Pathogenesis of Pulmonary Arterial Hypertension

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Abstract:

The pathogenic mechanisms by which pulmonary arterial hypertension (PAH) develops and progresses, are complex and not fully elucidated. Considerable heterogeneity in disease pathological features, some of which are unique and some common amongst the PAH spectrum of diseases, including idiopathic and scleroderma associated PAH, adds further to this complexity. Our understanding so far has significantly progressed and has underpinned some of the successful therapeutics that are being used to treat PAH resulting in marked improvement in survival. In this revised chapter, we explore the biological processes and mechanistic insights that have advanced the field, the genetic and epigenetic components, the role of inflammation and metabolism, the cellular and molecular pathways, and their importance in the context of disease heterogeneity.

Pulmonary arterial hypertension (PAH) is a progressive, incurable pulmonary vascular disease characterized by structural remodelling of the small pulmonary arteries and increased blood pressure in the pulmonary circulation, culminating in right ventricular failure. With an estimated prevalence of up to approximately 50 per million population [1,2], PAH represents a form of precapillary pulmonary hypertension (PH), also categorized as group I Pulmonary Hypertension by the World Health Organization classification system[3].

Our understanding of PAH has been rapidly expanding in recent years. Since the first edition of this chapter, there have been several gains in new knowledge of cellular and molecular insights in the mechanism of the disease, based on technological breakthroughs in omics and next generation sequencing to characterise the disease, with a significant translation of much of this knowledge for therapeutic advantage of PAH patients.

The field of PAH has continued to evolve, marking the past decade with notable advancements[4]. The establishment of bone morphogenetic protein (BMP) type 2 receptor (BMPRII) and transforming growth factor–β (TGF-β) superfamily signalling as promising therapeutic targets is an example, one that particularly underscores the importance of a close interplay between clinical and fundamental research scientists. Not very long after the recognition of BMPRII mutations in PAH patients [5–8], benefit of restoring BMP9-BMPRII signalling was shown in animal studies [9–11] and the promising role of sotatercept, a ligand trap with affinity for activin and growth and differentiation factor (GDF), has now been demonstrated in a phase 2 clinical trial [12]. Tacrolimus, which acts on the same pathway, is now emerging as another potential therapeutic option [13,14]. Although optimal ways to re-balance TGF-β superfamily signaling pathways are still being elucidated [15–17], BMP9-BMPRII augmentation represents a novel, anti-remodeling approach to managing PAH patients, beyond the paradigms of pulmonary vasodilation.

The past few years have also been marked by attempts to re-evaluate diagnostic and therapeutic strategies in PAH management. At the 6th World Symposium in Pulmonary Hypertension, held in 2018 (Nice, France), revising the hemodynamic definitions of PH was suggested, specifically lowering the mean pulmonary artery pressure (mPAP) cut-off from ≥25 to >20 mmHg, without a corresponding change in the cut-offs for pulmonary artery wedge pressure (≤15 mm Hg) or pulmonary vascular resistance (⩾ 3 Wood units) [3,18]. This new diagnostic recommendation more accurately reflects physiologic mPAP values in healthy individuals[19], and it considers the prognostic importance of borderline elevated mPAP [20–22] and early treatment initiation in PAH [23–26]. Although criticized for limited clinical impact and the paucity of data guiding treatment [27–29], the newly suggested mPAP cutoff of >20mmHg, together with studies examining the inclusion of prostacyclin analogues as an initial therapy [30–32], represents collective effort to improve PAH outcomes by intervening at an early stage of disease, with upfront aggressive treatments.

The recent efforts to refine management of PAH patients is particularly fitting for PAH associated with SSc (SSc-PAH), which carries a particularly grim prognosis, with a shorter expected survival than other causes of PAH and even other subtypes of connective tissue disease-associated PAH (CTD-PAH) [37–40]. While SSc-PAH might also benefit from early, intensive therapy[41–46], SSc-PAH remains a major cause of death in SSc patients [47–51]. The currently incurable nature of PAH and the particularly poor outcome of SSc-PAH patients indicate the need for a deeper understanding of the PAH pathobiology, and an appreciation for its pathologic heterogeneity, which we further discuss in the following text. The development of pulmonary arterial hypertension (PAH) represents a serious co-morbidity in collagen vascular diseases (CVD). This complication is commonly observed in SSc patients where the risk of developing PAH persists throughout the disease. Affecting up to 10% of patients, SSc appears to act as a susceptibility factor in the development of PAH [2;3]. Whilst

significant progress has been made in understanding the pathological mechanisms that contribute to the development and progression of heritable and idiopathic forms of PAH, the relative pathogenic mechanisms that contribute to the development of PAH in SSc patients remain less appreciated. Indeed SSc-PAH patients have a significantly poorer prognosis compared to other forms [4]. Here we discuss the key pathological findings in PAH and fundamental pathobiological mechanisms implicated in the development and progression of PAH, particularly in SSc patients.

Although a few therapeutic candidates, such as anti-IL-6 tocilizumab [52,53] and B lymphocyte-depleting rituximab [54], recently showed absent or inconclusive clinical benefits, the PAH field continues to explore novel treatments [4] (Table 1). These include serotonin synthesis inhibition with rodatristat (NCT04712669), anti-estrogen therapies with anastrozole[55] and tamoxifen (NCT03528902), and progenitor cells [56] NCT03001414). While once associated with subdural hematomas and other safety concerns [57–59], imatinib mesylate is being reconsidered for PAH management (NCT04416750), particularly as an inhaled therapy (NCT05036135, NCT04903730). Seralutinib is another tyrosine kinase inhibitor being trialed (NCT04456998/NCT04816604). It is important to note that each of these treatment modalities represents the ongoing endeavor by the PAH field to uncover molecular and pathobiological underpinnings of the disease that underly individual pulmonary vascular lesions. Amid exciting clinical trials, appreciation of the pathobiological complexity and the pathologic heterogeneity of PAH will remain the cornerstone of novel therapies and improved outcomes.

Group 1 PAH contains, in addition to IPAH (and its closely related familial/genetic counterpart), related but also disparate diseases, including collagen vascular disease, HIV infection, anorectic and addiction-related drugs, and schistosomiasis [3]. While all of the forms of PAH have some shared pathology, there are structural differences in

the scope of pulmonary vascular remodeling, which may underlie different pathogenetic processes. For instance, while pulmonary media and intima remodeling can occur in IPAH and collagen vascular disease-associate PAH (CVD-PAH) such scleroderma (SSc) associated PAH, an association with interstitial lung disease is predominantly seen in the latter. All forms of PAH have significant inflammation; however, the specific type of inflammatory cells and cytokines and how they affect pathogenesis may differ, such as between such as between portopulmonary hypertension *vs* schistosomiasis-associated PAH. It is becoming apparent that a TH1/M1 driven inflammation (characterized by activation of interferon-γ, TNF-α, IL12/IL18) or TH2/M2 inflammation (characterized by IL4/IL13, TGF-β, among others) may ultimately affect how a particular cause of PAH ultimately results in pulmonary vascular disease.

In studying PAH, we have to rely on the knowledge derived from shared and distinct insights gained from the conglomerate investigations in each subtype of disease. Indeed, despite the significant insights into PAH pathogenesis[33–36], we have still a lot to learn especially about which disease features are shared and which are unique. I addition, many key pathogenetic processes that are based mostly on investigations centered around IPAH may also appear to be shared, and it is imperative that we recognize the heterogeneity of the conditions in Group1. In the present review, the authors chose to focus on key pathological features of PAH and then relate these alterations with key pathogenetic process. These include the role of growth factors, metabolic reprogramming, and inflammation.

In the current edition, we use the term "associated PAH," or APAH, to designate PAH arising secondary to systemic rheumatic diseases. APAH is also commonly referred to as connective tissue disease-associated PAH (CTD-PAH) and collagen vascular disease-associated PAH (CVD-PAH), all of which are synonymous with each other. In addition to the routinely used and widely established clinical subgroups of PAH (e.g.,

IPAH, FPAH, APAH, SSc-PAH, drug-induced PAH...etc), in our discussion of disease heterogeneity we introduce an additional categorization of pathology-based PAH patterns into four distinctive types: PAH-like pattern, FPAH-like pattern, APAH-like pattern, and VOD-like pattern.

A. Pathology of scleroderma-associated pulmonary arterial hypertension

In the prior edition of this chapter, we have described in detail the spectrum of the pulmonary vascular pathology of SSc-PAH[60]. Central to considering the pathological spectrum of pulmonary vascular lesions in SSc-PAH is the understanding of the structure of the pulmonary circulation. We strongly recommend that the reader refers to a more detailed description of this unique vascular architecture as it relates to pulmonary vascular physiology[61,62]. In summary, a handful of three-dimensional casting-reconstruction studies demonstrated that a single pulmonary artery branch successively forms a fractal structure with 17 orders of vascular segments. The smallest identifiable segments by this approach are in the order of 25 μm diameter small arteries, which amount to approximately 25 million segments (the more distal segments down to the capillaries cannot be evaluated because of limitations of the casting method).

In 2012, we summarized our experience with 12 cases of CTD-PAH, including SSc-PAH[63] . In contrast to our initial impression that SSc-PAH has a more distinct pathology, with less or no plexiform lesions, the pathologic spectrum and frequency of the lesions were overall similar to the 48 cases of IPAH [64] in this cohort of the Pulmonary Hypertension Breakthrough Initiative (PHBI) (Figure 1). Remarkably, we did not observe pulmonary venous disease as previously reported [65].

B. Comparative pathology of CTD-PAH vs. other forms of PAH.

We have recently highlighted that a critical challenge in the field of PAH is the fact that the pathology of pulmonary vascular lesions is highly heterogeneous [66], both among lungs with similar underlying pathological patterns and among different regions of the same PAH lung. In this section, it is our goal to further underscore this heterogeneity, which involves a reassessment of the clinical vs. pathological characteristics found in the study of 2012. To achieve this goal, the published dataset was re-evaluated using Principal Component Analyses using the R package. These data are best interpreted in the context of the original data [63].

The PHBI cohort was assembled with multiple clinical parameters pertinent to pulmonary hemodynamics, laboratory data, and patient demographics, ultimately leading to a tentative clinical diagnosis, such as idiopathic IPAH, associated PAH (APAH), and PAH-associated with congenital heart malformations, familial or hereditary PAH, and drug-induced PAH.

Based on our experience of the spectrum of the pathology of pulmonary vascular disease [61,67,68], we opted in the original publication for reporting the final diagnosis based on the pathological pattern rather than the underlying clinical diagnosis in Stacher et al [63] (Figure 2). The various clinical subtypes of PAH were pathologically categorized into the following three patterns: IPAH-like pattern, APAH-like pattern, and venoocclusive disease-like pattern.

The study on the modern pathology of PAH involved: characterization of the pulmonary vascular remodeling based on measurements of the intima, media, combined intima+media, and adventitia thickening in circular profiles of pulmonary arteries; assessment of the number of profiles of plexiform lesions; and a semi-quantitative assessment of perivascular inflammation [63] (Figure 3). Controls consisted of donor lungs that were rejected from the option of lung transplantation. We noted that these control lungs were segregated into two groups, one younger (22 lungs) and an older one, above 50 years of age, with more marked pulmonary vascular

remodeling (6 lungs) (Figures 4A, 4B, 4D). As it is apparent from the principal component analysis (PCA) of the control lungs, despite some superposition of the clustering of younger (CTL) and older groups,4 older lungs (CTL2) separated from the younger control cluster (Figure 5). It is of interest that the variance of the intima is the largest when the variance of the media is the lowest, while the variance of the adventitia thickening is intermediate between the intima and media. The variance of the intima tracks closely with that of the sum of the thickness of the intima and media. The implications of the increased remodeling, including aspects related to pulmonary vascular adaption to age and decreased pulmonary vascular reserve, are discussed in more detail in the prior edition of this chapter [60].

The PAH cases had more marked intima, media, and combined intima+media thickening (Figures 3, 4A-D). The degree of these parameters of pulmonary vascular remodeling is overall similar between pathologically diagnosed APAH vs. IPAH.

Plexiform lesions were detected in both pathological IPAH and APAH patterns (Figure 4C). As a group, IPAH had a mean of 10-fold higher number of plexiform lesion profiles per area sampled than the APAH pattern ($p<0.001$). This supports our impression that plexiform lesions are not as frequently observed in APAH as in IPAH (7). Notably, both IPAH and APAH demonstrated significant heterogeneity in the number of profiles of the plexiform lesion (as illustrated in Fig. E10 in Stacher et al [63]).

The important association of perivascular inflammation with pulmonary vascular remodeling and pulmonary hemodynamics was underscored by the previous analyses of the PHBI cohort [63]. However, there were no significant differences between the IPAH- vs. APAH-pathological patterns (Figure 4D). Again, as formerly underscored[63], there is significant heterogeneity in the inflammation score among the cases in the PAH group.

Examination of the pulmonary vascular remodeling data and a more detailed pathological analysis based on concordance vs. discordance between the pathological patterns and clinical diagnoses provide interesting insights into pathologic heterogeneity in PAH. The concordance between the pathological pattern and clinical diagnosis was observed in 5(12) APAH, all 5 familial/hereditary cases (as the IPAH morphology is like sporadic vs. hereditary PAH), and 27(34) sporadic PAH (Figure 2). The discordance between the pathological vs. clinical diagnosis was more evident in cases of APAH, which was clinically interpreted as IPAH (4/12), drug-induced (amphetamine use) (2/12), or venocclusive disease (VOD) (1/12). Of the cases clinically diagnosed as APAH, three were due to scleroderma, 1 due to Lupus, and 1 due to rheumatoid arthritis. Interestingly, 2/4 of clinically diagnosed VODs had an APAH and an IPAH pattern (Figure 2).

The discordant cases of APAH pathological patterns with a clinical diagnosis of drug-induced PH (n= 2) fell within the range of intima thickness seen in the concordant APAH cases (Figure 2). A similar overall pattern was observed with media thickness as well (Figure 6); it is noteworthy that the most prominent media remodeling was found in a case of IPAH pattern with the clinical diagnosis of chronic thromboembolic disease (I-C in Figure 6) and IPAH pattern with the clinical diagnosis of VOD (I-V in Figure 6).

Notwithstanding the novel findings related to perivascular inflammation in the PHBI study and its correlation with remodeling and hemodynamics, there were no significant differences based on concordance vs. discordance of pathological vs. clinical diagnosis (Figure 7).

The PCA provided further interesting insights into the overall characteristics of the control vs. PAH cases. The PCA of control vs. PAH lungs (irrespective of the specific pathological pattern, i.e., all cases of APAH, IPAH, and VOD were considered as PAH) reveal some degree of shared clustering between the controls, and aged controls

(labeled as CTL2), and the PAH lungs. There is a significant degree of heterogeneity in PAH cases as illustrated by the 8 lungs that are plotted beyond the .68 ellipse probability. The variability is accounted mostly by the morphological parameters of the intima, media, intima+media, and adventitia thickness. Three of these 8 lungs were APAH, while the remaining were IPAH patterns. Interestingly, 2 of the 5 IPAH patterns had a clinical diagnosis of APAH.

The PCA for the pathological patterns of APAH and IPAH reveals a marked sharing of the main clusters (Figure 8). Ten APAH lungs (10/12) were also included in the 0.68 probability ellipse for IPAH, underscoring the marked shared pathological parameters between both pathological parameters. On the other hand, fourteen of 48 IPAH pathological cases did not fall within the IPAH probability ellipse, highlighting the angle of heterogeneity of IPAH pathology. It is of interest that 7/12 APAH lungs were included in the IPAH probability ellipse, underscoring their shared variability with the morphological parameters. Intima, media, intima+media, and adventitia contributed most to the variability accounted in principal component or dimension 1 (dim1), while plexiform lesions and perivascular inflammation accounted for most of the variability in principal components or dimensions 2 and 3 (dim2 and dim3) (Figure 9).

Eleven 11(53) cases fell outside the APAH and IPAH probability ellipses, i.e., those lungs with the highest levels of loading scores and therefore variability along with principal components 1 and 2. Only one of 11 had a discrepant APAH pathological vs. IPAH clinical diagnosis (with one that was APAH concordant). Interestingly, three of 5 FPAH fell outside the probability ellipses. In our original report, we documented the more marked remodeling in FPAH cases when compared with the sporadic IPAH pattern [63].

In conclusion, it is apparent from the original reporting and this reassessment that there is significant pathological heterogeneity in PAH, most notably in IPAH pathology.

Moreover, there is a significant number of cases of APAH and IPAH pathology with a discrepant clinical diagnosis.

As we have outlined in our perspective on the cancer hypothesis of PAH [66], this pathological heterogeneity requires the incorporation of molecular tools to elucidate if the lesions in PAH have a similar molecular signature or their variability among different patients in both IPAH and APAH. Novel approaches reliant on digital special profiling are now available to answer these pressing questions.

C. Pathogenetic insights of PAH

For many decades, the main concepts underlying the pathogenesis of pulmonary hypertension involved vasoconstriction of pulmonary vessels and vascular remodeling and the role of hypoxia. Levels of mediators such as endothelin increased in pulmonary hypertension[69] and coupled with decreased levels of nitric oxide or prostacyclin which resulted in reduced pulmonary vasodilation, and further enhanced vasoconstriction. The challenge was that patients with PAH are largely unresponsive to vasodilators, notably calcium channel blockers, which suggested that established disease is largely independent of vasoconstriction.

The focus remained on vasoconstrictors like endothelin and vasodilators like nitric oxide and prostacyclin and their mechanism of action in PAH. The body of work led to two of the main therapies for PAH, based on endothelin receptor blockers or phosphodiesterase 5 inhibitors, respectively[70]. Ongoing work in targeting these pathways has resulted in new therapeutics such as selexipag, an oral selective IP prostacyclin-receptor agonist [71] (Table 1).

Other investigators have concentrated their efforts on mediators of vascular remodeling such as platelet-derived growth factor (PDGF) and TGFβ, among others. More recently, insights into the interplay of metabolism and gene mutations have

expanded considerably the scope of key pathogenetic mechanisms involved in pulmonary hypertension. As with multiple chronic diseases, inflammation is a likely factor, promoting an interface between the pulmonary vascular compartment and growth signals affecting pulmonary vascular remodeling. Pulmonary vascular remodeling has been considered the result of abnormal injury/repair. In mild/moderate pulmonary hypertension, hypoxia is thought to initiate pulmonary vascular remodeling. However, what initiates remodeling in severe cases, including PAH, is still unclear.

The potential for endothelial cell apoptosis as the key triggering factor in severe PH is based data from animal models. Auto-antibodies may be causal and underlie severe pulmonary hypertension in the setting of autoimmune diseases like in scleroderma. Many of these potential mechanisms also share key features with malignant processes.

The concept that pulmonary vascular remodeling shares molecular and pathological features with cancer is now approximately 25 years old[72–74] and came about from the finding that the expansion of endothelial cells in plexiform lesions in IPAH were monoclonal, i.e. they originated from a single progenitor cell[75]. This finding implied a selection process based on genetic events which were subsequently validated with the findings of BMPR2 mutations[6,7] and TGFβ receptor 2 somatic instability[76]. Additional evidence of somatic genetic loss became evident when comparing primary cultures of pulmonary vascular cells from IPAH lungs with explanted lungs [77].

It is however worth mentioning that these events are restricted to IPAH; there is no current evidence that similar genetic processes affected other forms of PAH, including CVD-PAH. This difference implies that pulmonary vascular remodeling, involving pathologically similar lesions (see above), is stereotypic regardless of potentially diverse trigger factors. The concept that these lesions may arise from the de-repression of progenitor cells residing in the pulmonary vascular wall may well fit into this overall concept; genetic events would occur within a single cell, giving rise to a

monoclonal expansion in IPAH. On the other hand, non-genetic hits would recruit multiple progenitor cells, giving rise to a polyclonal growth in PAH due to left to right heart shunt; whether in SSc-PAH or Schistosomiasis-associated PAH, plexiform lesions are monoclonal or polyclonal remains unclear.

a. Genetic basis of PAH

Current knowledge of idiopathic PAH assumes that approximately 25-30% of patients have an underlying genetic cause for the disease and are classified as heritable PAH (HPAH)[34]. Genetic studies on heritable PAH have identified a significant number of genes associated with susceptibility to developing the disease, which was first heralded by the identification of bone morphogenetic protein receptor type II (BMPR2), mutations, a member of the TGFβ superfamily, in PAH patients in the early 2000s [787]. Next-generation sequencing has more recently expanded the genetic landscape by broadening the mutation spectrum in known genes and identifying novel genetic risk alleles[79].

BMPR2 loss-of-function mutations account for the majority of heritable cases (82%) and 17% of IPAH cases and were the first mutations to be associated with PAH[80–82] . BMPR2 mutations exhibit incomplete penetrance and are inherited in an autosomal dominant fashion. Only 24% of carriers develop PAH[83] suggesting that other, as yet unknown genetic, epigenetic and/or environmental factors may be required before the disease can manifest in carriers of BMPR2 mutations. More than 300 mutations have been described throughout the BMPR2 gene to date, including several frame-shifts, missense and nonsense mutations in critical regions, causing loss-of-function and resulting in PAH. Collectively, these mutations result in reduced expression of functional BMPR2 on the cell surface, and consequently in reduced signalling downstream of the receptor[84]. BMPR2 signalling inhibits the proliferation of vascular smooth muscle cells, whilst promoting the survival of pulmonary arterial endothelial

cells, thereby protecting the pulmonary artery from damage and adverse inflammatory responses [85].

Other members of the TGFβ superfamily, including activin receptor-like kinase 1 (*ALK1 or ACVRL1*), endoglin (*ENG*), *SMAD1, SMAD4, SMAD8* and *BMP9*, have been reported to contain mutations. Although these mutations are less frequent, they further establish the BMP signaling pathway in PAH pathogenesis [34]. Like BMPR2 mutations, these less common mutations exhibit incomplete penetrance and are also inherited in an autosomal dominant fashion. *ALK1* complexes with BMPR2 to form a functional receptor. Mutations in *ALK1* were more prevalent in 10% of paediatric PAH cases compared to adults PAH. In addition, mutations in the developmental *TBX4* gene are among the most common genetic causes of paediatric PAH suggesting an underlying developmental lung disease component[86]. Two new candidate genes with known functions in vasculogenesis and remodeling, *FBLN2* and *PDGFD* were also identified using rare variant analysis of a large international consortium[87] .

Signal transducers and transcriptional modulators such as SMAD1 and SMAD8 act downstream of BMPR2[88]. Once activated, BMPR2 phosphorylates SMAD1 and SMAD8, which translocate to the cell nucleus. SMAD1 and SMAD8, along with SMAD4 transcriptionally regulate many genes, including those that encode inhibitor of DNA binding (ID) proteins[88]. In pulmonary arterial smooth muscle cells, ID proteins mediate the anti-proliferative effects of BMPs and BMPR2 signalling[89]. BMPR2 complexed with ALK1 can be preferentially activated by BMP9 in the pulmonary endothelium to maintain pulmonary vascular homeostasis and quiescence. Loss of BMP9 is thought to cause endothelial dysfunction and result in pulmonary vascular remodelling[10].

Rare variants have been identified in potassium (K^+) channels or accessory K^+ channel subunits are also associated with PAH. In 2013, novel mutations in KCNK3, the gene encoding the two-pore domain potassium channel TASK-1, were identified in patients

with IPAH and HPAH, describing the first channelopathy in PAH. These heterozygous autosomal dominant KCNK3 mutations have a higher penetrance than BMPR2 mutations. Carriers of KCNK3 mutations develop a severe form of PAH, with raised mean pulmonary arterial pressures at right heart catheterisation which failed to respond to acute vasodilator challenge[90]. Rare variants in ABCC8, which encodes sulfonylurea receptor 1 (SUR1), a regulatory subunit of the ATP-sensitive potassium channel associated with PAH, have also been identified resulting in decreased ATP-sensitive potassium channel function, which was pharmacologically recovered[91] .

The advent of whole genome sequencing (WGS) and whole exome sequencing (WES) has increased our understanding of genes associated with predisposition and progression of PAH. To identify the missing heritability, WES was carried out on a collaborative European cohort of adult patients with IPAH and familial PAH. The study identified rare variants in *ATP13A3, AQP1* and *SOX17*, a potential role of *GDF2*, which encodes a BMPR2 ligand, and a familial segregation of mutations in *AQP1* and *SOX17* in PAH[92]. In 2019, Rhodes et al also reported in a large genome-wide association study that common genetic variation at loci in an enhancer near *SOX17* and in *HLA-DPA1/DPB1* is associated with PAH and propose that impairment of *SOX17* function maybe more prevalent in PAH than suggested by rare mutations in *SOX17* [93].

In the last 15 years, genetic and genomics studies of SSc patients have identified a number of genes associated with vascular complications, including the development of PAH. These genes include *IL23R*[94]*, TNIP1* and *TNFAIP3*[95], *uPAR* [96],*TLR2* [97], *CAV*[98]*, PPAR*γ[99], *ATP8B4*[100], *MIF*[101]*, NKX2-5* [102]. Variants can broadly be classified in those affecting immune or inflammatory pathways and mechanisms and those which affect vascular structure and function.

Reported Interleukin 23 receptor gene (IL23R) polymorphisms identified susceptibility which is associated with anti-topoisomerase (ATA)-positive scleroderma and is protective against the development of pulmonary hypertension suggesting complex

immunopathogenesis of SSc pulmonary hypertension[94]. Furthermore, polymorphisms in ubiquitin-modifying enzyme TNFAIP3, a key regulator of the NF-κB inflammatory signaling pathway, are associated with the development of diffuse cutaneous SSc (dcSSc), fibrosing alveolitis and PAH[95]. More recently, studies of the Toll-like receptor genes (TLR) in scleroderma identified a rare functional polymorphism in the TLR2 gene associated with (ATA)-positive SSc patients and with the development of SSc-PAH. This polymorphism resulted in increased activation of IL-6 and TNFα by monocytes in response to TLR agonists[97]. More recently, whole-exome sequencing identified *ATP8B4,* which codes for ATPase phospholipid transporting 8B4 protein in immune cells, to be associated with a significant increase in the risk of SSc pulmonary complications and SSc-PAH. It catalyzes the hydrolysis of ATP coupled to the transport of aminophospholipids [100]. Macrophage migration inhibitory factor (MIF) gene encodes a constitutively expressed protein with an important role in autoimmune and inflammatory processes. A polymorphism in the promoter region of MIF is associated SSc-PAH resulting in increased MIF protein levels and is linked to several immune-mediated diseases[101].

Genetic variation in the promoter region of the Urokinase-type plasminogen activator receptor (uPAR) is associated with SSc vascular complications including digital ulceration and SSc-PAH has been identified. UPAR encodes a pleiotropic receptor, which is involved in fibrosis, immunity, angiogenesis, as well as vascular remodeling[96].

Caveolin-1 (CAV1) is essential for the formation of caveolae and is an inhibitor of tissue fibrosis. A protective polymorphism was associated with increased CAV1 protein expression in tissues of SSc patients [98]. Mutations in CAV1 that lead to a reduction in protein expression have also been identified in heritable PAH patients. CAV1 appears to modify TGFβ signalling, including the reduction in BMP signalling[98].

The multifunctional nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) has potent anti-fibrotic effects, and its expression and activity are

impaired in SSc patients. A case-control and meta-analysis study provided evidence of a polymorphism found in a con-coding region, which associates with pulmonary hypertension in a French cohort. The role of PPAR-γ dysfunction has been implicated in diverse cardiovascular pathologies [99].

NKX2-5 is a homeobox transcription factor that is required for the formation of the heart and vessels during development, with significant postnatal down-regulation and reactivation in disease states, characterized by vascular remodeling. Disease-associated polymorphisms located in non-coding regions of *NKX2-5*, increase *NKX2-5* transcriptional activity and are associated with SSc-PAH [102].

Multiple genetic association studies in scleroderma have identified potential risk alleles, that play a role in adaptive and innate immune responses as well as fibrosis. It is noteworthy that currently the genetic/genomic landscape of SSc-associated PAH consists of genes regulating inflammation and vascular function which are markedly different from the genes observed in heritable and IPAH which are mainly associated with a reduction in BMPR2/SMAD activity. To date, no association to members of the TGFβ superfamily of receptors, including BMPR2 and ALK1, has been detected in SSc-PAH patients[103]. More research is required in assessing the relationship between genetic determinants, vascular phenotype and the severity of SSc-PAH. At the dawn of the era of 'Big data' technologies, activities are likely to focus on integrating clinical data, imaging and multi-omic data in advancing our understanding in this area[104].

b. Pathogenic factors and signaling pathways implicated in the development of PAH

Multiple proteins, lipids and gases have been implicated in the various molecular mechanisms that result in PAH. Over the last two decades, the role of mediators such as endothelin, nitric oxide and prostacyclin have been established and detailed

mechanisms of their action in PAH have been delineated **(Table 1).** More recently, as our knowledge of the pathogenesis of PAH expanded, our understanding of mediators such as BMPs, Activin, PDGF, serotonin, and epigenetic components such as microRNAs (miRNAs) has also increased and has led to novel factors and emerging pathways that can be targeted for therapeutic advantage. Furthermore, new areas of study in PAH, such as inflammation and metabolism, are now providing mechanistic insights into the intricate and complex cross-talk between different cell types, factors, and pathways that drive disease pathology. We direct readers to several excellent reviews[61,82,88,105] and shall focus on some well-established and some key emerging mediators.

(i) The TGF-β/BMP axis:

The transforming growth factor (TGF-β) superfamily comprises more than 35 structurally related genes, which include bone morphogenetic proteins (BMP); and growth/differentiation factors and activins [88,106–108]. The role of TGF-β, which in itself promotes the pro-fibrotic effects in numerous pathologies including SSc[109]**,** is well described. In general terms, in vascular remodelling is caused by the imbalance between various TGF-β family members resulting dysregulated signaling that results in vascular cell proliferation. The equilibrium is disturbed by overactive pro-proliferative SMAD2/3 signaling as well as deficient anti-proliferative SMAD1/5/, ultimately resulting in the unrestricted proliferation of vascular endothelial and smooth muscle cells[110]. TGF-β has been implicated in promoting several of the cellular changes associated with vascular remodeling, including SMC proliferation matrix deposition, and alterations in cell growth, particularly of endothelial cells whilst BMPs maintain vascular homeostasis [105,111–118] .

BMPR2 is a transmembrane serine/threonine kinase, which serves as a receptor for bone morphogenetic proteins (BMPs)[88]. BMPR2 is expressed on the surface of a wide range of cell types, including pulmonary arterial endothelial cells (PAECs) and

Pulmonary arterial smooth muscle cells (PASMCs) [88]. In association with a co-receptor, BMPR2 can signal through multiple pathways, including the canonical SMAD1/5/8-dependent pathway[83,88]. In normal PASMCs, ligation and subsequent activation of BMPR2 inhibits proliferation and promotes apoptosis [119]. PAH PASMCs harboring BMPR2 mutations are resistant to the growth-suppressive effects of BMPs, namely BMP2 and BMP4. Loss of responsiveness to the growth-suppressive effects of BMPs has also been reported in PASMCs derived from PAH patients lacking BMPR2 mutations, primarily as a consequence of downregulated BMPR2 expression or post-translational BMPR2 cleavage[9]. In PAECs, the BMPR2/ALK1 receptor complex signals selectively in response to the circulating ligands BMP9 and BMP10. BMP9 has been demonstrated to attenuate apoptosis in PAECs and strengthen the barrier function of the pulmonary endothelial monolayer. These effects are lost in PAECs isolated from PAH patients with BMPR2 mutations[10].

Although mutations in BMPR2 have not been identified in SSc patients, many proteins with a biased activity in favour of TGFβ or impaired BMP actions have been reported[120,121]. Accumulative evidence in support is provided by, TGF-β-driven pre-clinical models of SSc which spontaneously develop PAH and exhibit endothelial cell damage and a pulmonary vasculopathy[122]. Furthermore, there is a decrease of BMPR2 levels in SSc patients, associated with enhanced activity in downstream signalling components of the TGFβ pathways including Smad2 and MAPK[123]. The accumulation of evidence of decreased BMP signalling in SSc-PAH, albeit not from mutations in BMPR2, and increased TGFβ activity may still provide the underlying mechanism underpinning SSc-PAH although further mechanistic research is required in this area.

Much research has been caried out to address and restore the balance between BMPs and TGFβ activity. For example, the treatment of animals with BMP9 has been shown to reverse established PAH in preclinical models[10]. Although the role of BMP9 in PAH

is becoming evident, its significance in SSc-PAH as yet remains unknown [10,124–127] . GDF-15 may be a useful biomarker in PAH associated with SSc. GDP-15 may have a role in regulating inflammatory pathways by regulating apoptosis, cell repair and cell growth, which are biological processes observed in cardiovascular and neoplastic disorders. Its presence in lung tissue may suggest a role in the pathology of the disease[128]**.**

Other studies have used targeted gene delivery of BMPR2 to attenuate PAH[129] and inhibition of the TGF-β receptor ALK5 also prevents the development and progression of PAH[130]. As TGF-β signaling is crucial for many physiological functions, prolonged inhibition might lead to harmful side effects, although it has proved beneficial in persisting with more refined strategies to normalize TGF-β activity in PAH and other diseases[131,132].

Recent studies have attempted to overcome the significant challenges in targeting the the TGF-β/BMP axis, by focusing on a proposed mechanism that involves rebalancing pro-proliferative and anti-proliferative signalling. The BMPR2-Smad1/5/8 pathway is down-regulated in PAH leading to increased production of activin ligands, such as activin A, growth differentiation factor 8 (GDF8), and GDF11. As a result, the activin receptor type IIA (ActRIIA)–Smad2/3 pathway is upregulated. Increased phosphorylated Smad (pSmad)2/3 activity promotes expression of the endogenous BMP antagonists gremlin-1 and noggin. Gremlin-1 and noggin further reduce BMP–Smad1/5/8 signalling. The overall result is that anti-proliferative signalling is reduced, shifting the balance toward pro-proliferative activin–Smad2/3 signalling, which leads to pulmonary vascular remodelling[11].

Sotatercept (Table 1), a fusion protein, sequesters excess activin ligands and growth differentiation factors, thereby reducing ActRIIA–Smad2/3 signalling and restoring balance between growth-promoting and growth-inhibiting signaling pathways[12]. Furthermore, Joshi et al demonstrated that activin-class ligands are key mediators of

inflammatory and immune responses in a model severe experimental PAH. These authors clearly demonstrated that activin ligands may be important regulators of macrophage activation and perivascular infiltration in PAH lungs [133], suggesting the importance of these ligands in PAH inflammation (see inflammation below).

(ii) Serotonin (5-HT):

The 'serotonin hypothesis of PAH' arose over 40 years ago when patients taking the anorexigen aminorex fumarate were associated with an increased risk of developing PAH[134]. In the 1990s, it was reported that there was increased plasma serotonin in some patients with primary PH associated with platelet storage pool defect[135]. In PAH, platelet-driven and locally produced serotonin from lung tissue and arterial endothelial cells is thought to promote both vasoconstriction and remodeling of the pulmonary vasculature by inducing proliferation of pulmonary arterial fibroblasts and SMC [136,137]. One way that serotonin promotes pulmonary smooth muscle cell proliferation and contraction is through receptor mediated (5HT1B) vasoconstriction and receptor-independent uptake via the serotonin transporter (SERT)[136,138]. SERT has been implicated in both clinical and experimental PAH, functionally activating the PDGFβ receptor to induce SMC proliferation[139]**.** Recently, SERT has been implicated in the pathogenesis of pulmonary hypertension in patients with chronic-obstructive pulmonary disease (Group 3) [140] . 5HT1B receptor expression is upregulated in PASMCs from female PAH patients potentially exacerbating the impact of increased serotonin levels[141] . A substantial upregulation of 5-HT(2B) receptor expression was noted in the pulmonary arteries in both animal models and IPAH patients[142,143]. Serotonin has also been shown to induce ECM synthesis in interstitial fibroblasts from SSc patients via the 5-HT(2B) receptors in a TGFβ dependent manner[144]. Although some studies have highlighted the dysregulation of components of the serotonin pathway in SSc and the links between vascular disease and tissue fibrosis [139] mechanistic details remain of the serotonin pathway unclear in the vasculature of SSc-

PAH remains unclear. Several studies aimed to target the serotonin pathway including targeting receptor antagonists (e.g., ketanserin and terguride), receptor agonists (e.g., sumatriptan), or transporter reuptake inhibitors (e.g., fluoxetine) with disappointing results [145–148].

The rate limiting step in serotonin biosynthesis production of 5-hydroxytryptophan from tryptophan by isoforms of tryptophan hydroxylase (TPH). TPH1 is associated with the production of serotonin in the periphery (outside the brain) and is overexpressed in the lungs and pulmonary arterial endothelial cells of patients with PAH and leads to excess local serotonin production [149]. Targeting TPH1 presents a logical strategy to improve vascular remodelling and treat PAH. Currently, a promising Phase 2b, double-blind, clinical trial (ELEVATE 2, NCT04712669) using rodatristat ethyl, a potent inhibitor of TPH1 is underway. Rodatristat efficacy has been demonstrated in monocrotaline and SUGEN hypoxia nonclinical models of PAH and robust dose-dependent reductions of 5-hydroxyindoleacetic acid, the major metabolite of serotonin in plasma and urine of healthy human subjects[150].

(iii) Platelet-Derived Growth Factor:

In the last 25 years, many studies have highlighted the platelet-derived growth factor (PDGF) pathway as a major regulator of pulmonary vascular remodeling and PAH development[151–153]. PDGF is composed of two polypeptide chains A, B, C and D leading to the formation of a dimeric protein (PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD and PDGF-AB)[154], the best-studied being PDGF-AA and BB. Initially identified in platelets, PDGF isoforms are secreted from several cell types including macrophages, endothelial cells, vascular smooth muscle cells and fibroblasts. PDGF is a potent mitogen and chemoattractant for fibroblasts, vascular smooth muscle cells and endothelial cells [154]. It has often been implicated in PAH with increased circulating levels of PDGF-BB are elevated in IPAH patients[153] but not in SSc-PAH[155]. Conversely PDGF receptor β (PDGFRβ) expression in SSc-PAH is broader and more intense in

small- and post-capillary vessels compared to IPAH[156]. Furthermore, Baroni et al reported that stimulatory autoantibodies against PDGFR may have a causal role in the pathogenesis of SSc[157]. In contrast, it remains unclear whether PDGFRα, which is a marker of mesenchymal stem cells and vascular progenitor cells, has a role in PAH [158].

Inhibition of the PDGF pathway is an attractive therapeutic strategy for the treatment of PAH. In animal studies, the PDGFR-β tyrosine kinase inhibitor Imatinib can reverse vascular remodeling via reduction in serotonin through inhibition of tryptophan hydroxylase 1 expression[159]. Other animal studies have demonstrated that imatinib reverses vascular remodeling in severe experimental pulmonary hypertension regardless of the initiating stimulus[160] .

Administration of imatinib to PAH patients in two clinical trials showed a significant decrease in pulmonary vascular resistance (PVR) compared to a placebo and reported a significant improvement at 24 weeks in functional capacity[57,161]. However, sever adverse events in the Imatinib group raised concern over safety. Currently, a new study (Clinicaltrials.gov id: NCT05036135) in IPAH and CTD-PAH is evaluating the safety and efficacy of AV-101 (a dry powder inhaled imatinib).

c. Inflammation in PAH

In the last decade, inflammation has emerged as a driving factor of the development of PAH. Extensive infiltration of T and B lymphocytes, monocytes, dendritic and mast cells in the remodeled arteries and elevated levels of pro-inflammatory (e.g. interleukin-1β (IL-1β), IL-6, tumor necrosis factor α (TNFα) and chemotactic (e.g. monocyte chemotactic protein-1 (MCP-1), IL-8) cytokines in the sera of PAH patients provides a growing body of evidence that early and persistent inflammation and altered immune responses underlie PAH pathophysiology[162,163].

It is generally accepted that endothelial injury and concomitant upregulation of adhesion molecules are thought to promote the transmigration of these inflammatory cells into the vascular wall – the so-called 'inside-out' theory[164]. This is being increasingly contested, however, by the 'outside-in' hypothesis, which proposes that inflammation is initiated in the adventitial and perivascular layers and progresses inwards towards the intima. Activated fibroblasts are thought to create a microenvironment permissive for the recruitment of circulating leukocytes through the production and retention of soluble factors, such as chemokines, cytokines, and growth factors in the adventitia of injured arteries [165]. Perivascular and adventitial accumulation of monocytes and macrophages as well as increased expression of proinflammatory cytokines and chemokines (GM-CSF, CCL2, CX3CL1, CXCL12, and IL6), have been consistently reported in patients with PAH and animal models. Frid et al demonstrated recently a mechanism where immunoglobulin-driven dysregulated complement activation regulates proinflammatory and pro-proliferative processes in animal models of PAH and demonstrated that signaling of complement is a critical determinant of clinical outcome in PAH[166]

Amongst ongoing debate, the field has moved ahead with attempting to target inflammation clinically. One example of more selective targeting of pathological inflammation is the ongoing study of rituximab in scleroderma-associated PAH (ClinicalTrials.gov identifier: NCT01086540). Another example is anti-IL-6 therapy using tocilizumab [52,53]. In SSc patients, tocilizumab reversed activation of TGF-β in dermal fibroblasts[167] and preserved lung function of patients with SSc interstitial lung disease [168]. However, a clinical trial on a group of IPAH and CTD-PAH patients, treatment with tocilizumab showed no significant effects on haemodynamic parameters of IPAH patients. A potential improvement was noted in the small subgroup of patients with CTD-associated PAH which requires further investigation [53].

BMPR2 has an anti-inflammatory role in pulmonary endothelial cells and therefor with reduced levels of BMPR2 would potentially lead to an increase of inflammation. Tian et al found that an acute inflammatory insult caused endothelial-mesenchymal transdifferentiation (EndMT) of endothelial cells via canonical SMAD2/3 signaling. This can be reversed in by ALK5 or Smad3 knockdown or by ALK5 inhibition in Bmpr2 mutant rats[169]. This implies that targeting ALK5 or SMAD3 could potentially inhibit activin/GDF8/GDF11 signaling and implicate activin-driven inflammation in pulmonary vascular remodelling in PAH [133].Joshi et al also showed that Sotatercept reverses pulmonary vascular remodelling in severe experimental PAH by acting on perivascular macrophages which are activated by activin A. PAH patients have been shown to produce high levels of Activin A. Sotatercept also normalized IL6 levels induced by activin A, activin B, and GDF11 [133].

In the future, it may be useful to target inhibition of inflammation of at-risk individuals (such as those with SSc-PAH) to prevent the development of pulmonary vascular disease. Overall, blocking inflammation remains an attractive target in pulmonary hypertension.

d. Epigenetics of PAH:

Since our last update and the emergence of non-genetic mechanisms which contribute to the cellular changes and the development of PAH, the pathological relevance in the context of SSc-PAH is also being explored.

Germ line pathological mutations have been identified in 17 genes thus far in PAH patients, the most prevalent occurring in ~25% of idiopathic PAH patients is in the BMPR2 gene[7]. In contrast genetic association in PAH patients with connective tissue diseases such as SSc is far from clear cut. Studies have highlighted the apparent phenocopying of SSc-PAH to disease-causing mutations identified in PAH. For

example, we have previously shown the BMPRII protein in patients with SSc-PAH to be reduced[170]. More recently mutations in the TET methyl-cytosine dioxygenase 2 (TET2) an important enzyme in DNA methylation were identified in PAH patients, and expression in PBMCs of TET2 was shown to be decreased in 86% of SSc-PAH patients relative to healthy subjects[171]. Interesting extensive CpG sites methylation in the BMPRII promoter region has been noted in SSc microvascular endothelial cells (SSc-MVECs), and these cells were more sensitive to apoptotic triggers than are control-MVECs [172]. It awaits future studies to assess if this is true for the pulmonary vasculature.

Histone acetylation is a key mechanism by which cell proliferation and survival is regulated. Zhao et. al. demonstrated elevated protein levels of histone deacetylase (HDAC) 1 and 5 in PAH lungs. Administration of HDAC inhibitors, reduced proliferation of PAH vascular fibroblasts and PDGF-stimulated growth of SMCs[173]. HDAC5 is also significantly increased in dermal ECs and fibroblasts from patients with SSc compared and leads to a reduction in expression of many genes including CYR61 resulting in dysregulated angiogenesis by ECs. In SSc fibroblasts, overexpression of CYR61 exhibits an antifibrotic effect, reducing the expression of profibrotic genes, including COL1A1 [174]. The pulmonary relevance of HDAC5 in SSc remains to be determined.

Micro RNAs (miRs) have been proposed as secreted biomarkers and mediators in the development of PAH[175,176]. The growing relevance of miRNAs in modulating the pathogenic processes that contribute to the development of PAH is becoming more apparent in recent years. However, the relevance of these miRNAs in the development and progression of SSc-PAH lags. Within the context of FPAH and IPAH, a number of differentially expressed miRNAs have been identified that have relevance to PAH. Expression miR-96 is reduced in PASMCs from female patients with PAH, and negatively regulates the expression of the serotonin receptor 5-HT1BR. Restoration of miR-96 expression reduces the development of hypoxia-induced PH in mice[141].

Hypoxia can promote broader changes in miR expression impacting cellular functions. For example, hypoxia leads to the up-regulating of miR-210 in SMC inhibiting apoptosis. Whereas miR-98 reduced in PAECs from PAH patients, is repressed by hypoxia and regulates ET-1 expression and PAEC proliferation[175,177].

More recently, Sindi et al demonstrated that reduced KLF2 signalling is a common feature of human PAH and that KLF2-induced exosomal microRNAs, miR-181a-5p and miR-324-5p act together to attenuate pulmonary vascular remodelling[178]. Further epigenetic studies by the same group showed that endothelium-targeted miR-150 delivery prevented the disease in Sugen/hypoxia mice, while endothelial knockdown of miR-150 had adverse effects miR-150 target genes revealed significant associations with PAH pathways, including proliferation, inflammation, and phospholipid signaling[179].

The utility of long noncoding RNAs (lncRNAs) in SSc as novel candidate biomarkers to support diagnosis and discriminate disease subtypes has recently been explored and ANCR and SPRY4-IT1 were found to positively correlate with SSc-PAH [180].

A deeper understanding of the epigenetic contribution to susceptibility and progression of PAH in SSc patients is needed.

e. Metabolism in PAH:

Another area of pathogenetic significance in PAH is dysregulated metabolism. A large body of literature on PAH in humans and pulmonary hypertension (PH) in animal models have underscored an important role of altered pulmonary vascular cell metabolism in disease pathogenesis [181–183]. The significance of metabolic pathways as key promoters of cell proliferation and disease pathogenesis was first described in studies on cancer, a group of diseases that shares many other pathobiological similarities with PAH [73,74,184]. For example, cancer and PAH demonstrate upregulation of the transcription factor hypoxia inducible factor-1 (HIF-1), which activates genes

involved in glycolysis as well as cell proliferation [185–187]. This connection between cell proliferation and metabolism has long been recognized in the cancer field, in which various metabolism-altering therapies are clinically approved [188].

Based on the similarities between cancer and the proliferative pulmonary vascular lesions in PAH [63], there have been growing efforts to understand the potential contribution of metabolic pathways to the PAH pathobiology and their therapeutic targetability[181–183]. Most heavily investigated is pulmonary vascular glucose metabolism, specifically a shift toward glycolysis coupled with suppressed glucose carbon oxidation via the mitochondrial tricarboxylic acid (TCA) cycle in PAH [189–191] , a phenomenon known as the Warburg effect[72,192,193]. One potential mechanism of glycolysis-induced cell proliferation is NADPH generation from glucose carbons via the pentose phosphate pathway, thereby facilitating glutathione production, and nucleotide and lipid membrane synthesis [194,195].

While its exact relevance specifically to SSc-PAH remains unclear, recent studies strongly suggest a link between glycolysis and pulmonary vascular cell proliferation. The presence of upregulated HIF-1 signaling, and increased glycolysis has been established in the whole lungs (e.g., increased ^{18}F-FDG uptake) [189–191] and pulmonary arterial cells of human PAH [186,187,189,191], largely in idiopathic PAH. Systemic induction of mitochondrial glucose oxidation and suppression of glycolytic lactate production, achieved with the pharmacologic agent dichloroacetate, protected animals from pulmonary hypertension [196–198], and the same therapeutic strategy benefited a small number of carefully selected susceptible IPAH patients[199]. Other interventions to reduce glycolysis specifically in the vascular wall constituents, the endothelium and the smooth muscle cells, protected mice from hypoxia-induced pulmonary hypertension, which notably is distinct from PAH[200,201]. Recent data indicate that HIF-1-dependent glycolysis in myeloid cells may also contribute to PH pathobiology, at least in the specific context of hypoxia-induced pulmonary hypertension [202,203].

Metabolic substrates and pathways other than glucose metabolism likely promote PAH pathobiology. TCA cycle metabolites have been shown to promote cell proliferation [204–207] with numerous studies demonstrating mitochondrial dysregulation in diseased pulmonary arteries of PAH patients and animals with experimental pulmonary hypertension [186,189,208–210](61, 65, 83-86). Fatty acids represent an alternative carbon source for the mitochondrial TCA cycle, termed anaplerosis, the pathogenetic significance of which has long been recognized in cancer [211]. Fatty acid catabolism may be both a source of energy as well as carbons for macromolecule biosynthesis. For instance, fatty acid oxidation is necessary for endothelial cell proliferation [212] and likely contributes to the excessive endothelial growth seen in PAH [63,73,213]. Pulmonary vascular metabolism of amino acids, such as glutamine, represents another key pathogenetic process [214–216]. The aggregate of these observations underscores the individual and combine significance of the various metabolic pathways in promoting pulmonary vascular proliferation.

There are shortcomings however, applicable to the field of PAH in general in addition to SSc-PAH, which require further clarification before metabolism-altering therapies can be considered as a reliable therapeutic strategy. One largely unexamined area of critical importance regards how the various coexisting metabolic pathways interact with each other and affect the mitochondria to cause pulmonary vascular diseases. For instance, inhibition of fatty acid oxidation reciprocally induces glucose oxidation in the mitochondria, a phenomenon known as the Randle cycle [217], which in turn secondarily suppresses glycolysis. The combined and relative contributions of fatty acid oxidation and glycolysis to pulmonary vascular diseases remain unknown. Similarly, differential effects of the multiple carbon sources of TCA cycle intermediates (glucose, fatty acids and glutamine) remain undefined.

Another area for future research is interrogation of metabolic pathways in a disease-specific and lesion-specific manner. Despite the cumulative data, evidence supporting

a pathogenetic role of dysregulated pulmonary vascular metabolism specifically in SSc-PAH is lacking and merits future studies [187]. As discussed above, the varying frequency of plexiform lesions in SSc-PAH lungs compared to IPAH lungs suggests that, although similar in morphology, metabolic dysregulation might play a disease-specific, and even a patient or lesion-specific role in promoting pulmonary vascular proliferation. High-throughput quantification of metabolic targets in a spatially resolved manner with intact pulmonary vascular anatomy (e.g., in individual plexiform lesions), now enabled by new commercially available tools, is anticipated to provide key insights into how metabolic pathways contribute to SSc-PAH. Using omic strategies to determine metabolic profiles most informative to diagnosis or risk stratification is potentially another way to progress in this area [72,218].

Concluding remarks:

In conclusion, the field of PAH has seen major recent advancements, adding to our understanding of the disease pathogenesis and identifying new avenues of therapeutic approaches. These insights stem from the discovery of genetic variants, various cell-signaling pathways, and epigenetic factors, collectively contributing to pulmonary vascular proliferation and clinically evident PAH.

Moving forward, addressing the following areas of uncertainty will be critical in enabling clinical applications of the new findings. The relevance of the abovementioned biologic pathways needs to be defined over the time course of disease development and in each subgroup of PAH. For instance, understanding the role of BMPR2 and TGF-β signaling at the onset of PAH as opposed during later stages of the disease will help determine when they can be optimally targeted to improve outcome. One challenge in studying PAH is the scarcity of human lung tissues in the early stages of the disease; methods allowing indirect assessment of the pulmonary arteries, for example using blood samples or imaging studies, will prove invaluable tools. Considering the pathobiologic and pathologic heterogeneities

of PAH, significance of the molecular and genetic discoveries in IPAH will require validation in other cohorts, including SSc-PAH patients. Interactions and the collective impact of the various biologic pathways, for instance whether metabolic dysregulation affect inflammation, remain to be clarified. As the PAH field accrues multi-omic data, researchers and clinicians will benefit from establishing optimal ways to integrate various omics data with clinical data.

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Table 1: Pathways targeted for clinical intervention in PAH. This is an abridged list with only approved treatments (blue), trials with positive endpoints or ongoing trials included.

Pathway	Drug	Target	Status	Ref or Clinicaltrials.gov ID
Endothelin-1 (ET-1)	Bosentan	ETA & ETB receptor	Approved	219
	Macitentan	ETA & ETB receptor	Approved	220
	Ambrisentan	ETA receptor	Approved	221
Prostacyclin (PGI_2)	Epoprostenol	PGI_2 receptor	Approved	222
	Treprostinil	PGI_2 receptor	Approved	223,224
	Iloprost	PGI_2 receptor	Approved	222
	Selexipag	PGI_2 receptor	Approved	71
Nitric Oxide (NO)	Riociguat	soluble Guanylate Cyclase	Approved	225
	Sildenafil	Phosphodiesterase type 5	Approved	226

	Tadalafil	Phosphodiesterase type 5	Approved	[227]
Bone morphogenic protein (BMP) / Activin	Sotatercept	Activin (ligand trap)	Phase 2	[12]
BMP Receptor 2 signalling	Tacrolimus (FK506)	Calcineurin / BMPR2	Phase 2a	[14]
Tyrosine kinase /PDGF signalling	Imatinib (inhaled)	Tyrosine Kinase receptors, PDGFR	Phase 2b/3	NCT05036135
Tyrosine kinase /PDGF signalling	Seralutinib	Tyrosine Kinase receptors, PDGFR	Phase 2	NCT04456998 NCT04816604
Serotonin	Rodatristat Ethyl	Tryptophan Hydroxylase 1	Phase 2b	NCT04712669 [228]
Inflammation	Rituximab	B-lymphocyte antigen CD20	Phase 2	[54]
Inflammation	Elafin	Elastase-specific protease	Phase 1	[229]
Inflammation	Sulfasalazine	Cystine transporter subunit (xCT)	Phase 1	NCT04528056
Metabolism	Metformin	AMP-activated Protein kinase	Phase 2	[230]
Metabolism	Bardoxolone	Nrf2, NFkB	Phase 2	NCT02657356
Oestrogen signalling	Anastrozole	Aromatase	Phase 2	NCT03229499
Oestrogen signalling	Tamoxifen	Oestrogen receptor	Phase 2	NCT03528902

Angiotensin	Recombinant ACE2	angiotensin-converting enzyme (ACE)	Phase 2	NCT01884051
Aldosterone	Spironolactone	aldosterone receptor blocker	Phase 2	[231]
DNA repair	Olaparib	poly(ADP-ribosyl)transferase	Phase 1b	NCT03782818
Epigenetic regulation	Apabetalone	Bromodomain-Containing Protein 4	Phase 1	NCT04915300
Dopamine	Zamicastat	Dopamine Beta-Hydroxylase	Phase 2	NCT04316143
Vasoactive Intestinal Peptide	Pemziviptadil	Vasoactive intestinal polypeptide receptor 1 & 2	Phase 2	NCT03795428

Figure 1

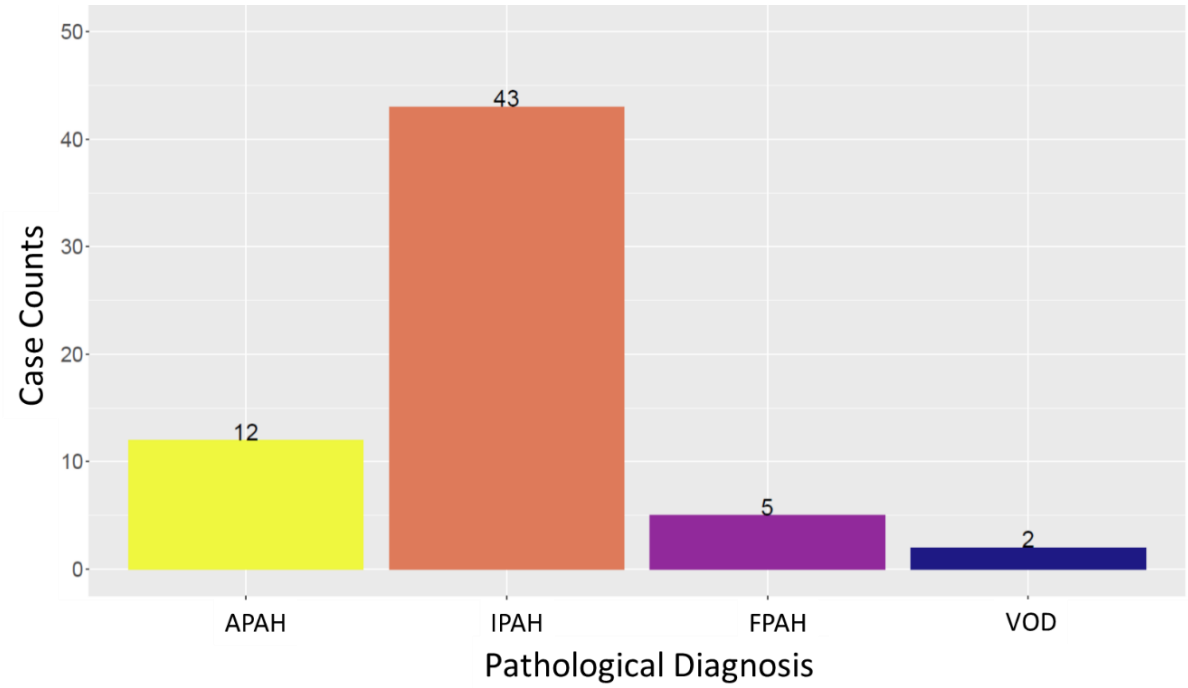

Figure 1. Overall frequency of histopathological PAH patterns in the cohort. The IPAH pattern includes lungs with idiopathic PAH, PAH with congenital heart malformations (n=9), drug and-associated. The familial or hereditary cases are included separately, though also with an IPAH morphology (FPAH). VOD (venocclusive disease). (Based and adapted on [63] with permission of the American Thoracic Society. Copyright © 2022 American Thoracic Society. All rights reserved. The American Journal of Respiratory and Critical Care Medicine is an official journal of the American Thoracic Society. Readers are encouraged to read the entire article for the correct context at [63]. The authors, editors, and The American Thoracic Society are not responsible for errors or omissions in adaptations).

Figure 2

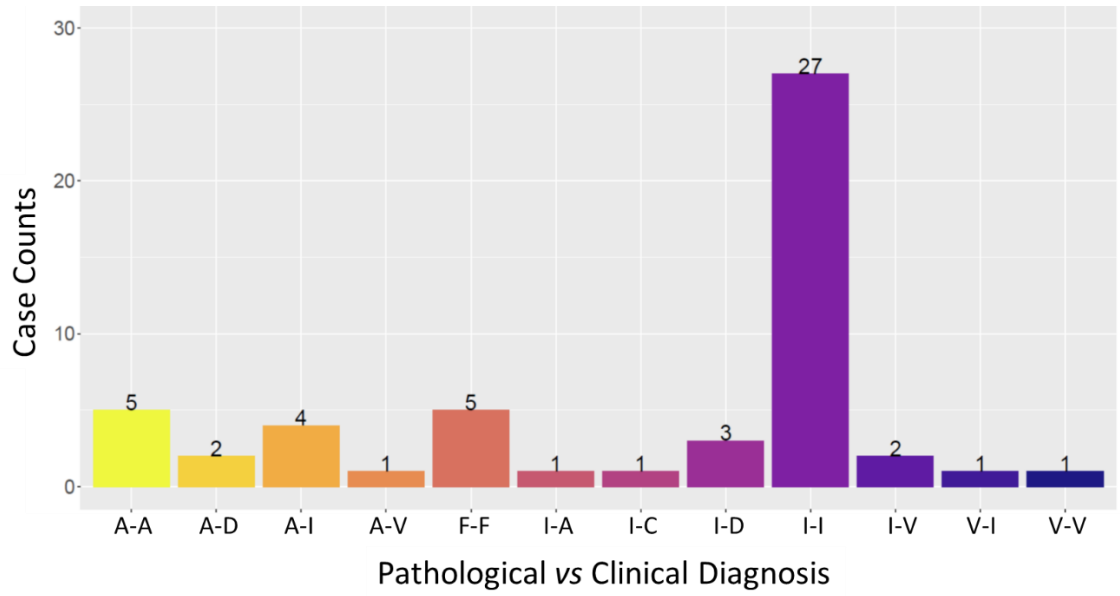

Figure 2. Distribution of lungs based on the status of the concordance or discordance between the pathological pattern (First Letter= A: APAH, F: FPAH, I: IPAH, V: venocclusive disease) and the clinical diagnosis (second letter= A: APAH, D: drug induced PH, I: idiopathic PAH, C: Chronic Thromboembolic Disease, F: FPAH; V: venocclusive disease).

Figure 3

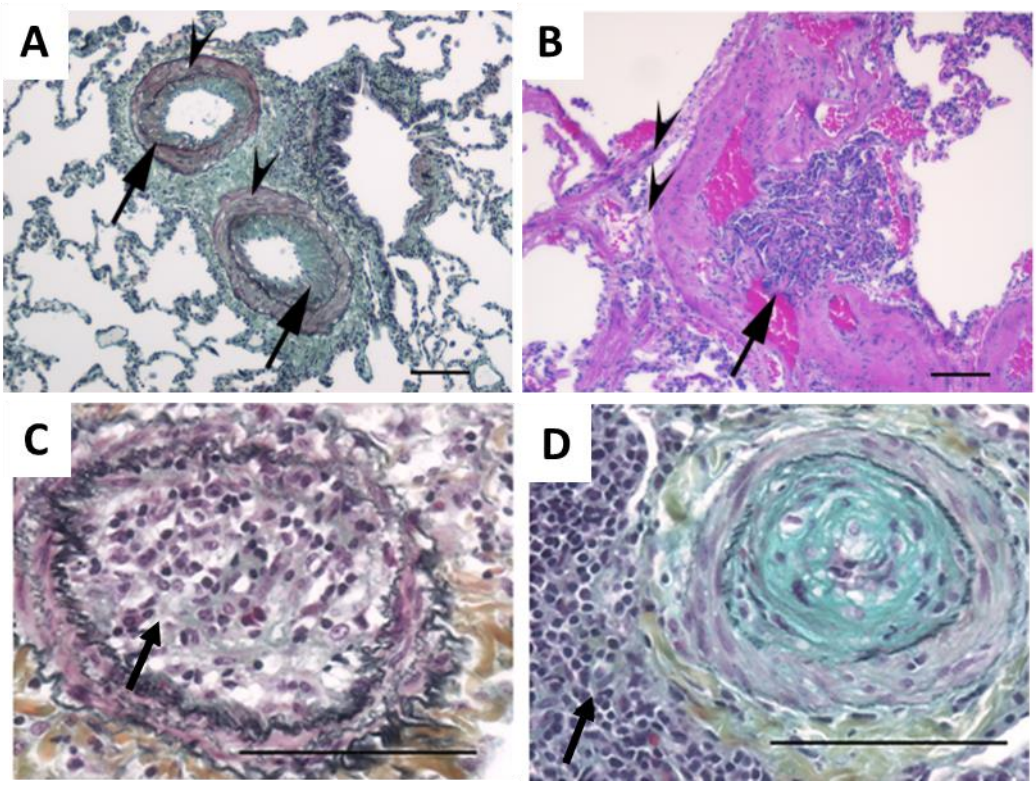

Figure 3: Histopathological parameters indicative of pulmonary vascular remodeling in pulmonary arterial hypertension. A. Intima thickening, media thickening (long arrows), and adventitia. The quantification of these parameters in control and PAH lungs is described in [63]. B. Plexiform lesion (arrows), characteristic lesion in PAH. C. Obliterative lesion with intravascular and perivascular inflammation (arrow). D. Concentric lesion with lymphoid aggregate in the adventitia (arrow). (Based on [63] and reproduced with permission of the American Thoracic Society. Copyright © 2022 American Thoracic Society. All rights reserved. The American Journal of Respiratory and Critical Care Medicine is an official journal of the American Thoracic Society. Readers are encouraged to read the entire article for the correct context at [63]. The authors, editors, and The American Thoracic Society are not responsible for errors or omissions in adaptations).

Figure 4

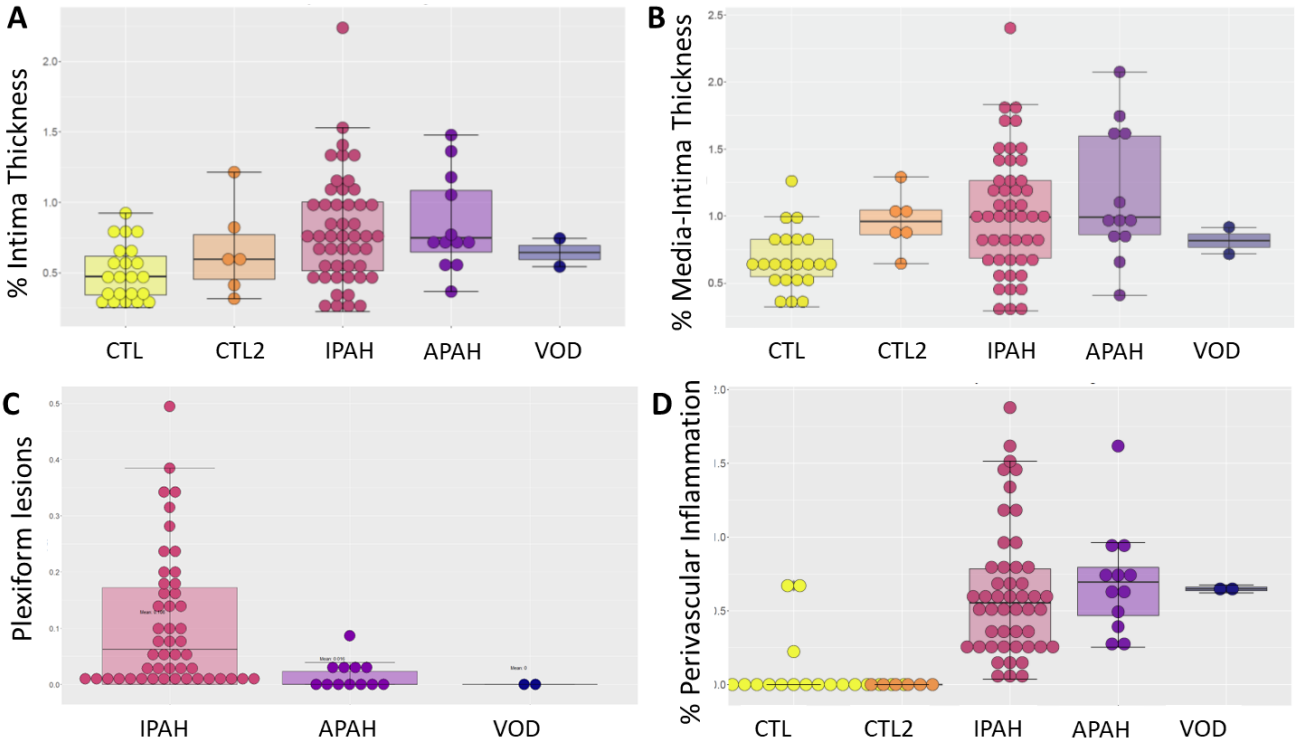

Figure 4. Pulmonary vascular remodeling A. Intima thickness in control (CTL), aged controls (CTL2), IPAH, APAH, and venocclusive disease (VOD). B. Media+intima thickening. C. Number of profiles of plexifom lesion (note that control cases do not have plexiform lesion and were not included in the graph). D. Perivascular inflammation score (based in [63]).

Figure 5

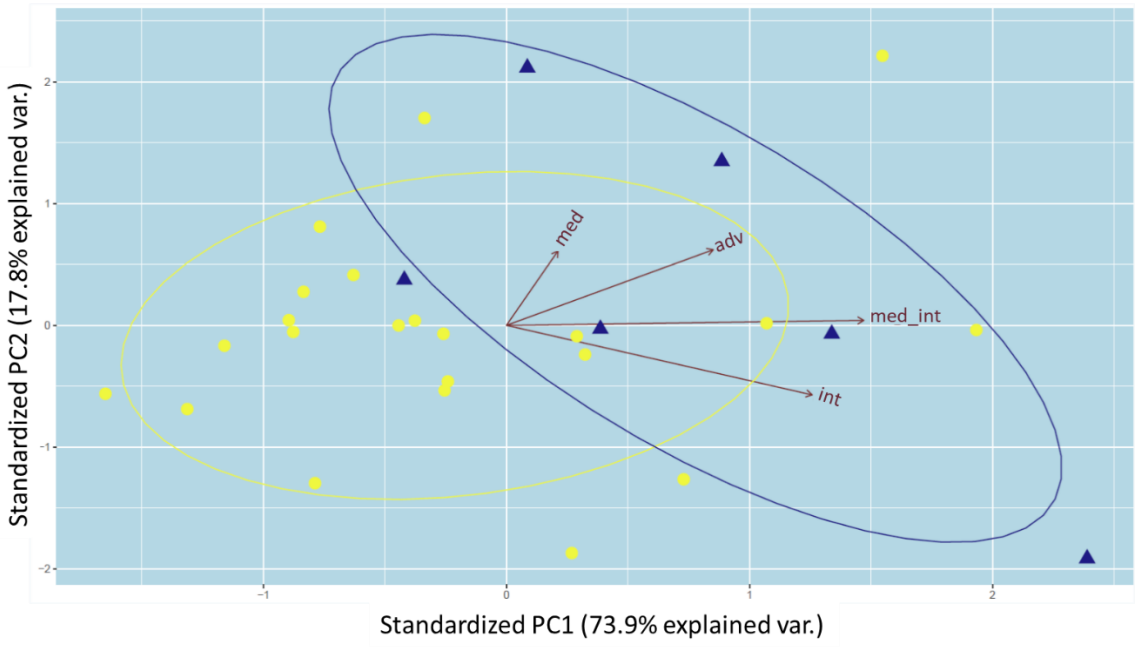

Figure 5. Principal component analyses (PCA) of control (CTL, yellow) and aged controls (CTL2, blue) (based in [63]). Vectors indicate measure of variance along PC1 and PC2, involving intima thickness (int), media thickness (med), intima+media thickness (int_med), adventitia thickness (adv), number of profiles of plexiform lesions (plx) and perivascular inflammation score (inf). Each point represents the values of single lungs. (based in [63]).

Figure 6

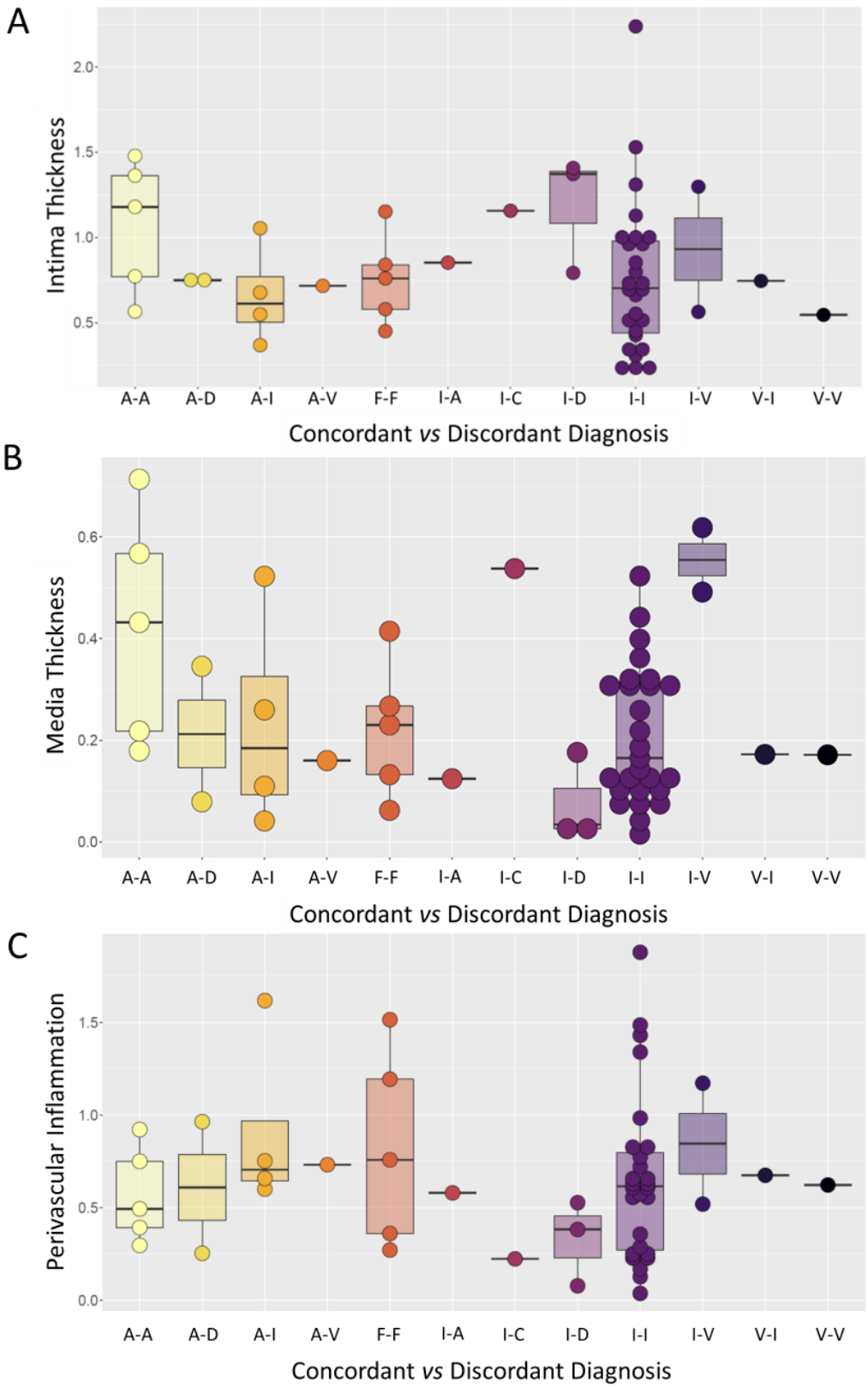

Figure 6. A. Intima thickening, B. Media thickening, C: Perivascular inflammation in concordant vs. discordant cases. (First Letter= A: APAH, F: FPAH, I: IPAH, V: venocclusive disease) and the clinical diagnosis (second letter= A: APAH, D: drug induced PH, I: idiopathic PAH, C: Chronic Thromboembolic Disease, F: FPAH; V: venocclusive disease).

Figure 7

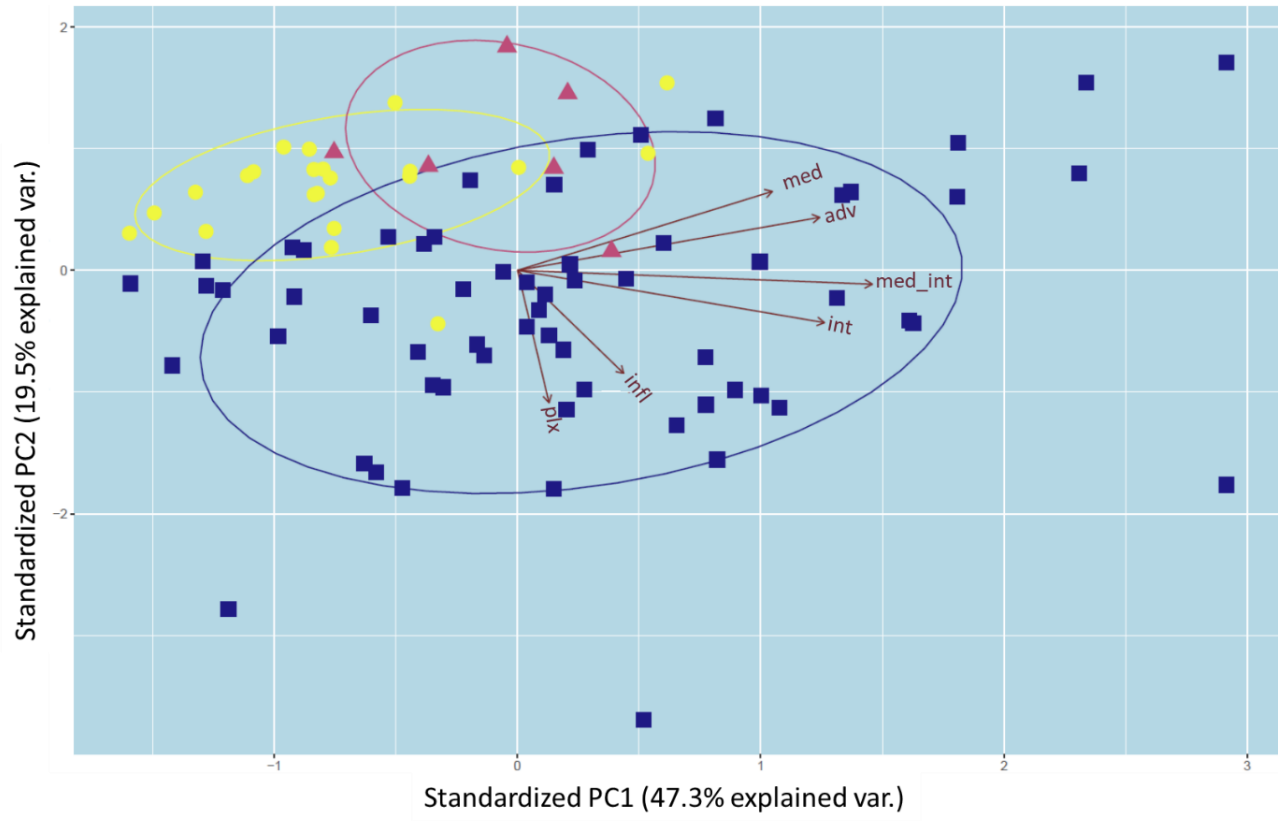

Figure 7: PCA for PAH and control lungs (CTL, yellow) and aged controls (CTL2, blue) (based in [63]). Vectors indicate measure of variance along PC1 and PC2, involving intima thickness (int), media thickness (med), intima+media thickness (int_med), adventitia thickness (adv), number of profiles of plexiform lesions (plx) and perivascular inflammation score (inf) (based in [63]). Each point represents the values of single lungs.

Figure 8:

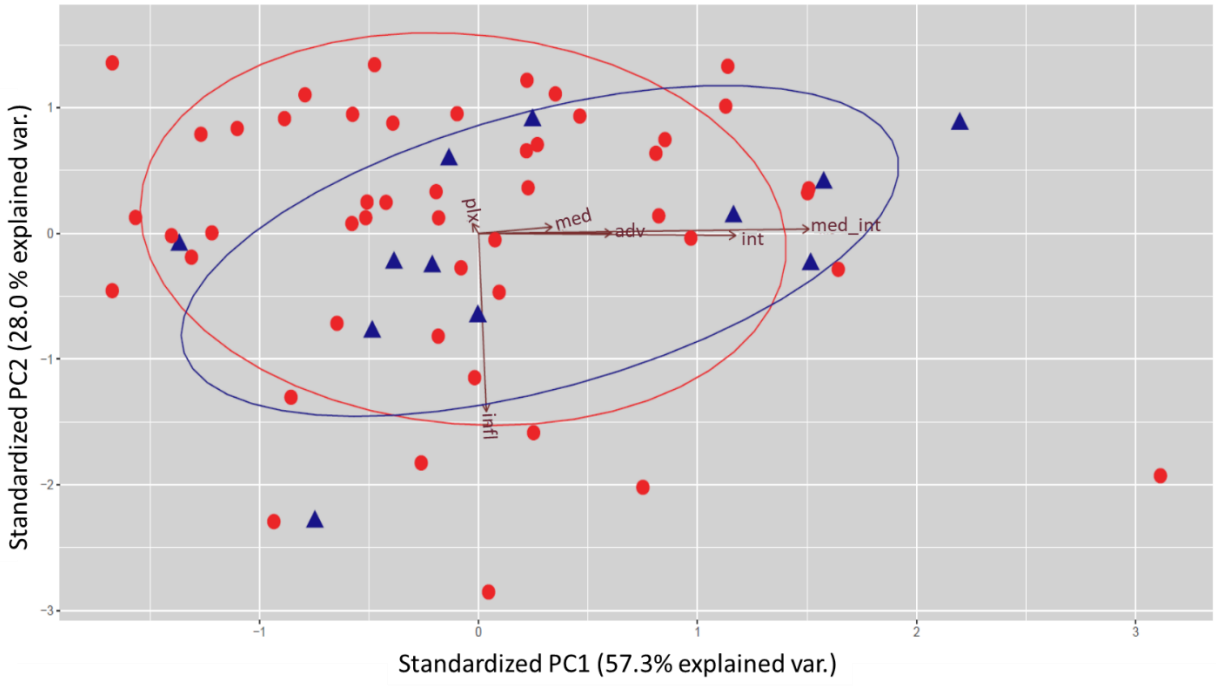

Figure 8: PCA for APAH and IPAH morphological patterns. Principal component analyses (PCA) of APAH (Blue) and IPAH (red) (based in [63]). Vectors indicate measure of variance along PC1 and PC2, involving intima thickness (int), media thickness (med), intima+media thickness (int_med), adventitia thickness (adv), number of profiles of plexiform lesions (plx) and perivascular inflammation score (inf) (based in [63]). Each point represents the values of single lungs.

Figure 9

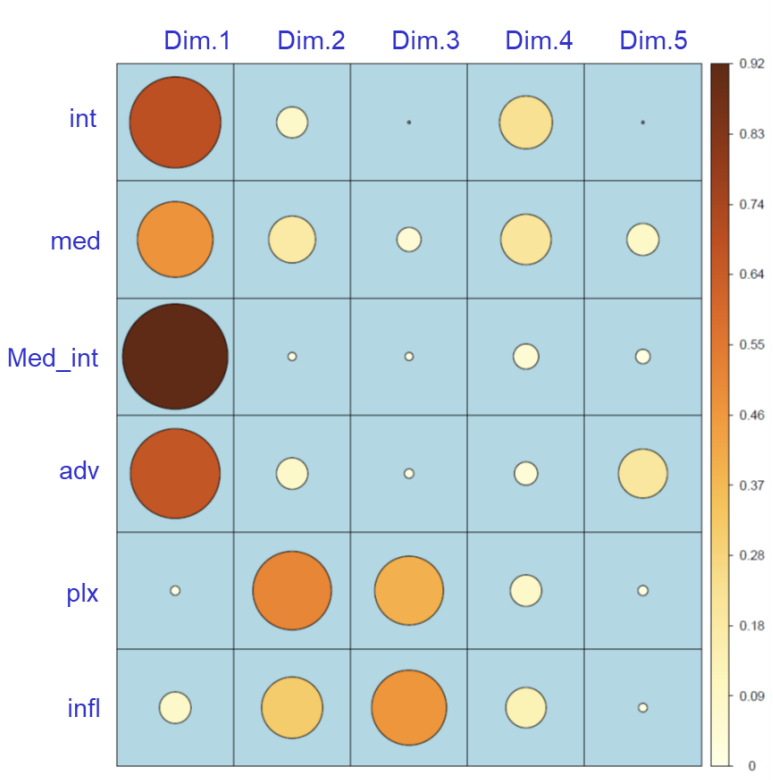

Figure 9. Contribution of each histopathological parameter to components (dimensions) 1-5 of the PCA of APAH vs. IPAH pathological pattern. The relative size is a quantitative measure of the contribution (based in [63]).