CXCL12α is associated with a poorer prognosis in pulmonary arterial hypertension. PloS One 2015;10(4):e0123709.

[88] Dahal BK, Heuchel R, Pullamsetti SS, Wilhelm J, Ghofrani HA, Weissmann N, et al. Hypoxic pulmonary hypertension in mice with constitutively active platelet-derived growth factor receptor-β^2. Pulm Circ 2011 April;1(2):259–68.

[89] Cipriani P, Franca MA, Liakouli V, Pacini A, Manetti M, Marrelli A, et al. Differential expression of stromal cell-derived factor 1 and its receptor CXCR4 in the skin and endothelial cells of systemic sclerosis patients: Pathogenetic implications. Arthritis Rheum 2006 September;54(9):3022–33.

[90] Manetti M, Liakouli V, Fatini C, Cipriani P, Bonino C, Vettori S, et al. Association between a stromal cell-derived factor 1 (SDF-1/CXCL12) gene polymorphism and microvascular disease in systemic sclerosis. Ann Rheum Dis 2009 March;68(3):408–11.

[91] Sowa G. Caveolae, caveolins, cavins, and endothelial cell function: new insights. Front Physiol 2012;2:120.

[92] Chen F, Barman S, Yu Y, Haigh S, Wang Y, Black SM, et al. Caveolin-1 is a negative regulator of NADPH oxidase-derived reactive oxygen species. Free Radic Biol Med 2014 August;73:201–13.

[93] Zhao YY, Zhao YD, Mirza MK, Huang JH, Potula HH, Vogel SM, et al. Persistent eNOS activation secondary to caveolin-1 deficiency induces pulmonary hypertension in mice and humans through PKG nitration. J Clin Invest 2009 July;119(7):2009–18.

[94] Wang KY, Lee MF, Ho HC, Liang KW, Liu CC, Tsai WJ, et al. Serum Caveolin-1 as a novel biomarker in idiopathic pulmonary artery hypertension. Biomed Res Int 2015;2015:173970.

[95] Cipriani P, Di BP, Capece D, Zazzeroni F, Liakouli V, Ruscitti P, et al. Impaired Cav-1 expression in SSc mesenchymal cells upregulates VEGF signaling: a link between vascular involvement and fibrosis. Fibrogenesis Tissue Repair 2014;7:13.

[96] Chan SY, Rubin LJ. Metabolic dysfunction in pulmonary hypertension: from basic science to clinical practice. Eur Respir Rev 2017 December 31;26(146).

[97] Michelakis ED, Gurtu V, Webster L, Barnes G, Watson G, Howard L, et al. Inhibition of pyruvate dehydrogenase kinase improves pulmonary arterial hypertension in genetically susceptible patients. Sci Transl Med 2017 October 25;9(413).

[98] Yuan K, Shao NY, Hennigs JK, Discipulo M, Orcholski ME, Shamskhou E, et al. Increased pyruvate dehydrogenase kinase 4 expression in lung Pericytes is associated with reduced endothelial-Pericyte interactions and small vessel loss in pulmonary arterial hypertension. Am J Pathol 2016 September;186(9):2500–14.

[99] Paulin R, Dromparis P, Sutendra G, Gurtu V, Zervopoulos S, Bowers L, et al. Sirtuin 3 deficiency is associated with inhibited mitochondrial function and pulmonary arterial hypertension in rodents and humans. Cell Metab 2014 November 4;20(5):827–39.

[100] Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood 2005 January 15;105(2):659–69.

[101] Weidemann A, Johnson RS. Biology of HIF-1alpha. Cell Death Differ 2008 April;15(4):621–7.

[102] Tuder RM, Voelkel NF. Plexiform lesion in severe pulmonary hypertension: association with glomeruloid lesion. Am J Pathol 2001 July;159(1):382–3.

[103] Fijalkowska I, Xu W, Comhair SA, Janocha AJ, Mavrakis LA, Krishnamachary B, et al. Hypoxia inducible-factor1alpha regulates the metabolic shift of pulmonary hypertensive endothelial cells. Am J Pathol 2010 March;176(3):1130–8.

[104] Tamby MC, Humbert M, Guilpain P, Servettaz A, Dupin N, Christner JJ, et al. Antibodies to fibroblasts in idiopathic and scleroderma-associated pulmonary hypertension. Eur Respir J 2006 October;28(4):799–807.

[105] Arends SJ, Damoiseaux J, Duijvestijn A, Brus-Palmans L, Boomars K, Broers B, et al. Prevalence of anti-endothelial cell antibodies in idiopathic pulmonary arterial hypertension. Eur Respir J 2010 April;35(4):923–5.

[106] Liang S, Desai AA, Black SM, Tang H. Cytokines, chemokines, and inflammation in pulmonary arterial hypertension. Adv Exp Med Biol 2021;1303:275–303.

[107] Jones RJ, De Bie EMDD, Groves E, Zalewska KI, Swietlik EM, Treacy CM, et al. Autoimmunity is a significant feature of idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med 2022 July 1;206(1):81–93.

[108] Vegh J, Szodoray P, Kappelmayer J, Csipo I, Udvardy M, Lakos G, et al. Clinical and immunoserological characteristics of mixed connective tissue disease associated with pulmonary arterial hypertension. Scand J Immunol 2006 July;64(1):69–76.

[109] Becker MO, Kill A, Kutsche M, Guenther J, Rose A, Tabeling C, et al. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. Am J Respir Crit Care Med 2014 October 1;190(7):808–17.

[110] Guo L, Li M, Chen Y, Wang Q, Tian Z, Pan S, et al. Anti-endothelin receptor type a autoantibodies in systemic lupus erythematosus-associated pulmonary arterial hypertension. Arthritis Rheumatol 2015 September;67(9):2394–402.

[111] Svegliati S, Amico D, Spadoni T, Fischetti C, Finke D, Moroncini G, et al. Agonistic anti-PDGF receptor autoantibodies from patients with systemic sclerosis impact human pulmonary artery smooth muscle cells function in vitro. Front Immunol 2017;8:75.

[112] Dib H, Tamby MC, Bussone G, Regent A, Berezné A, Lafine C, et al. Targets of anti-endothelial cell antibodies in pulmonary hypertension and scleroderma. Eur Respir J 2012 June;39(6):1405–14.

[113] Tamby MC, Chanseaud Y, Humbert M, Fermanian J, Guilpain P, Garcia-de-la-Pe-Lefebvre, et al. Anti-endothelial cell antibodies in idiopathic and systemic sclerosis associated pulmonary arterial hypertension. Thorax 2005 September;60(9):765–72.

[114] Arends SJ, Damoiseaux JG, Duijvestijn AM, Debrus-Palmans L, Vroomen M, Boomars KA, et al. Immunoglobulin G anti-endothelial cell antibodies: inducers of endothelial cell apoptosis in pulmonary arterial hypertension? Clin Exp Immunol 2013 December;174(3):433–40.

[115] Sasaki N, Kamataki A, Sawai T. A histopathological study of pulmonary hypertension in connective tissue disease. Allergol Int 2011 December;60(4):411–7.

[116] Liu XD, Guo SY, Yang LL, Zhang XL, Fu WY, Wang XF. Anti-endothelial cell antibodies in connective tissue diseases associated with pulmonary arterial hypertension. J Thorac Dis 2014 May;6(5):497–502.

[117] Xing Y, Zhao J, Zhou M, Jing S, Zhao X, Mao P, et al. The LPS induced pyroptosis exacerbates BMPR2 signaling deficiency to potentiate SLE-PAH. FASEB J 2021 December;35(12):e22044.

[118] Riemekasten G, Philippe A, Nather M, Slowinski T, Müller DN, Heidecke H, et al. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis 2011 March;70(3):530–6.

[119] Gunther J, Kill A, Becker MO, Heidecke H, Rademacher J, Siegert E, et al. Angiotensin receptor type 1 and endothelin receptor type a on immune cells mediate migration and the expression of IL-8 and CCL18 when stimulated by autoantibodies from systemic sclerosis patients. Arthritis Res Ther 2014 March 11;16(2):R65.
[120] Kill A, Tabeling C, Undeutsch R, Kühl AA, Günther J, Radic M, et al. Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. Arthritis Res Ther 2014 January 28;16(1):R29.
[121] Tabeling C, González Calera CR, Lienau J, Höppner J, Tschernig T, Kershaw O, et al. Endothelin B receptor Immunodynamics in pulmonary arterial hypertension. Front Immunol 2022;13:895501.
[122] Hénault J, Tremblay M, Clément I, Raymond Y, Senécal JL. Direct binding of anti-DNA topoisomerase I autoantibodies to the cell surface of fibroblasts in patients with systemic sclerosis. Arthritis Rheum 2004 October;50(10):3265–74.
[123] Favoino E, Grapsi E, Barbuti G, Liakouli V, Ruscitti P, Foti C, et al. Systemic sclerosis and primary biliary cholangitis share an antibody population with identical specificity. Clin Exp Immunol 2023 April 7;212(1):32–8.
[124] Terrier B, Tamby MC, Camoin L, Guilpain P, Broussard C, Bussone G, et al. Identification of target antigens of antifibroblast antibodies in pulmonary arterial hypertension. Am J Respir Crit Care Med 2008 May 15;177(10):1128–34.
[125] Chizzolini C, Raschi E, Rezzonico R, Testoni C, Mallone R, Gabrielli A, et al. Autoantibodies to fibroblasts induce a proadhesive and proinflammatory fibroblast phenotype in patients with systemic sclerosis. Arthritis Rheum 2002 June;46(6):1602–13.
[126] Fineschi S, Cozzi F, Burger D, Dayer JM, Meroni PL, Chizzolini C. Anti-fibroblast antibodies detected by cell-based ELISA in systemic sclerosis enhance the collagenolytic activity and matrix metalloproteinase-1 production in dermal fibroblasts. Rheumatology (Oxford) 2007 December;46(12):1779–85.
[127] Perosa F, Prete M, Di LG, Ostuni C, Favoino E, Valentini G. Anti-centromere protein a antibodies in systemic sclerosis: significance and origin. Autoimmun Rev 2016 January;15(1):102–9.
[128] Perosa F, Favoino E, Favia IE, Vettori S, Prete M, Corrado A, et al. Subspecificities of anticentromeric protein a antibodies identify systemic sclerosis patients at higher risk of pulmonary vascular disease. Medicine (Baltimore) 2016 June;95 (25):e3931.
[129] Jiang Y, Turk MA, Pope JE. Factors associated with pulmonary arterial hypertension (PAH) in systemic sclerosis (SSc). Autoimmun Rev 2020 September; 19(9):102602.
[130] Senécal JL, Hoa S, Yang R, Koenig M. Pathogenic roles of autoantibodies in systemic sclerosis: current understandings in pathogenesis. J Scleroderma Relat Disord 2020 June;5(2):103–29.
[131] van Leeuwen NM, Boonstra M, Bakker JA, Grummels A, Jordan S, Liem S, et al. Anticentromere antibody levels and isotypes and the development of systemic sclerosis. Arthritis Rheumatol 2021 December;73(12):2338–47.
[132] Senécal JL, Koenig M, Archambault G, Hoa S. Criteria for the pathogenicity of anticentromere (anti-CENP-B) autoantibodies in systemic sclerosis: comment on the article by van Leeuwen et al. Arthritis Rheumatol 2022 September;74(9): 1606–7.
[133] Robitaille G, Christin MS, Clément I, Senécal JL, Raymond Y. Nuclear autoantigen CENP-B transactivation of the epidermal growth factor receptor via chemokine receptor 3 in vascular smooth muscle cells. Arthritis Rheum 2009 September;60 (9):2805–16.
[134] Cavazzana I, Ceribelli A, Taraborelli M, Fredi M, Norman G, Tincani A, et al. Primary biliary cirrhosis-related autoantibodies in a large cohort of italian patients with systemic sclerosis. J Rheumatol 2011 October;38(10):2180–5.
[135] Steelandt A, Benmostefa N, Avouac J, Mouthon L, Allanore Y. Ethnic influence on the phenotype of French patients with systemic sclerosis. Joint Bone Spine 2021 March;88(2):105081.
[136] Bass RD, Moore DF, Steen VD. Prevalence and characteristics of systemic sclerosis patients fulfilling the 2019 EULAR/ACR classification criteria for systemic lupus erythematosus. Arthritis Care Res (Hoboken) 2023 September 10. Epub ahead of print. PMID: 37691427.
[137] Dima A, Jurcut C, Baicus C. The impact of anti-U1-RNP positivity: systemic lupus erythematosus versus mixed connective tissue disease. Rheumatol Int 2018 July; 38(7):1169–78.
[138] Huang C, Li M, Liu Y, Wang Q, Guo X, Zhao J, et al. Baseline characteristics and risk factors of pulmonary arterial hypertension in systemic lupus erythematosus patients. Medicine (Baltimore) 2016 March;95(10):e2761.
[139] Zhang N, Li M, Qian J, Wang Q, Zhao J, Yang Z, et al. Pulmonary arterial hypertension in systemic lupus erythematosus based on a CSTAR-PAH study: baseline characteristics and risk factors. Int J Rheum Dis 2019 May;22(5):921–8.
[140] Lei Y, Zhang X, Feng Y, Wang J, Luo R. Risk factors of pulmonary arterial hypertension in patients with systemic lupus erythematosus. Cardiol Young 2021 October;31(10):1619–24.
[141] Kuwana M, Kaburaki J, Okano Y, Tojo T, Homma M. Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. Arthritis Rheum 1994 January;37(1):75–83.
[142] Hashimoto A, Endo H, Kondo H, Hirohata S. Clinical features of 405 Japanese patients with systemic sclerosis. Mod Rheumatol 2012 April;22(2):272–9.
[143] Xiang W, Dong R, Li M, Liu B, Ma Z, Yang Q. The role of anti-U1 RNP antibody in connective tissue disease-associated pulmonary arterial hypertension: a systematic review and Meta-analysis. J Clin Med 2022 December 20;12(1).
[144] Okawa-Takatsuji M, Aotsuka S, Fujinami M, Uwatoko S, Kinoshita M, Sumiya M. Up-regulation of intercellular adhesion molecule-1 (ICAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and class II MHC molecules on pulmonary artery endothelial cells by antibodies against U1-ribonucleoprotein. Clin Exp Immunol 1999 April;116(1):174–80.
[145] Okawa-Takatsuji M, Aotsuka S, Uwatoko S, Takaono M, Iwasaki K, Kinoshita M, et al. Endothelial cell-binding activity of anti-U1-ribonucleoprotein antibodies in patients with connective tissue diseases. Clin Exp Immunol 2001 November;126 (2):345–54.
[146] Castaño JAT, Hernández-Gonzalez I, Gallego N, Pérez-Olivares C, Ochoa PN, Arias P, et al. Customized massive parallel sequencing panel for diagnosis of pulmonary arterial hypertension. Genes (Basel) 2020 September 30;11(10).