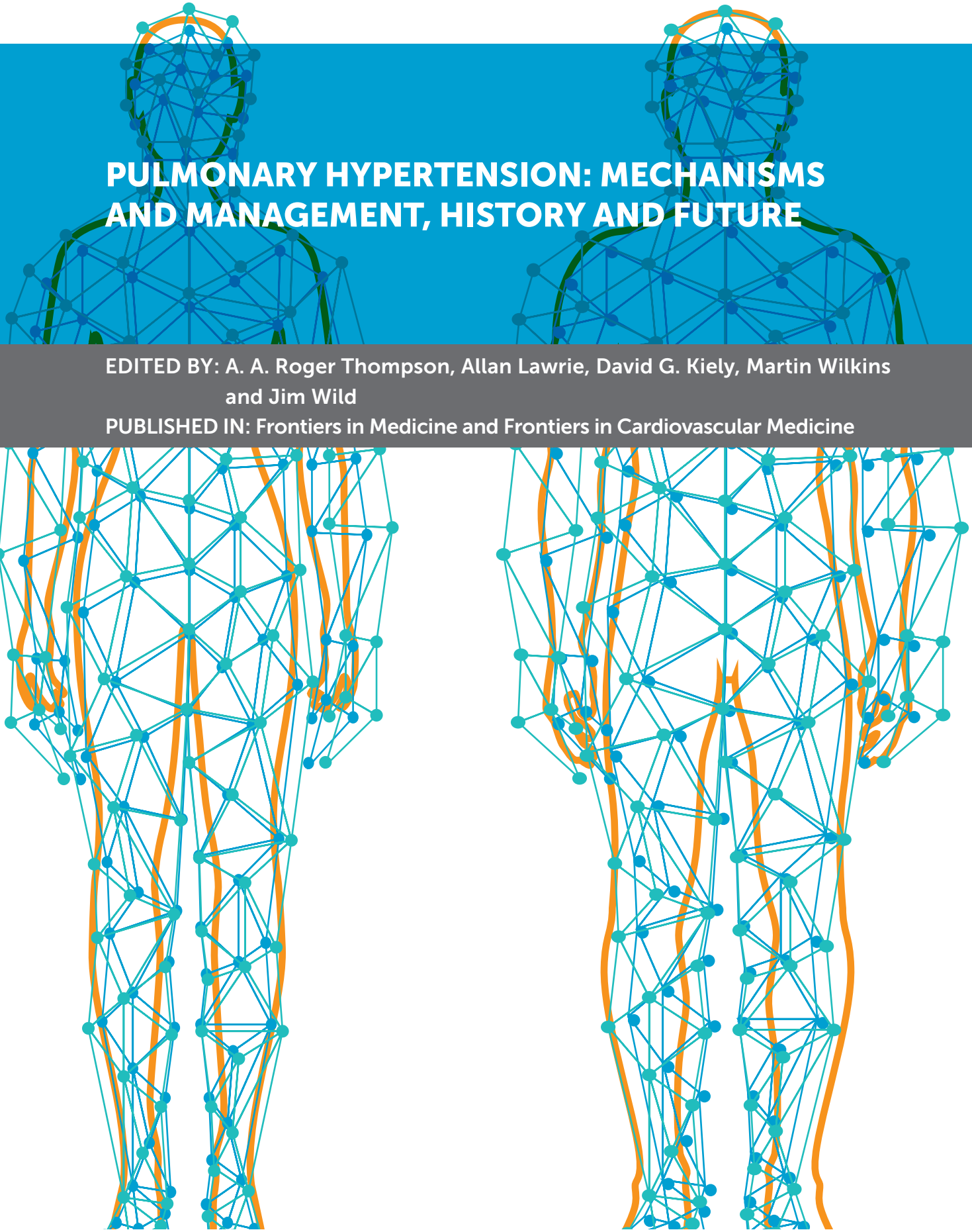


PULMONARY HYPERTENSION: MECHANISMS AND MANAGEMENT, HISTORY AND FUTURE

EDITED BY: A. A. Roger Thompson, Allan Lawrie, David G. Kiely, Martin Wilkins and Jim Wild

PUBLISHED IN: Frontiers in Medicine and Frontiers in Cardiovascular Medicine

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-790-4
DOI 10.3389/978-2-88963-790-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

PULMONARY HYPERTENSION: MECHANISMS AND MANAGEMENT, HISTORY AND FUTURE

Topic Editors:
A. A. Roger Thompson, University of Sheffield, United Kingdom
Allan Lawrie, University of Sheffield, United Kingdom
David G. Kiely, Sheffield Teaching Hospitals NHS Foundation Trust, United Kingdom
Martin Wilkins, Imperial College London, United Kingdom
Jim Wild, University of Sheffield, United Kingdom

We are grateful for the support of Actelion – a sponsor of this Research Topic – whose cooperation has contributed to fostering scientific discovery by reducing article publishing costs for some authors. We hereby state publicly that Actelion has had no editorial input in articles included in this research topic, thus ensuring that all aspects of this Research Topic were evaluated objectively, unbiased by any specific policy or opinion of Actelion.

Actelion is part of the Johnson & Johnson Family of Companies. We are leaders in the science and medicine of pulmonary arterial hypertension (PAH), with over 15 years of experience in this devastating cardiovascular disorder.

Citation: Thompson, A. A. R., Lawrie, A., Kiely, D. G., Wilkins, M., Wild, J., eds. (2020). Pulmonary Hypertension: Mechanisms and Management, History and Future. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-790-4

Table of Contents

frontiers in Medicine

EDITORIAL
published: 21 April 2020
doi: 10.3389/fmed.2020.00125

Editorial: Pulmonary Hypertension: Mechanisms and Management, History and Future

A. A. Roger Thompson[1,2]*, *Martin R. Wilkins*[3], *Jim M. Wild*[1,4,5], *David G. Kiely*[1,2,5] and *Allan Lawrie*[1,5]

[1] Department of Infection, Immunity and Cardiovascular Disease, The University of Sheffield, Sheffield, United Kingdom, [2] Sheffield Pulmonary Vascular Disease Unit, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom, [3] Department of Medicine and National Heart and Lung Institute, Imperial College London, London, United Kingdom, [4] POLARIS, Academic Unit of Radiology, Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, United Kingdom, [5] INSIGNEO Institute for in silico Medicine, The University of Sheffield, Sheffield, United Kingdom

Keywords: pulmonary hypertension, screening tools, biomarkers, heart failure, therapeutics, imaging

Editorial on the Research Topic

Pulmonary Hypertension: Mechanisms and Management, History and Future

From pulmonary hypertension observed at high altitude to pulmonary hypertension associated with left heart disease, this Frontiers Research Topic illustrates the diversity of clinical and pathological phenotypes associated with elevated pressure in the pulmonary circulation. The topic articles highlight the breadth of ongoing research into understanding this multi-faceted disease and are based on the content of a symposium that drew inspiration from the work of the British pathologist, Donald Heath, who published his manuscript on hypertensive pulmonary vascular disease after working as junior doctor in Sheffield (1, 2).

Despite advances in therapy over the last two decades, most forms of pulmonary hypertension (PH) have a poor prognosis. The greatest improvements in outcome have been observed in patients with CTEPH (3) where surgery may provide a cure, and pulmonary arterial hypertension (PAH) where younger patients with idiopathic disease (IPAH) in the UK now have a 5-year survival in excess of 80% (4). Intuitively, earlier diagnosis and institution of treatment may slow vascular remodeling and improve outcomes. However, despite increased awareness and the availability of new therapies the time from symptom onset to diagnosis remains unchanged at 2–3 years, suggesting that new approaches need to be taken to improve disease detection (5). This highlights the importance of identifying easily performed and scalable tests that can be deployed in the investigative pathway. Three of the studies in this Research Topic highlighted different modalities used in the diagnosis of pulmonary hypertension: exercise, serological biomarkers, and imaging. Billings et al. reported the utility of exercise testing in newly diagnosed, treatment naïve patients with pulmonary hypertension. Although this was a large retrospective study, only a small number of patients (1% of 895) were diagnosed while in WHO FC I. These data are consistent with early vascular changes preceding the development of symptoms and highlight the need for effective and sensitive screening tools for early disease. Billings et al. provide evidence that an incremental shuttle walk distance (ISWD) of <80% predicted identified 8 out of 9 patients with pulmonary hypertension in WHO FC I, while diffusing capacity (DLCO) measurements, using a cut-off of 80% predicted, only identified 4 of these patients. Further work is required to assess whether the simple and adaptable incremental shuttle walk test is a suitable field walking test to identify exercise limitation and to aid screening of high-risk populations, perhaps in combination with other biomarkers.

Edited and reviewed by:
Argyrios Tzouvelekis, Alexander Fleming Biomedical Sciences Research Center, Greece

***Correspondence:**
A. A. Roger Thompson
r.thompson@sheffield.ac.uk

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *04 December 2019*
Accepted: *20 March 2020*
Published: *21 April 2020*

Citation:
Thompson AAR, Wilkins MR, Wild JM, Kiely DG and Lawrie A (2020) Editorial: Pulmonary Hypertension: Mechanisms and Management, History and Future. Front. Med. 7:125. doi: 10.3389/fmed.2020.00125

One such high risk group are those with connective tissue diseases, particularly systemic sclerosis (6). Hickey et al. discuss putative blood-based or lung tissue biomarkers for the identification of systemic sclerosis patients who have developed pulmonary hypertension. A significant issue with potential biomarkers is heterogeneity of patient phenotype even within this sub-group of PAH patients (7). Notably, the contribution of co-morbid interstitial lung disease is difficult to unravel. Hickey et al. also point out limitations associated with NT-proBNP, perhaps the foremost blood-based biomarker for PH in clinical use. While NT-proBNP features as an important component of early screening algorithms for SSc-PAH (8), it lacks specificity and carries the inherent disadvantage that the right ventricular strain, driving its release, is not a feature of early disease.

The third study with a focus on enhancing diagnosis, Chin et al., reports a strong correlation between CT measurement of pulmonary artery diameter and mean pulmonary artery pressure in the presence or absence of interstitial lung disease. This report implies that use of simple CT parameters could help predict presence of pulmonary hypertension in patients with lung disease, contrasting with previous reports suggesting that PA diameter was a less useful indicator of PH in patients with interstitial lung disease. Nonetheless, however useful our predictive tools, the armory of treatment of PH in these patients with interstitial lung disease remains frustratingly empty.

Work on the role of hypoxia in vascular remodeling offers potential promise for patients with PH associated with lung disease. At a molecular level, the importance of hypoxia inducible factors in vascular cell phenotypes is becoming clearer. These oxygen sensors, the discovery of which was recently acknowledged by the 2019 Nobel prize award to three HIF-biologists, are discussed in a review by Young et al. and the specific impact of altitude hypoxia on the pulmonary circulation is highlighted. While hypoxia has a defining role in PH associated with hypoxia and lung disease, the relevance of hypoxia at early stages of the pathogenesis of other forms of PH is less clear.

One important mediator of vascular remodeling with a potentially ubiquitous role across all forms of PH is neutrophil elastase. Neutrophils are considered the dominant source of elastase *in vivo* but as noted by Taylor et al., they are a relatively understudied cell in PH. Nonetheless, an excess of neutrophils (or high neutrophil to lymphocyte ratio) is reportedly correlated with poor outcomes in patients with PH (9) and inhibition of neutrophil elastase reversed disease in mouse models (10). The effect of elastase on turnover of the vascular extracellular matrix, and specifically changes resulting in stiffening of the large and small pulmonary vessels, is the subject of the review by Sun and Chan. Recent work from this group has shown that changes in arterial stiffness precede proliferative remodeling and that mechanosensitive pathways play important roles in regulating vascular cell expansion (11).

The complexity of pro-proliferative signaling in the pulmonary vasculature is further illustrated by the role of TRAIL, reviewed by Braithwaite et al.. Cleavage of TRAIL, a transmembrane protein, results in a soluble cytokine that can signal via a number of cell surface receptors but can also bind to decoy receptors. TRAIL canonically induces apoptosis in cancer cells and in virus-infected epithelial cells but paradoxically promotes vascular smooth muscle cell proliferation. These contrasting effects are mediated by at least two distinct signaling pathways and but exactly how cell and context-specific effects of TRAIL are mediated requires further study. Notably, the importance of TRAIL in infection, fibrosis and cancer might complicate therapeutic targeting of this pathway. However, other mediators known to interact with TRAIL, such as osteoprotegerin, have shown therapeutic promise (12).

Mechanistic insights are increasingly being generated by omics studies (13, 14) and this burgeoning field is reviewed by Hemnes. Blood-based or genetic signatures to enhance the granularity of patient classification or simply to facilitate diagnosis would have great utility, although relatively small patient numbers limit power and validation of predictive signatures. Furthermore, identifying whether omics are capable of detecting changes that precede pulmonary vascular remodeling will be challenging. Nonetheless, omics approaches could not only be used to stratify or diagnose PH but could also provide more personalized treatment approaches. Following interesting work on patients with positive vasodilator responses (15), Hemnes suggests using omics data to identify signatures defining a good response with other treatments. The concept of enriching clinical studies using specific omics signatures and then refining those signatures based on clinical response is proposed. Such strategies might increase the efficiency of later phase clinical trials.

While the majority of topic articles focus on the pulmonary circulation, Charalampopoulos et al. provide a comprehensive overview of PH due to left heart disease. Echocardiographic features that favor left heart disease over PAH are highlighted and the authors discuss studies reporting use of fluid challenges or exercise during right heart catheterization to identify occult left heart disease. The question of whether pulmonary vasodilators have any role in these patients or in patients with combined pre- and post-capillary pulmonary hypertension is the subject of ongoing clinical trials (e.g., SERENADE, NCT02246634) but unfortunately for this large group of patients, there is as yet, no clear answer.

The final review in the topic, by Middleton et al., notes the lack of specific guidelines for arrhythmia management in PAH. Indeed, there is a paucity of data on arrhythmia prevalence and on how frequently arrhythmia contributes to death in PAH patients. Deterioration in the context of atrial arrhythmia is a common clinical scenario that is potentially reversible. Therefore, exciting advances in monitoring technology, including wearable or implantable monitors, are an important avenue for future research. Indeed, our group are examining the utility of invasive pulmonary artery pressure and implantable rhythm monitors.

The broad and systematic approaches covered by the manuscripts included in this Research Topic highlight many of the current challenges to improve patient outcome and well-being in PH. Earlier diagnosis of patients with PH remains a major challenge. Despite advances in treatment and increasing awareness of the disease over the last 2–3 decades, many patients

arrive at specialist centers after a significant diagnostic delay (16). While not reviewed in this topic, recent work from our center has sought to improve this issue by highlighting the potential use of artificial intelligence to interrogate routine healthcare data in order to identify patients at high risk of idiopathic PAH at an earlier stage (5). Furthermore, the topic only alludes to the use of novel tools to phenotype these high-risk patients. Multi-omic profiling and imaging of cardiac and lung structure and function (17, 18) including the use of emerging techniques such as hyperpolarised gases (19) or 4-dimensional magnetic resonance imaging (20) will likely hold the key to developing more sensitive and specific assessment tools. Such tools should generate new insights into molecular targets that alter vascular remodeling and could provide better clinical trial endpoints for assessment of treatment efficacy. The development of biomarkers and imaging tools using patients with earlier disease should be a priority for improving the treatment of PH in the precision medicine era.

AUTHOR CONTRIBUTIONS

AT and AL drafted the editorial with input from MW, JW, and DK.

FUNDING

AT is supported by a British Heart Foundation Intermediate Clinical Fellowship (FS/18/13/33281). AL is supported by British Heart Foundation Senior Basic Science Research Fellowship (FS/13/48/30453).

ACKNOWLEDGMENTS

The authors gratefully acknowledge topic contributors and the sponsorship of the topic by Actelion, a Janssen Pharmaceutical Company. The sponsor played no role in the writing of this editorial.

REFERENCES

1. Heath D. Travellers on a Hidden River. *J Med Biogr*. (1998) 6:105–113. doi: 10.1177/096777209800600208
2. Heath D, Whitaker W. Hypertensive pulmonary vascular disease. *Circulation*. (1956) 14:323–43. doi: 10.1161/circ.14.3.323
3. Quadery SR, Swift AJ, Billings CG, Thompson AAR, Elliot CA, Hurdman J, et al. The impact of patient choice on survival in chronic thromboembolic pulmonary hypertension. *Eur Respir J*. (2018) 52:1800589. doi: 10.1183/13993003.00589-2018
4. National Audit of Pulmonary Project Board. *National Audit of Pulmonary Hypertension, 10th Annual Report - NHS Digital*. (2019). Available online at: https://digital.nhs.uk/data-and-information/publications/statistical/national-pulmonary-hypertension-audit/2019 (accessed November 24, 2019).
5. Bergemann R, Allsopp J, Jenner H, Drage E, Samyshkin Y, Schmitt C, et al. High levels of healthcare utilization prior to diagnosis in idiopathic pulmonary arterial hypertension support the feasibility of an early diagnosis algorithm: the SPHInX project. *Pulm Circ*. (2018) 8:1–9. doi: 10.1177/2045894018798613
6. Hurdman J, Condliffe R, Elliot CA, Davies C, Hill C, Wild JM, et al. ASPIRE registry: Assessing the Spectrum of Pulmonary hypertension Identified at a. *Eur Respir J*. (2012) 39:945–55. doi: 10.1183/09031936.00078411
7. Ramjug S, Hussain N, Hurdman J, Billings C, Charalampopoulos A, Elliot CA, et al. idiopathic and systemic sclerosis-associated pulmonary arterial hypertension: a comparison of demographic, hemodynamic, and MRI characteristics and outcomes. *Chest*. (2017) 152:92–102. doi: 10.1016/j.chest.2017.02.010
8. Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D, et al. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis*. (2014) 73:1340–9. doi: 10.1136/annrheumdis-2013-203301
9. Harbaum L, Baaske KM, Simon M, Oqueka T, Sinning C, Glatzel A, et al. Exploratory analysis of the neutrophil to lymphocyte ratio in patients with pulmonary arterial hypertension. *BMC Pulm Med*. (2017) 17:72. doi: 10.1186/s12890-017-0407-5
10. Cowan KN, Heilbut A, Humpl T, Lam C, Ito S, Rabinovitch M. Complete reversal of fatal pulmonary hypertension in rats by a serine elastase inhibitor. *Nat Med*. (2000) 6:698–702. doi: 10.1038/76282
11. Bertero T, Oldham WM, Cottrill KA, Pisano S, Vanderpool RR, Yu Q, et al. Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. *J Clin Invest*. (2016) 126:3313–35. doi: 10.1172/JCI86387
12. Arnold ND, Pickworth JA, West LE, Dawson S, Carvalho JA, Casbolt H, et al. A therapeutic antibody targeting osteoprotegerin attenuates severe experimental pulmonary arterial hypertension. *Nat Commun*. (2019) 10:5183. doi: 10.1038/s41467-019-13139-9
13. Gräf S, Haimel M, Bleda M, Hadinnapola C, Southgate L, Li W, et al. Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. *Nat Commun*. (2018) 9:1416. doi: 10.1038/s41467-018-03672-4
14. Rhodes CJ, Ghataorhe P, Wharton J, Rue-Albrecht KC, Hadinnapola C, Watson G, et al. Plasma metabolomics implicates modified transfer RNAs and altered bioenergetics in the outcomes of pulmonary arterial hypertension. *Circulation*. (2017) 135:460–75. doi: 10.1161/CIRCULATIONAHA.116.024602
15. Hemnes AR, Trammell AW, Archer SL, Rich S, Yu C, Nian H, et al. Peripheral blood signature of vasodilator-responsive pulmonary arterial hypertension. *Circulation*. (2015) 131:401–9. doi: 10.1161/CIRCULATIONAHA.114.013317
16. Armstrong I, Billings C, Kiely DG, Yorke J, Harries C, Clayton S, et al. The patient experience of pulmonary hypertension: a large cross-sectional study of UK patients. *BMC Pulm Med*. (2019) 19:67. doi: 10.1186/s12890-019-0827-5
17. Swift AJ, Capener D, Johns C, Hamilton N, Rothman A, Elliot C, et al. Magnetic resonance imaging in the prognostic evaluation of patients with pulmonary arterial hypertension. *Am J Respir Crit Care Med*. (2017) 196:228–39. doi: 10.1164/rccm.201611-2365OC
18. Johns CS, Kiely DG, Rajaram S, Hill C, Thomas S, Karunasaagarar K, et al. Diagnosis of pulmonary hypertension with cardiac MRI: derivation and validation of regression models. *Radiology*. (2019) 290:61–68. doi: 10.1148/radiol.2018180603
19. Arai TJ, Horn FC, Sá RC, Rao MR, Collier GJ, Theilmann RJ, et al. Comparison of quantitative multiple-breath specific ventilation imaging using colocalized 2D oxygen-enhanced MRI and hyperpolarized 3 He MRI. *J Appl Physiol*. (2018) 125:1526–35. doi: 10.1152/japplphysiol.00500.2017
20. Fidock B, Barker N, Balasubramanian N, Archer G, Fent G, Al-Mohammad A, et al. A systematic review of 4D-Flow MRI derived mitral regurgitation quantification methods. *Front Cardiovasc Med*. (2019) 6:103. doi: 10.3389/fcvm.2019.00103

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

REVIEW
published: 24 May 2018
doi: 10.3389/fmed.2018.00157

Using Omics to Understand and Treat Pulmonary Vascular Disease

*Anna R. Hemnes**

Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN, United States

Pulmonary arterial hypertension (PAH) is a devastating disease for which there is no cure. Presently this condition is differentiated from other diseases of the pulmonary vasculature by a practitioner's history, physical examination, and clinical studies with clinical markers of disease severity primarily guiding therapeutic choices. New technologies such as next generation DNA sequencing, high throughput RNA sequencing, metabolomics and proteomics have greatly enhanced the amount of data that can be studied efficiently in patients with PAH and other rare diseases. There is emerging data on the use of these "Omics" for pulmonary vascular disease classification and diagnosis and also new work that suggests molecular markers, including Omics, may be used to more efficiently match patients to their own most effective therapies. This review focuses on the state of knowledge on molecular classification and treatment of PAH. Strengths and weaknesses of current Omic technologies are discussed and how these new technologies can be used in the future to improve diagnosis of pulmonary vascular disease, more effectively treat patients with existing and future drugs, and generate new understanding of disease pathogenesis and mechanisms underlying treatment success or failure. Bioinformatic methods to analyze the large volumes of data are developing rapidly, but still present major challenges to interpretation of potential Omic findings in pulmonary vascular disease, with low numbers of patients studied and a potentially high false discovery rate. With more experience, precise and established drug response definitions, this field with move forward and will likely be a major component of the clinical care of PH patients in the future.

Keywords: pulmonary hypertension, omics-technologies, precision medicine, right ventricle, OMICS data

OPEN ACCESS

Edited by:
A. A. Roger Thompson, University of Sheffield, United Kingdom

Reviewed by:
Bertrand De Meulder, European Institute for Systems Biology and Medicine (EISBM), France
Christopher J. Rhodes, Imperial College London, United Kingdom
Charaka Hadinnapola, University of Cambridge, United Kingdom

***Correspondence:**
Anna R. Hemnes
anna.r.hemnes@vanderbilt.edu

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *30 January 2018*
Accepted: *04 May 2018*
Published: *24 May 2018*

Citation:
Hemnes AR (2018) Using Omics to Understand and Treat Pulmonary Vascular Disease. Front. Med. 5:157. doi: 10.3389/fmed.2018.00157

INTRODUCTION

Despite major advances in the last several decades, pulmonary vascular disease is a major source of morbidity and mortality. Primarily manifest as elevated pulmonary arterial pressure or pulmonary hypertension (PH), pulmonary vascular disease also includes pulmonary embolism and vascular malformations. PH has been subdivided in the current classification scheme into conditions that have a primary pulmonary vasculopathy, e.g., pulmonary arterial hypertension (PAH) and chronic thromboembolic PH (CTEPH) and conditions in which PH is considered to be a secondary phenomenon (1). For instance, left atrial hypertension raises pulmonary venous pressure and subsequently pulmonary arterial pressure, but if the left atrial pressure were removed, the PH would regress, theoretically. Similarly, parenchymal lung disease such as chronic obstructive pulmonary disease is associated with PH occasionally, but pathologic specimens do not uniformly demonstrate pulmonary arterial vasculopathy. Many of these conditions predispose to right ventricular failure

and ultimately result in death, despite new treatments, making improved diagnostic and therapeutic options potentially highly clinically impactful.

Some forms of PH, e.g., PAH and chronic thromboembolic pulmonary hypertension (CTEPH), have specific medical or surgical therapies (2, 3), however the majority of PH and pulmonary vascular disease in general have no therapy aside from supportive care. Further complicating this group of diseases is the lack of specific diagnostic material. Lung biopsy is generally considered high risk in patients with PH or other forms of pulmonary vascular disease (4, 5), making diagnosis reliant on clinical factors, radiographic findings and invasive hemodynamics. This leaves clinicians with a major challenge of potentially missing a treatable condition such as PAH or CTEPH when they diagnose other forms of PH. Alternatively, PAH-directed therapy has been shown to potentially harm patients with non-PAH PH (6, 7), thus a practice pattern of simply treating all PH as PAH is potentially dangerous. In short, the field is in need of improved diagnostic modalities and more efficient mechanisms through which to identify patients who respond to a specific treatment.

Recent advances in biomedical research and bioinformatics approaches are now available to apply to pulmonary vascular disease and hold tremendous promise to improve diagnostic accuracy and improve treatments of all forms of pulmonary vascular disease. The term "Omics," hereafter referred to Omics, refers to the field of study generally ending in "-omics" such as genomics, proteomics, and transcriptomics. Broadly speaking, Omics suggests study of the entire field of proteins, metabolites or genes that may contribute to a disease state or phenomenon. Although early work in Omics included studies of genomics, transcriptomics, metabolomics, and proteomics, more recent work has added other fields such as radiology (radiomics) and cell biology (cell biomics). The use of transcriptomics and genomics in pulmonary vascular disease is most established, but recent work has drawn attention to how other Omics may be used to understand and optimally treat pulmonary vascular disease. This review will focus on these two key areas: First, use of Omics to classify PH and, second, use of Omics to optimize therapy in PAH and PH more broadly.

OMICS TO CLASSIFY PH

Originally developed in the second World Symposium on Pulmonary Hypertension in 1998, the classification of PH attempts to categorize patients by similar clinical and hemodynamic patters that correlate with specific pathologic findings and response to therapies (8). Although modifications have been made, the classification of PH continues to rely on clinical metrics (1). One notable exception to this is heritable PAH in which genetic mutations such as those in bone morphogenetic protein receptor type 2 (BMPR2) and other mutations are well-described (9–13) and may be used to assist in classification of patients.

Although the classification is generally quite useful in predicting response to PAH-directed therapies, many patients have features of multiple forms of PH, so-called "mixed" or "combined" PH. An example of a patient with combined group 1 and 2 PH is the 75 year old woman, an aunt in the blood line of a patient with heritable PAH who has normal lungs by chest imaging and spirometry, no evidence of chronic thromboembolic PH by ventilation perfusion lung scan, and a right heart catheterization demonstrating a pulmonary arterial wedge pressure of 20 mmHg and pulmonary arterial pressure of 95/50 mmHg with a normal cardiac output. Her elevated pulmonary arterial wedge pressure suggests group 2 PH, however she has a wide diastolic pressure gradient, suggesting underling pulmonary vasculopathy. Further, she likely carries a BMPR2 mutation, making PAH possible. After the 2012 World Symposium on PH, this patient would be called combined pre- and post-capillary PH (Cpc-PH) (14) and her case demonstrates a common clinical scenario of a patient with multiple potential etiologies of PH. As lung biopsies are not routinely obtained due to risk of bleeding and increased surgical risk in PH patients (3, 4), precise classification of many patients' pulmonary vascular disease may not be possible. Further, since most medical therapy is reserved for patients with exclusively PAH (2, 3, 15), patients with indicators they may have other forms of PH in addition to PAH may not be recommended for life-saving therapies. Thus, there are significant limitations to the current clinical classification of PH.

Although specific gene sequencing for mutations in BMPR2, ALK1, and other genes can, in the right clinical context, confirm a diagnosis of heritable PAH, these are specific, targeted tests and only applicable to small number of even patients with PAH, and a minute fraction of PH in general (9, 16, 17). Recently there has been interest in using Omic technologies to enhance the current classification of PH, thus moving beyond tests of single gene mutations. Early work in this field has used multiple Omic technologies to understand primarily how PAH patients are different from healthy controls. Given that PAH can be challenging to diagnose and is a relatively heterogeneous group of diseases including congenital heart disease and multiple different connective tissue diseases, some of the earliest Omic work focused on heritable PAH, in which the diagnosis is firm and the disease etiology less heterogeneous. West and colleagues used microarray analysis on cultured peripheral blood lymphocytes to define RNA transcript patterns in patients with heritable PAH compared with controls (18). Cultured lymphocytes offer the advantage of several weeks of *ex vivo* culture, thereby removing environmental influences on the cells. These data demonstrated alterations in estrogen metabolism, actin organization, growth, and apoptosis signaling with significant differences between heritable PAH and control patients. These early studies used a very well-defined disease phenotype to compare to controls and served as a prototype for future Omic analysis, focusing on tight phenotype and pathway or gene ontology analysis.

More recently, Rhodes and colleagues performed a comprehensive study of 1,416 plasma metabolites using ultraperformance liquid chromatography mass spectrometry in patients with PAH and healthy controls (19). They included only

idiopathic and heritable PAH patients, again ensuring a relatively homogenous population and compared with both healthy and disease controls. Using an unbiased approach to analysis, they identified metabolites that discern PAH from controls and, after correcting for various factors, found 20 metabolites that distinguish PAH from healthy and disease controls. A network analysis of these metabolites highlights alterations in amino acid, nucleoside and glucose and lipid metabolism. They further developed a discriminant score using seven metabolites to separate PAH from healthy and disease controls. Taken together, these data demonstrate that metabolomics can be used to define detectable, metabolic differences between PAH and control patients and perhaps point to pathways key to development or maintenance of PAH.

Our own group has used genomics to understand whether patients with Cpc-PH have genetic variant patterns that are more similar to PAH than to group 2 PH with isolated post-capillary PH (Ipc-PH). Assad and colleagues first defined demographics in the three groups of patients and showed the Cpc-PH is distinct and characterized by younger age than Ipc-PH patients with more severe pulmonary hemodynamics (20). Using a DNA biorepository with pre-existing data on single nucleotide variants linked to de-identified clinical data, distinct gene variant patterns in Cpc-PH were identified that were more similar to PAH than to Ipc-PH patients and were in pathways known to be of relevance to PAH such as extracellular matrix and immune function. These data show that there may be genetic variant patterns that could be used to both understand etiology of pulmonary vascular disease, but also to define phenotypes and endophenotypes of pulmonary vascular disease.

Other groups have used similar methodologies to explore expression patterns in the lung, clearly a highly relevant tissue to pulmonary vascular disease. Geraci and colleagues demonstrated different expression patterns in PAH compared to controls in lung tissue (21). Others have used Omics in tissue and peripheral blood to study differences in RNA expression patterns in scleroderma-associated PAH (22, 23). Taken together, these data show the broad applicability of RNA expression pattern studies to pulmonary vascular disease, both in the peripheral blood and the affected tissues. Unfortunately, due to low numbers of patients undergoing lung transplantation or autopsy, studies of tissues are limited in number, thereby potentially limiting data generated and increasing false discovery rate. Nonetheless, when available, tissue can provide key insights into disease pathology and also critical confirmatory information of peripheral blood findings.

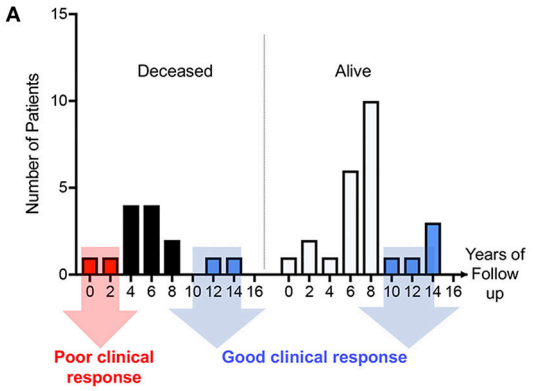
There is much enthusiasm that Omics will 1 day either augment or replace our current clinical classification of PH. With large numbers of patient enrollees, new molecular classifications discovered using Omics, may allow for more precise diagnoses, similar to progress made in cancer diagnostics. With homogenous diagnostic categories, discovery of molecular etiologies, identification of new therapeutics and more targeted clinical trials will all be possible (24). The National Institutes of Health/National Heart, Lung and Blood Institute (NHLBI) has funded a multi-center United States cohort study to use Omics, including genomics, transcriptomics, proteomics, and metabolomics, among other Omics, to advance understanding and classification of PH patients and is presently enrolling (NCT02980887) (25). Studies such as these potentially will change the classification and diagnosis of PH type to one that incorporates measurement of blood-based metrics into clinical parameters to define disease etiology and treatment recommendations.

OMICS TO IMPROVE THERAPY IN PH

PAH is a specific form of PH in which the distal pulmonary arterioles develop occlusive lesions, termed plexiform lesions, as well as other changes resulting in elevations in pulmonary vascular resistance generally with right heart failure and death within 3–5 years if untreated. While major progress has been made in therapy of PAH in the last several decades, it remains a highly mortal disease, with a recent publication showing that despite modern therapy, 40% of newly diagnosed patients die within 5 years (26), survival similar to early stage lung cancer. There are 10 FDA-approved therapies in three broad classes, however, clinicians have a very limited capacity to determine which patients are likely to respond to which drug class. This leads to costly prescription of multiple classes of medications, greater exposure to side effects of medications and greater burden of therapy, without clear therapeutic benefit. While the combination of tadalafil and ambrisentan has been shown to improve time to clinical worsening (27), there is very limited data on the efficacy of other combinations of medications. Additionally, PAH therapy is expensive, with annual costs ranging from $20,000 to $1,000,000 per patient. Use of costly and potentially ineffective drugs is untenable for healthcare systems. Thus, there is a pressing need to develop novel treatment strategies in PAH, ideally using drugs that are presently FDA-approved in the short term, and applying these strategies to future pivotal trials of drugs in all forms of PH.

Omics technologies have been used to improve patient care through identification of markers or patterns of markers that indicate positive responses to specific therapies, often drugs. While occasionally the same marker that defines a disease state can be used to predict treatment responses, e.g., the presence of cystic fibrosis with G551D mutation responds to treatment with ivacaftor (28), it is possible and perhaps likely that markers of drug responsiveness are different from those of disease diagnosis. Here we focus on Omic predictors of responses to PAH therapies, as there are presently no data for other forms of PH.

Some forms of PAH may provide clues as to how we can more effectively and efficiently treat PH. It has long been recognized that about 5–10% of patients with PAH have an acute pulmonary vasodilatory response when confronted with vasodilator therapy with normalization or near-normalization of pulmonary arterial pressures and preservation of cardiac output (29). Patients meeting these criteria can be treated safely with calcium channel blocker medications with substantially improved mortality and a much cheaper drug treatment cost (30). The identification of acute vasodilator response as a predictor of long term response



B

	Poor clinical response	Good clinical response
Change in 6MWD	<30m	>100m
RV function on Echo	Severe RV dysfunction	Normalizes
Change in FC	FC III or IV	FC I or II
Cardiac Index	<2	Normalizes
BNP	Persistently elevated	Normalizes
Survival	<3 years	>5 years
Transplant	Within 3 years	Never

6MWD = 6 minute walk distance; RV = right ventricle, BNP = brain natriuretic peptide, FC = functional class

FIGURE 1 | (A) Histograms of survival in ongoing survivors ($n = 25$) vs. deceased patients ($n = 14$). Y axis depicts years since initiation of IV epoprostenol. Patients with good response may be considered those still alive after 8 years of drug or those who are deceased but after 8 years of IV epoprostenol therapy. Eight years is the median survival in our cohort (31). **(B)** Table showing proposed single or combined metrics of response to parenteral prostacyclin therapy.

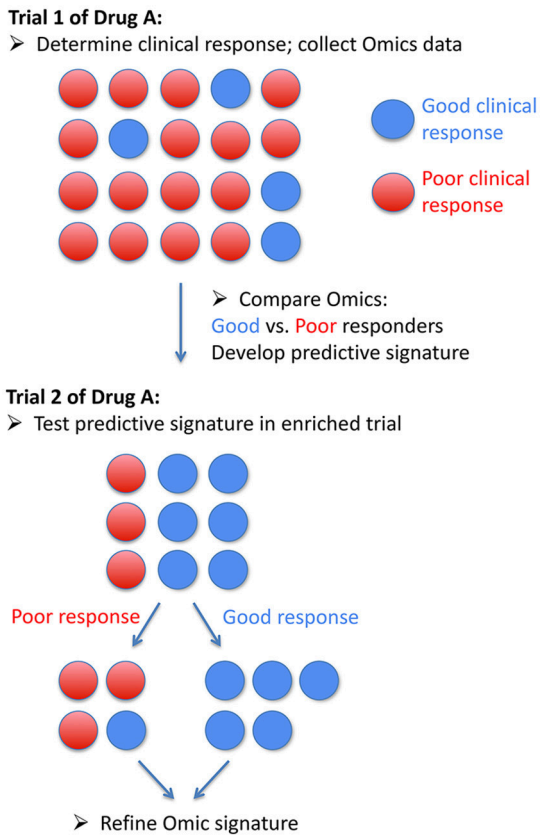


FIGURE 2 | Schematic of how Omic data could be used in a precision medicine trial of a drug in pulmonary arterial hypertension.

to calcium channel blocker therapy was a key development in the therapy of PAH as it (1) was the first foray into precision medicine in the field of pulmonary vascular disease and (2) defined a phenotypically homogenous group of drug responders that later could be studied to understand molecular etiology and markers of drug responsiveness in PAH.

Years after the discovery of this distinct phenotype, Omic technologies had advanced such that their use to find predictors of drug responsiveness now seemed plausible. Our group used transcriptomics to determine if the clinical response to calcium channel blocker therapies could be predicted using molecular markers (32). A major limitation to studying this condition is its extreme rarity making study of untreated patients infeasible. We overcame this limitation using cultured lymphocytes as above. Because the lymphocytes were cultured *ex vivo* for weeks, potential confounding by exposure to calcium channel blockers was minimized. We used microarray to measure mRNA expression levels and found that there were distinct patterns of RNA expression in the vasodilator-responsive PAH patients compared to those that were not. Since our primary goal was to define a simple RNA expression pattern to predict vasodilator responsive PAH, we confirmed the most differentially expressed genes using whole blood RNA. After doing this, we developed an algorithm of RNA expression patterns that predicted this drug-responsive PAH phenotype and validated it in an external cohort. The strengths of this approach are that the RNAs were selected agnostic of their biologic plausibility, allowing the selection of the strongest predictors and the use of cultured lymphocytes to remove environmental influences on RNA patterns. These data suggested that in a cohort of tightly phenotyped drug responders, with a clear definition of drug response, Omic predictors might potentially be used to predict drug responses.

The most appealing Omic field for predicting drug responses is genomics. Generally not impacted by external forces or intercurrent illness, DNA is also more stable facilitating transfer to different facilities and readily available through blood draws or buccal mucosa swab. In addition, DNA would not be expected to change if a patient is already started on PAH-directed therapy, whereas metabolomics, proteomics, and transcriptomics may be altered by the therapy itself. Given these appealing qualities, there have been a few recent attempts to predict drug responses using genomics. First, Benza and colleagues used targeted DNA sequencing to study gene variants in the endothelin signaling pathway (33). They identified several variants that predict clinical outcomes with endothelin receptor antagonist therapy in PAH. The authors approach was limited to only genes known to be in the endothelin pathway and it is possible that with

a discovery-based approach they would have identified even stronger predictors of endothelin receptor antagonist therapy outcomes.

Our group used whole exome sequencing in PAH patients with and without acute vasodilatory response. Again using this distinct endophenotype as a proof of the concept that genomics can be used to predict drug responses. We did not identify one single gene or gene variant that predicts calcium channel blocker responses, however we did find over 1,500 DNA variants unique to PAH patients. Next generation DNA sequencing is well known to identify hundreds of variants of undetermined significance, a limitation we attempted to avoid using pathway analysis of identified variants, comparing patients with acute response to vasodilators and those without. After filtering out gene variants associated with the presence of idiopathic pulmonary fibrosis and those not predicted to be deleterious using online resources, we studied the variants based on their frequency and found that there were generally more gene variants in patients with vasodilator-responsive PAH and also these genes were enriched in pathways associated with smooth muscle cell contraction. In the case of vasodilator-responsive PAH, we learned that there likely is not a single gene that differentiates PAH from control, but more likely a pattern of gene variants. Perhaps with greater numbers, these data can be refined and a gene variant risk score for PAH may be developed. Presently, these drug Omic methodologies are not appropriate for clinical care, though hopefully their use is not far away.

Future areas in which Omics may be useful in PAH therapeutics include development of genetic or other markers of predictors of response to endothelin receptor antagonists, phosphodiesterase type 5 inhibitors, riociguat, and prostacyclin pathway therapeutics. Prostacyclins may be particularly appealing for this sort of analysis as excellent clinical response are well described, and endpoints such as death and transplant are unfortunately relatively common, making numbers of enrollees required to generate useful data relatively low (34). Examples of how groups may be defined, either singly or in combination, are shown in **Figure 1**, including a demonstration of how survival (**Figure 1A**) or clinical metrics (**Figure 1B**) could be used to define good or poor clinical response. **Figure 2** outlines a schema of how Omics could be used to refine future trials in pulmonary hypertension medications, both existing and future.

CHALLENGES IN THE USE OF OMICS IN PULMONARY VASCULAR DISEASE

In order to move this field forward, we need a commonly agreed upon definition of "good response" to drugs to allow segregation of groups. A key feature that made analysis of RNA expression patterns in PAH patients with and without acute vasodilator responses possible was the distinct acute drug responses and long term survival data. This clear endophenotype could be exploited on a molecular level. However, most clinical drug responses are less well-established, with perhaps the exception of the occasional exceptional prostacyclin response. Moreover, use of a single drug to treat PAH is becoming less common, making identification of molecular predictors of response to a single drug less likely. Further, while Omics may select one or several biomarkers of prognosis in PAH, they must be carefully tested in their predictive value against traditional, highly predictive clinical models of survival in PAH such as the REVEAL risk score (26, 35, 36).

Further, we need to refine our pipeline for analysis of Omics data. Each methodology generates at a minimum, hundreds and, more commonly, thousands of data points that have to be filtered and prioritized. The use of biologic plausibility may lose strongly predictive Omic metrics and alternatively a simple "strength of expression" analysis may miss biologically important molecules with low level expression. Finally, integration of data across Omic platforms is presently challenging and required advanced computational skills with different analytic techniques. This has the potential to bias the final data and its interpretation. Thus different methods for analyzing data may yield very different results, each with its own strengths and weaknesses. Recent developments such as predictive modeling and machine learning, reviewed here (37) and here (38), may be a powerful way forward to integrate and understand these large datasets. Detailed discussion of these methodologies and their applications are outside the scope of this manuscript.

CONCLUSION

In conclusion, Omic technologies hold tremendous promise to improve the diagnosis and treatment of PH. There are major limitations to their present use, however, including potentially high false discovery rate with low numbers of patients, imprecise phenotypes and bioinformatics challenges. With more experience, precise and established drug response definitions, this field with move forward and will likely be a major component of the clinical care of PH patients in the future.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

FUNDING

NIH 1 U01 HL125212-01 (PI AH), P01 HL108800 (PI James Loyd and AH).

REFERENCES

1. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* (2013) **62**(25 Suppl.):D34–41. doi: 10.1016/j.jacc.2013.10.029
2. Galie N, Corris PA, Frost A, Girgis RE, Granton J, Jing ZC, et al. Updated treatment algorithm of pulmonary arterial hypertension. *J Am Coll Cardiol.* (2013) **62**(25 Suppl.):D60–72. doi: 10.1016/j.jacc.2013.10.031
3. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. *Rev Esp Cardiol.* (2016) **69**:177. doi: 10.1016/j.rec.2016.01.002

4. Castro M, Krowka MJ, Schroeder DR, Beck KC, Plevak DJ, Rettke SR, et al. Frequency and clinical implications of increased pulmonary artery pressures in liver transplant patients. *Mayo Clin Proc.* (1996) **71**:543–51.
5. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Respir J.* (2009) **34**:1219–63. doi: 10.1183/09031936.00139009
6. Kalra PR, Moon JC, Coats AJ. Do results of the ENABLE (Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure) study spell the end for non-selective endothelin antagonism in heart failure? *Int J Cardiol.* (2002) **85**:195–7. doi: 10.1016/S0167-5273(02)00182-1
7. Montalescot G, Drobinski G, Meurin P, MacLouf J, Sotirov I, Philippe F, et al. Effects of prostacyclin on the pulmonary vascular tone and cardiac contractility of patients with pulmonary hypertension secondary to end-stage heart failure. *Am J Cardiol.* (1998) **82**:749–55.
8. Simonneau G, Galie N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, et al. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* (2004) **43**(12 Suppl. S):5S—12S. doi: 10.1016/j.jacc.2004.02.037
9. Newman JH, Wheeler L, Lane KB, Loyd E, Gaddipati R, Phillips JA, III, et al. Mutation in the gene for bone morphogenetic protein receptor II as a cause of primary pulmonary hypertension in a large kindred. *N Engl J Med.* (2001) **345**:319–24. doi: 10.1056/NEJM200108023450502
10. Best DH, Sumner KL, Austin ED, Chung WK, Brown LM, Borczuk AC, et al. EIF2AK4 mutations in pulmonary capillary hemangiomatosis. *Chest* (2013) **145**:231–6. doi: 10.1378/chest.13-2366
11. Germain M, Eyries M, Montani D, Poirier O, Girerd B, Dorfmuller P, et al. Genome-wide association analysis identifies a susceptibility locus for pulmonary arterial hypertension. *Nat Genet.* (2013) **45**:518–21. doi: 10.1038/ng.2581
12. Ma L, Roman-Campos D, Austin ED, Eyries M, Sampson KS, Soubrier F, et al. A novel channelopathy in pulmonary arterial hypertension. *N Engl J Med.* (2013) **369**:351–61. doi: 10.1056/NEJMoa1211097
13. Austin ED, Ma L, LeDuc C, Berman Rosenzweig E, Borczuk A, Phillips JA, III, et al. Whole exome sequencing to identify a novel gene (caveolin-1) associated with human pulmonary arterial hypertension. *Circ Cardiovasc Genet.* (2012) **5**:336–43. doi: 10.1161/CIRCGENETICS.111.961888
14. Vachiery JL, Adir Y, Barbera JA, Champion H, Coghlan JG, Cottin V, et al. Pulmonary hypertension due to left heart diseases. *J Am Coll Cardiol.* (2013) **62**(25 Suppl.):D100–8. doi: 10.1016/j.jacc.2013.10.033
15. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, et al. ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. *J Am Coll Cardiol.* (2009) **53**:1573–619. doi: 10.1016/j.jacc.2009.01.004
16. Machado RD, Pauciulo MW, Thomson JR, Lane KB, Morgan NV, Wheeler L, et al. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *Am J Hum Genet.* (2001) **68**:92–102. doi: 10.1086/316947
17. 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) **37**:741–5. doi: 10.1136/jmg.37.10.741
18. West J, Cogan J, Geraci M, Robinson L, Newman J, Phillips JA, et al. Gene expression in BMPR2 mutation carriers with and without evidence of pulmonary arterial hypertension suggests pathways relevant to disease penetrance. *BMC Med Genomics* (2008) **1**:45. doi: 10.1186/1755-8794-1-45
19. Rhodes CJ, Ghataorhe P, Wharton J, Rue-Albrecht KC, Hadinnapola C, Watson G, et al. Plasma metabolomics implicates modified transfer RNAs and altered bioenergetics in the outcomes of pulmonary arterial hypertension. *Circulation* (2017) **135**:460–75. doi: 10.1161/CIRCULATIONAHA.116.024602
20. Assad TR, Hemnes AR, Larkin EK, Glazer AM, Xu M, Wells QS, et al. Clinical and biological insights into combined post- and pre-capillary pulmonary hypertension. *J Am Coll Cardiol.* (2016) **68**:2525–36. doi: 10.1016/j.jacc.2016.09.942
21. Geraci MW, Moore M, Gesell T, Yeager ME, Alger L, Golpon H, et al. Gene expression patterns in the lungs of patients with primary pulmonary hypertension: a gene microarray analysis. *Circ Res.* (2001) **88**:555–62. doi: 10.1161/01.RES.88.6.555
22. Hsu E, Shi H, Jordan RM, Lyons-Weiler J, Pilewski JM, Feghali-Bostwick CA. Lung tissues in patients with systemic sclerosis have gene expression patterns unique to pulmonary fibrosis and pulmonary hypertension. *Arthritis Rheum.* (2011) **63**:783–94. doi: 10.1002/art.30159
23. Bull TM, Coldren CD, Moore M, Sotto-Santiago SM, Pham DV, Nana-Sinkam SP, et al. Gene microarray analysis of peripheral blood cells in pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2004) **170**:911–9. doi: 10.1164/rccm.200312-1686OC
24. Newman JH, Rich S, Abman SH, Alexander JH, Barnard J, Beck GJ, et al. Enhancing insights into pulmonary vascular disease through a precision medicine approach. A joint NHLBI-cardiovascular medical research and education fund workshop report. *Am J Respir Crit Care Med.* (2017) **195**:1661–70. doi: 10.1164/rccm.201701-0150WS
25. Hemnes AR, Beck GJ, Newman JH, Abidov A, Aldred MA, Barnard J, et al. PVDOMICS: a multi-center study to improve understanding of pulmonary vascular disease through phenomics. *Circ Res* (2017) **121**:1136–9. doi: 10.1161/CIRCRESAHA.117.311737
26. Farber HW, Miller DP, Poms AD, Badesch DB, Frost AE, Muros-Le Rouzic E, et al. Five-Year outcomes of patients enrolled in the REVEAL Registry. *Chest* (2015) **148**:1043–54. doi: 10.1378/chest.15-0300
27. Galie N, Barbera JA, Frost AE, Ghofrani HA, Hoeper MM, McLaughlin VV, et al. Initial use of ambrisentan plus tadalafil in pulmonary arterial hypertension. *N Engl J Med.* (2015) **373**:834–44. doi: 10.1056/NEJMoa1413687
28. Whiting P, Al M, Burgers L, Westwood M, Ryder S, Hoogendoorn M, et al. Ivacaftor for the treatment of patients with cystic fibrosis and the G551D mutation: a systematic review and cost-effectiveness analysis. *Health Technol Assess.* (2014) **18**:1–106. doi: 10.3310/hta18180
29. Rich S, Brundage BH. High-dose calcium channel-blocking therapy for primary pulmonary hypertension: evidence for long-term reduction in pulmonary arterial pressure and regression of right ventricular hypertrophy. *Circulation* (1987) **76**:135–41. doi: 10.1161/01.CIR.76.1.135
30. Sitbon O, Humbert M, Jais X, Ioos V, Hamid AM, Provencher S, et al. Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. *Circulation* (2005) **111**:3105–11. doi: 10.1161/CIRCULATIONAHA.104.488486
31. Brittain EL, Pugh ME, Wheeler LA, Robbins IM, Loyd JE, Newman JH, et al. Shorter survival in familial versus idiopathic pulmonary arterial hypertension is associated with hemodynamic markers of impaired right ventricular function. *Pulm Circ.* (2013) **3**:589–98. doi: 10.1086/674326
32. Hemnes AR, Trammell AW, Archer SL, Rich S, Yu C, Nian H, et al. Peripheral blood signature of vasodilator-responsive pulmonary arterial hypertension. *Circulation* (2015) **131**:401–9; discussion: 9. doi: 10.1161/CIRCULATIONAHA.114.013317
33. Benza RL, Gomberg-Maitland M, Demarco T, Frost AE, Torbicki A, Langleben D, et al. Endothelin-1 pathway polymorphisms and outcomes in pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2015) **192**:1345–54. doi: 10.1164/rccm.201501-0196OC
34. Halliday SJ, Hemnes AR. Identifying "super responders" in pulmonary arterial hypertension. *Pulm Circ.* (2017) **7**:300–11. doi: 10.1177/2045893217697708
35. 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) **142**:448–56. doi: 10.1378/chest.11-1460

36. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). *Circulation* (2010) **122**:164–72. doi: 10.1161/CIRCULATIONAHA.109.898122
37. Zeng ISL, Lumley T. Review of statistical learning methods in integrated omics studies (an integrated information science). *Bioinform Biol Insights* (2018) **12**:1177932218759292. doi: 10.1177/1177932218759292
38. Karczewski KJ, Snyder MP. Integrative omics for health and disease. *Nat Rev Genet.* (2018) **19**:299–310. doi: 10.1038/nrg.2018.4

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 06 June 2018
doi: 10.3389/fmed.2018.00174

Pathophysiology and Diagnosis of Pulmonary Hypertension Due to Left Heart Disease

Athanasios Charalampopoulos, Robert Lewis, Peter Hickey, Charlotte Durrington, Charlie Elliot, Robin Condliffe, Ian Sabroe and David G. Kiely*

Sheffield Pulmonary Vascular Disease Unit, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom

Pulmonary hypertension due to left heart disease (PH-LHD) is the most common type of pulmonary hypertension, although an accurate prevalence is challenging. PH-LHD includes PH due to systolic or diastolic left ventricular dysfunction, mitral or aortic valve disease and congenital left heart disease. In recent years a new and distinct phenotype of "combined post-capillary and pre-capillary PH," based on diastolic pulmonary gradient and pulmonary vascular resistance, has been recognized. The roles of right ventricular dysfunction and pulmonary vascular compliance in PH-LHD have also been elucidated recently and they appear to have significant clinical implications. Echocardiography continues to play a seminal role in diagnosis of PH-LHD and heart failure with preserved LV ejection fraction, as it can identify valve disease and help to distinguish PH-LHD from pre-capillary PH. Right, and occasionally left heart catheterization, remains the gold-standard for diagnosis and phenotyping of PH-LHD, although Cardiac Magnetic Resonance Imaging is emerging as a useful alternative tool in non-invasive diagnostic and prognostic assessment of PH-LHD. In this review, the latest evidence for more recent advances will be discussed, including the role of fluid challenge and exercise during cardiac catheterization to unravel occult post-capillary and the role of vasoreactivity testing. The use of many or all of these diagnostic techniques will undoubtedly provide key information about sub-groups of patients with PH-LHD that might benefit from medical therapy previously considered to be only suitable for pulmonary arterial hypertension.

Keywords: pulmonary hypertension, left heart disease, heart failure with preserved ejection fraction, left ventricular diastolic dysfunction, right heart catheterization

OPEN ACCESS

Edited by:
Mehdi Mirsaeidi, University of Miami, United States

Reviewed by:
Anup C. Katheria, Sharp Mary Birch Hospital for Women & Newborns, United States
Jonathan Beaudoin, Laval University, Canada
Kenichi Hongo, Jikei University School of Medicine, Japan

****Correspondence:***
Athanasios Charalampopoulos athanasios.charalampopoulos@sth.nhs.uk

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *05 January 2018*
Accepted: *18 May 2018*
Published: *06 June 2018*

Citation:
Charalampopoulos A, Lewis R, Hickey P, Durrington C, Elliot C, Condliffe R, Sabroe I and Kiely DG (2018) Pathophysiology and Diagnosis of Pulmonary Hypertension Due to Left Heart Disease. Front. Med. 5:174. doi: 10.3389/fmed.2018.00174

INTRODUCTION

Pulmonary hypertension due to left heart disease (PH-LHD) is the most common type of pulmonary hypertension (PH). The prevalence of PH in patients with heart failure varies significantly with diagnostic criteria from 25 to 83% (1–4). PH-LHD is defined by post-capillary hemodynamics at right heart catheterization (RHC); that is a mean pulmonary arterial pressure $\geq$25 mmHg and a mean pulmonary arterial wedge pressure (PAWP) > 15 mmHg. PAWP is a surrogate marker of left atrial pressure (LAP). An elevated LAP causing PH can occur in systolic and/or diastolic left ventricular (LV) dysfunction and in left-sided valvular disease. In the most recent clinical classification of PH (5) two additional etiologies of PH-LHD have been recognized: PH due to congenital or acquired left ventricular outflow tract

TABLE 1 | Definitions.

Pre-capillary PH	Mean PAP ≥ 25 mmHg, mean PAWP ≤15 mmHg
Isolated post-capillary PH	Mean PAP ≥ 25 mmHg, mean PAWP >15 mmHg, DPG < 7 mmHg and/or PVR ≤ 3 Wood units
Combined pre-capillary and post-capillary PH	Mean PAP ≥ 25 mmHg, mean PAWP >15 mmHg, DPG ≥ 7 mmHg and/or PVR > 3 Wood units

PAP, pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; DPG, diastolic pressure gradient; PVR, pulmonary vascular resistance. Modified from 2015 ESC/ERS Pulmonary Hypertension Guidelines (5).

obstruction and pulmonary vein stenosis. In the same guidelines, a new PH phenotype of "combined pre-capillary and post-capillary PH" (Cpc-PH) has been introduced on the grounds of a diastolic pressure gradient (DPG)-the difference between diastolic pulmonary arterial pressure and mean PAWP-equal or higher than 7 mmHg (**Table 1**). This new term came to replace the older "PH out of proportion to LHD." Although, pathophysiology of Cpc-PH is not entirely clear, a chronic elevation of LAP due to longstanding LHD is believed to cause a profound pulmonary artery remodeling and pulmonary vascular resistance (PVR) rise, which is not usually found in isolated post-capillary PH. Patients with Cpc-PH seem to be in the middle of a spectrum of which pre-capillary PH and isolated post-capillary PH are the two extremes, regarding their clinical and echocardiographic characteristics (6).The prevalence of Cpc-PH amongst patients with systolic and diastolic heart failure is believed to be within 12 and 14% (7). An adequate understanding of pathophysiology along with an accurate diagnosis and differentiation of PH-LHD from pre-capillary PH, such as pulmonary arterial hypertension (PAH) and chronic thromboembolic PH (CTEPH), are of paramount importance to select the appropriate treatment for the patient.

PATHOPHYSIOLOGY

The pathophysiological hallmark of PH-LHD is elevated LAP. LV systolic and diastolic dysfunction as well as aortic and/or mitral valve stenosis and/or regurgitation can raise left ventricular filling pressure and subsequently LAP over a period of time. LAP can then be transmitted backwards via pulmonary veins to the pulmonary vasculature leading to pulmonary arterial intimal thickening and medial hypertrophy and PH (**Figure 1**). Compliance in the pulmonary vasculature, unlike in the systemic circulation, is more evenly distributed across the pulmonary bed and the distal vessels are responsible for most of it (8). Hence, compliance is mostly determined by PVR. The relationship between PVR and compliance is an inverse hyperbolic one. "Passive" left-sided elevated pressures shift the hyperbole leftwards leading to an additional decline of compliance for a given PVR and thus enhanced pulmonary wave reflections which return during ventricular systole and increase the systolic (but not the diastolic) pulmonary artery pressure (9). In a recent study, pulmonary arterial compliance (or capacitance) defined as the ratio of stroke volume to pulmonary pulse pressure was the best predictor of mortality in PH-LHD associated with heart failure with preserved left ventricular ejection fraction (HFpEF) (10).

The Right Ventricle (RV)

The first compensatory mechanism of the RV to the elevated pulmonary pressure is hypertrophy. Thus, the RV can adapt with a 4- to 5-fold increase in myocardial contractility, a process which is described pathophysiologically as right ventricular-pulmonary artery (RV-PA) "coupling." In the next progressive stage the RV starts dilating, wall stress according to LaPlace's law increases, imbalance between oxygen demand and supply and RV ischemia occurs, contractility declines, the RV fails to maintain cardiac output and inevitably "uncoupling" and decompensation begins. The development of tricuspid (TR) and pulmonary regurgitation due to annular dilatation leads to RV volume overloading and decreases stroke volume even further. RV-PA coupling has been estimated by echocardiography as the ratio of tricuspid annular plane systolic excursion (TAPSE) to pulmonary artery systolic pressure (PASP) and a cut-off limit <0.36 has been shown to have prognostic value (11). Coupling has also been estimated by a hybrid method combining RHC and cardiac magnetic resonance (CMR) as the ratio of pulmonary artery effective elastance, which is a measure of arterial load, to the maximum end-systolic RV elastance, which is an index of contractility (12).

Up until recently the impact of PH-LHD on RV function had been underestimated. In recent years, however, its significance and prognostic value has been highlighted. RV systolic dysfunction assessed by echocardiography has been found in at least 1/5 of patients with HFpEF and potentially up to 30–50% (13). In addition, a decrease of TAPSE by 5 mm was associated with a 26% mortality rise (13), while in another study a decrease of RV fractional area change by 7% with a 2.2-fold increase of all-cause mortality after adjustment for pulmonary pressures in patients with HFpEF (14).

Ventricular Interdependence and Comorbidities

Other parameters such as ventricular interdependence may contribute to decreased cardiac output and heart failure. As RV pressure increases and interventricular dyssynchrony is established, the interventricular septum bows toward the LV moving "paradoxically" and leading to a decrease of LV diastolic filling and a further drop of stroke volume. In addition, atrial fibrillation, coronary artery disease via right coronary or circumflex artery stenosis leading to reduced RV oxygen supply, chronic obstructive pulmonary disease, obstructive sleep apnea, diabetes, and obesity, all comorbidities frequently seen in patients with LHD may increase pulmonary pressures and further compromise RV function.

The Role of DPG

DPG has been considered to be a more accurate indicator of pulmonary vascular remodeling in LHD than transpulmonary gradient (the difference between mean pulmonary artery pressure and mean PAWP [TPG]), as it is theoretically not influenced

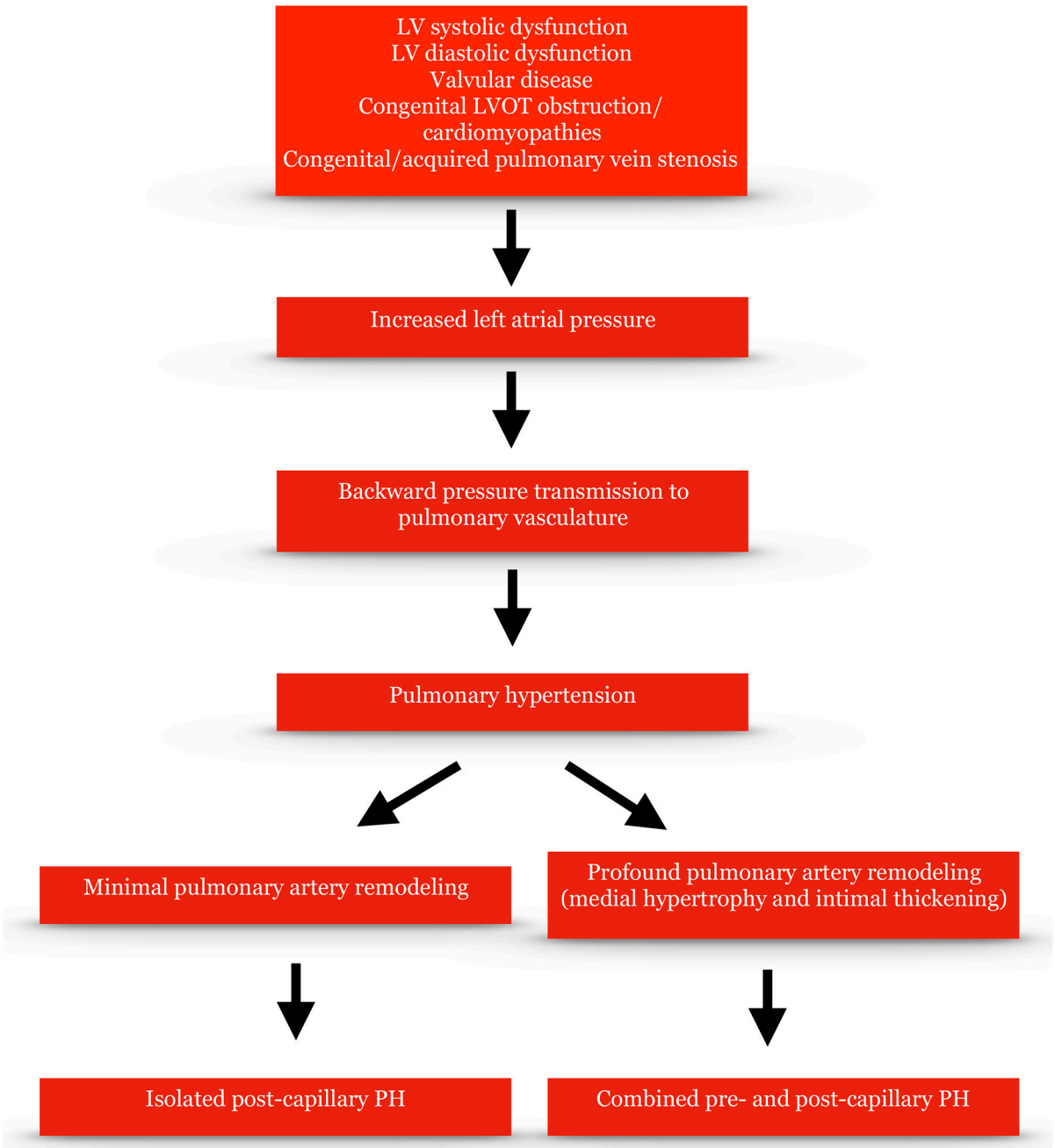


FIGURE 1 | Pathophysiological mechanisms of pulmonary hypertension due to left heart disease.

by the variability of cardiac output or the impact of LAP on pulmonary vascular compliance. In a retrospective large study in patients with PH-LHD an elevated DPG $\geq$ 7 mmHg was correlated with worse median survival compared with a DPG $<$ 7 mmHg and a high TPG $>$ 12 mmHg (15). In the same study, in a small number of patients high DPG was also correlated with greater pathological pulmonary vascular remodeling compared to patients with high TPG but normal DPG. Two further studies, retrospective as well, in patients who had undergone orthotopic heart transplantation and in patients with unexplained cardiomyopathy did not confirm the prognostic value of DPG (16, 17). In a more recent study the risk of death in Cpc-PH and isolated post-capillary PH after adjustment of relevant covariates was similar, however the researchers identified 75 common exonic single-nucleotide polymorphisms between Cpc-PH and pre-capillary PH in pathways pertinent with cell structure, extracellular matrix and immune function (6).

DIAGNOSIS

Risk Factors, Clinical Symptoms, and Signs

Features of metabolic syndrome such as obesity, systemic hypertension, diabetes and dyslipidemia, are more prevalent in patients with PH-LHD than PAH. Researchers have shown that two or more of these features were present in 94.1% of PH-LHD patients vs. only 34.3% of PAH (18). The findings were confirmed in another study, which showed that older age, the presence of systemic hypertension and coronary artery disease are more frequent in PH-LHD and can best differentiate from PAH along with other echocardiographic and hemodynamic parameters (19). In the same study there was a similarly high female predominance in the PH-LHD and PAH groups but more pronounced than in the HFpEF without PH group. In addition to these risk factors, the prevalence of permanent atrial fibrillation in PH-LHD seems to be significantly higher than in patients with PAH and risk factors for LHD (20).

The symptoms and clinical signs of patients with PH-LHD are these of heart failure. Exertional dyspnea, orthopnea, and/or paroxysmal nocturnal dyspnea, anginal chest pain typically on exertion, palpitations, dry cough, pre-syncope and/or syncope, hepatomegaly, ascites, elevated jugular venous pressure, and peripheral edema are the most common ones. Right ventricular heave, a loud pulmonary component of the second heart sound and a pansystolic murmur of tricuspid regurgitation may also be present. In a retrospective study which compared patients with PAH with or without cardiovascular comorbidities to PH-LHD, patients with PH-LHD were more likely to have peripheral edema, whilst there was a trend toward a worse functional class after adjustment for age but no difference in 6-min walking test distance (20). Patients with PH-LHD usually have normal resting oxygen saturations with a possible drop on exercise, unless if they also have respiratory comorbidity or a right-to-left shunt.

Chest X-Ray, ECG, and Pulmonary Function Test

The chest X-ray may show signs of cardiomegaly and enlarged pulmonary artery branches. Pleural effusions may be seen more frequently in PH-LHD than PAH, although they are not uncommon in severe PAH as well (21).

Left QRS axis deviation, atrial fibrillation and signs of left ventricular hypertrophy are more likely on ECG in PH-LHD than in pre-capillary PH, whereas right axis, sinus rhythm, R wave dominant in lead V1, ST-T segment depression, and T-wave inversion in the right precordial and inferior leads are more frequently seen in pre-capillary PH. Right bundle branch block does not appear to differentiate the two conditions (20, 22). These ECG findings have been recently incorporated in predictive score models to discriminate non-invasively between PH-LHD and PAH (23, 24).

It is well-known that pulmonary alveolar diffusion is impaired in patients with congestive heart failure. Gas transfer factor for carbon monoxide (TLco) is reduced in this population compared to controls (25). In addition, TLco and end-tidal CO_2 have been found to be lower in PAH patients compared to PH-LHD (26, 27). In the ASPIRE registry, mean TLco in the PAH group was 55 vs. 62% predicted in the PH-LHD one (26).

Natriuretic Peptides

Brain natriuretic peptide (BNP) and its N-terminal fragment NT-proBNP can both be elevated in heart failure with reduced LV ejection fraction and HFpEF. They can be secreted from the cardiomyocytes of both ventricles due to stretching from volume overload. The upper limit of normal for BNP is 35 pg/mL and for NT-proBNP 125 pg/mL (28). Natriuretic peptides may be elevated in elderly people, atrial fibrillation and renal impairment without heart failure. They can also be disproportionally low in obese patients. In addition, they cannot differentiate between pre-capillary PH and PH-LHD as they may be raised in both conditions. Their role in patients' follow-up, assessment of response to treatment and detection of progression of the underlying disease as well as their prognostic role can be very important.

Echocardiography

Echocardiography plays a seminal role in the detection of signs of PH-LHD. It can diagnose LV systolic and diastolic dysfunction, aortic and mitral valve disease, LV outflow tract obstruction, restrictive and hypertrophic cardiomyopathy and constrictive pericarditis. In all these conditions, LV diastolic dysfunction usually occurs and may lead to PH-LHD. The estimation of PASP, which raises the suspicion of PH when elevated, derives from applying continuous Doppler and the modified Bernoulli equation: *4 x peak TR velocity (TRV)*^{2} + *estimated right atrial pressure*. Right atrial pressure can be estimated from the size and collapsibility during respiration/sniff of the inferior vena cava on the subcostal view (29). According to the current ESC/ERS guidelines a threshold of peak TRV higher than 2.8 m/s is considered suspicious of PH with or without the presence of other echocardiographic signs of PH and risk factors for PH (5). However, peak TRV is angle and flow-dependent and it can frequently be under- or overestimated (30). Peak TRV should be measured from several different acoustic windows and views, while in atrial fibrillation an average of measurements from the view with the highest velocities should be taken and only a well-defined, spade-shaped, dense spectral profile should be measured. In cases with no or trivial TR when TRV cannot accurately be estimated, mean pulmonary artery pressure can be calculated instead from the peak velocity of a pulmonary regurgitant jet at the beginning of diastole or from the acceleration time (time from onset to peak) of the forward flow at the right ventricular outflow tract (29).

Diagnosing LV diastolic dysfunction with echocardiography sometimes can be challenging. Four parameters have been recently included in an algorithm to determine diastolic dysfunction: an average (of septal and lateral) E/e' > 14, a septal e' < 7 cm/s or lateral <10 cm/s, a TRV > 2.8 m/s and a left atrial volume index >34 ml/m^2. If <50% of these parameters are present, diastolic function is normal, if >50%, there is diastolic dysfunction and when exactly 50%, diastolic function is indeterminate (31). Other echocardiographic signs such as mitral "L" velocity >20 cm/s (ongoing LV filling in mid-diastole

due to markedly delayed LV relaxation), mitral inflow E/A wave velocity ratio and E wave deceleration time, as well as a higher diastolic wave velocity than the systolic one in the pulmonary vein Pulsed Doppler profile may also be useful (31). Unlike PH-LHD, significant LV diastolic dysfunction in PAH is very unlikely. In a small study, 88% of PAH patients demonstrated grade I (mild) diastolic dysfunction and 10% normal diastolic function (32).

Left atrial dilatation is considered to be one of the most useful indicators of LHD. However, in the presence of atrial fibrillation may not always represent elevated LAP. In atrial fibrillation, an average E/e' $\geq$11 instead of 14 should be used as a marker of diastolic dysfunction (31). Researchers in the past have described conditions such as severe mitral regurgitation with a dilated left atrium but normal LAP. Left atrial compliance seems to play a pivotal role in these cases (33). The absence of mid-systolic "notching" in the right ventricular outflow tract Pulsed Doppler envelope has also been shown to be highly predictive of a PVR < 3 Wood units and a PAWP >15 mmHg (34). On the other hand, a PASP > 70 mmHg is more likely to represent PAH than PH-LHD (35). The severity of TR cannot differentiate between PH-LHD and PAH. In a prospective cohort of patients with PAH and PH-LHD, 45% had mild, 34% moderate, and 21% severe TR. The mechanisms causing TR were annular dilatation, RV remodeling-tethering and inadequate tricuspid valve leaflet area (36). Finally, a RV end-diastolic diameter to LV end-diastolic diameter ratio (measured in the 4-chamber apical view at the tips of the atrioventricular valves) <1 and the absence of pericardial effusion seem to be more likely in PH-LHD than PAH (20). **Table 2** summarizes the distinct echocardiographic characteristics in pre-capillary PH and PH-LHD. An echocardiographic score based on LA dimension, mid-systolic notching and E/e' to differentiate between PH-LHD and PAH has been proposed (37). More recently another echocardiographic score, which seems to be useful in discriminating between isolated and Cpc-PH as well, has been published (38).

TABLE 2 | Echocardiographic features likely to be present in pre- and post-capillary pulmonary hypertension.

Pre-capillary PH	PH-LHD
Normal sized or small LV cavity	Normal sized or dilated LV cavity
No LV hypertrophy	LV hypertrophy
Preserved LVEF	Variable LVEF
Normal sized or small left atrium	Dilated left atrium
Grade I LV diastolic dysfunction or normal LV diastolic function	$\geq$ Grade II LV diastolic dysfunction
Presence of mid-systolic notching	Absence of mid-systolic notching
RV/LV ratio > 1	RV/LV ratio < 1
PASP > 70 mmHg	Typically PASP < 70 mmHg
Pericardial effusion	No pericardial effusion
No mitral and/or aortic valve disease	Mitral and/or aortic valve disease

PH-LHD, pulmonary hypertension due to left heart disease; LV, left ventricle; LVEF, left ventricular ejection fraction; RV/LV ratio, right ventricular to left ventricular end-diastolic diameter ratio; PASP, pulmonary artery systolic pressure. Modified from Roberts JD and Forfia PR. Pulm Circ. 2011;1:160-181.

CMR

CMR may play an important role in making diagnosis and identifying prognosis of PH in general and PH-LHD in particular. Especially in cases where visualization or accuracy of measurements is limited, CMR should be strongly considered. CMR remains the gold standard imaging modality for RV assessment (39). Beyond RV size and function, CMR can quantify the curvature of interventricular septum and more recently pulmonary arterial stiffness, which may detect early stages of pulmonary vascular remodeling (40, 41). In the ASPIRE registry, patients with PH-LHD had lower RV mass, a better-preserved cardiac function and less gadolinium hinge-point enhancement compared to pre-capillary PH (42). In addition, CMR is an excellent tool for myocardial tissue characterization and with the use of late gadolinium enhancement it can diagnose infiltrative cardiomyopathies e.g., amyloidosis, hypertrophic cardiomyopathy, as well as systolic and diastolic LV dysfunction. CMR can also play a significant role in diagnosis of congenital heart diseases, which may be related to both PAH and PH-LHD.

Predictive Non-invasive Score Models

Over the last few years, score models to assess the pre-RHC probability of PH-LHD have been created aspiring to avoid potentially unnecessary catheterizations. Bonderman et al. proposed a model based on RV strain on ECG and BNP (23). Jacobs et al. suggested a risk model including history of LHD, the sum of S deflection in V1 and R deflection in V6 on the ECG, left atrial dilatation and left valve disease worse than mild on echocardiogram (24). Finally, Richter et al. suggested a prediction score for elevated PAWP based on age >68 years, BMI > 30 kg/m^2, absence of RV enlargement and presence of left atrial dilatation on echocardiography (43).

RHC-Fluid Challenge-Exercise-Vasoreactivity Testing

The gold standard method for the diagnosis of PH-LHD remains RHC. All pulmonary pressures should be measured at end-expiration with the patient breathing spontaneously. The mean PAWP has been better correlated with mean LAP than the LV end-diastolic pressure (LVEDP) in patients with LV disease, except for very high PAWP over 25 mmHg (44, 45). However, a LVEDP > 15 mmHg on left heart catheterization can be used to define PH-LHD, when an accurate PAWP is not feasible (e.g., in chronic thromboembolic disease). An accurate PAWP may be challenging to obtain. A waveform consistent with atrial waveform, a PAWP $\leq$ diastolic pulmonary arterial pressure, blood easily aspirated and highly oxygenated in the wedge position and the placement of the tip of the catheter in West zone 3 (at the lung base, where pulmonary artery and venous pressure exceeds pulmonary alveolar pressure, which represents lung areas with the greatest blood flow rates), below left atrial level, can eliminate technical difficulties. Mitral regurgitation may cause large "v" waves that can be mistakenly interpreted as an elevated PAWP. This can be accounted for by reading the PAWP at the

time of the "a" wave. Patients with PH-LHD tend to have a higher mean right atrial pressure, cardiac output and right ventricular end-diastolic pressure, as well as lower PVR compared with PAH (19, 20).

Patients with clinical and echocardiographic features suggestive of PH-LHD may sometimes have pre-capillary PH, especially if they have been previously heavily diuresed. Fluid challenge, which is the rapid intravenous administration of a high volume of normal saline, is believed to be helpful to unpick cases of occult PH-LHD with resting pre-capillary hemodynamics and unmask LV diastolic dysfunction. The current ESC/ERS PH guidelines do not recommend fluid loading at RHC due to lack of standardization as well as the theoretical risk of a rapid significant PAWP elevation to result in pulmonary edema. Three studies with fluid challenge in PH patients have been conducted since the publication of these guidelines though. In the first one, the infusion of 500 mL of saline within 5–10 min led to the reclassification of 22.2% of patients as occult PH-LHD (defined as a mean PAWP > 15 mmHg), while in another small study in patients with systemic sclerosis a similar percentage of patients with pre-capillary baseline hemodynamics were found to have a LVEDP > 15 mmHg post-infusion of 500 mL within 5–10 min (46, 47). In the most recent one, rapid infusion of saline at a rate of 7 mL/kg reclassified 8% of patients with no PH and 6% of patients with pre-capillary PH at baseline. A cut-off limit of PAWP ≥ 18 mmHg was used in the latter study (48). In all three studies, fluid loading was safe. Of note, even in healthy subjects a rapid fluid infusion will lead to a PAWP > 15 mmHg in a percentage >60%, however with a larger than 500 mL volume of saline (49). Likewise, exercise during RHC may unmask diastolic dysfunction in patients with suspected HFpEF and a baseline PAWP < 15 mmHg. Exercise can be performed on a supine or upright bike (the latter would be feasible when the RHC is done from the neck or the arm) or with leg lifting. In normal subjects and athletes, an increase of PAWP up to 25 mmHg in a broadly linear fashion with cardiac output has been shown (50). However, significant increases of PAWP at a cardiac output <10 L/min is uncommon in normal individuals. Exercise during RHC seems to be particularly helpful in obese patients with a borderline, between 12 and 15 mmHg, PAWP (51).

According to the ESC/ERS guidelines vasoreactivity testing is only recommended in patients with suspected idiopathic, familial, or drug-induced PAH (5). In the context of PH-LHD vasodilator challenge can be used in the evaluation of patients for listing for cardiac transplantation, especially those with a TPG > 12–15 mmHg and/or PVR > 3 Wood units. The preferable vasodilator agent is inhaled nitric oxide. An increase of PAWP post-nitric oxide may be seen but pulmonary edema is very rare.

Treatment

PAH targeted therapies act on three distinct pathophysiological pathways. The first one is the nitric oxide-soluble guanylate cyclase (sGC)-cGMP pathway which includes drugs like phosphodiesterase type 5 inhibitors (Sildenafil, Tadalafil) and stimulators of cGC like Riociguat. The second one is the endothelin-1 pathway with drugs like endothelin receptor antagonists (Bosentan, Ambrisentan, and Macitentan) and the third is the prostacyclin pathway with prostacyclin analogs (Epoprostenol, Treprostinil, Iloprost) and an oral selective IP prostacyclin-receptor agonist (Selexipag). These drugs have proven to be efficacious in PAH and CTEPH patients in several randomized, controlled, double-blind trials. On the other hand, trials of the same drugs in patients with PH-LHD have not shown any benefit and some of them have also been related with increased mortality (52–54). In the recent SIOVAC trial (55), where Sildenafil was tested vs. placebo in patients with post-capillary PH and history of correction of valvular heart disease, median PVR in the Sildenafil group was 3.4 Wood units, TPG 16 mmHg, but DPG only 2 mmHg. Of note, the study did not meet its primary end-point. Further trials need to be conducted in patients with Cpc-PH hemodynamics to answer the question about a possible efficacy of pulmonary vasodilator drugs in this specific PH phenotype.

CONCLUSIONS

Up to now, no pulmonary vasodilator treatment has proven to be efficacious in PH-LHD. Some of the PAH drugs have even been detrimental when given in patients with LHD. Hence, it is apparent that the accurate identification of the major driver of PH in each patient is essential. In clinical practice the diagnosis and phenotyping of PH cannot be based on a single test, even if that test is the RHC, but rather on a combination of clinical data, imaging and hemodynamics. A better understanding of pathophysiology and further clinical trials are required to clarify whether Cpc-PH is a distinct clinical entity or part of a spectrum of PH phenotypes and identify potential treatments for the future.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Lam CS, Roger VL, Borlaug BA, Enders FT, Redfield MM. Pulmonary hypertension in heart failure with preserved ejection fraction: a community-based study. *J Am Coll Cardiol.* (2009) **53**:1119–26. doi: 10.1016/j.jacc.2008.11.051
2. Leung CC, Moondra V, Catherwood E, Andrus BW. Prevalence and risk factors of pulmonary hypertension in patients with elevated pulmonary venous pressure and preserved ejection fraction. *Am J Cardiol.* (2010) **106**:284–6. doi: 10.1016/j.amjcard.2010.02.039
3. Damy T, Goode KM, Kallvikbacka-Bennett A, Lewinter C, Hobkirk J, Nikitin NP, et al. Determinants and prognostic value of pulmonary arterial hypertension in patients with chronic heart failure. *Eur Heart J.* (2010) **31**:2280–90. doi: 10.1093/eurheartj/ehq245

4. Bursi F, McNallan SM, Redfield MM, Nkomo VT, Lam CS, Weston SA, et al. Pulmonary pressures and death in heart failure: a community study. *J Am Coll Cardiol.* (2012) **59**:222–31. doi: 10.1016/j.jacc.2011.06.076
5. Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J.* (2016) **37**:67–119. doi: 10.1093/eurheartj/ehv317
6. Assad TR, Hemnes AR, Larkin EK, Glazer AM, Xu M, Wells QS, et al. Clinical and biological insights into combined post- and pre-capillary pulmonary hypertension. *J Am Coll Cardiol.* (2016) **68**:2525–36. doi: 10.1016/j.jacc.2016.09.942
7. Gerges M, Gerges C, Pistritto AM, Lang MB, Trip P, Jakowitsch J, et al. Pulmonary hypertension in heart failure: epidemiology, right ventricular function, and survival. *Am J Respir Crit Care Med.* (2015) **192**:1234–46. doi: 10.1164/rccm.201503-0529OC
8. Saouti N, Westerhof N, Helderman F, Marcus JT, Stergiopulos N, Westerhof BE, et al. RC time constant of single lung equals that of both lungs together: a study in chronic thromboembolic pulmonary hypertension. *Am J Physiol Heart Circ Physiol.* (2009) **297**:H2154–60. doi: 10.1152/ajpheart.00694.2009
9. Tedford RJ, Hassoun PM, Mathai SC, Girgis RE, Russell SD, Thiemann DR, et al. Pulmonary capillary wedge pressure augments right ventricular pulsatile loading. *Circulation* (2012) **125**:289–97. doi: 10.1161/CIRCULATIONAHA.111.051540
10. Al-Naamani N, Preston IR, Paulus JK, Hill NS, Roberts KE. Pulmonary arterial capacitance is an important predictor of mortality in heart failure with a preserved ejection fraction. *JACC Heart Fail* (2015) **3**:467–74. doi: 10.1016/j.jchf.2015.01.013
11. Guazzi M, Bandera F, Pelissero G, Castelvecchio S, Menicanti L, Ghio S, et al. Tricuspid annular plane systolic excursion and pulmonary arterial systolic pressure relationship in heart failure: an index of right ventricular contractile function and prognosis. *Am J Physiol Heart Circ Physiol.* (2013) **305**:H1373–81. doi: 10.1152/ajpheart.00157.2013
12. Sanz J, García-Alvarez A, Fernández-Friera L, Nair A, Mirelis JG, Sawit ST, et al. Right ventriculo-arterial coupling in pulmonary hypertension: a magnetic resonance study. *Heart* (2012) **98**:238–43. doi: 10.1136/heartjnl-2011-300462
13. Gorter TM, Hoendermis ES, van Veldhuisen DJ, Voors AA, Lam CS, Geelhoed B, et al. Right ventricular dysfunction in heart failure with preserved ejection fraction: a systematic review and meta-analysis. *Eur J Heart Fail* (2016) **18**:1472–87. doi: 10.1002/ejhf.630
14. Melenovsky V, Hwang SJ, Lin G, Redfield MM, Borlaug BA. Right heart dysfunction in heart failure with preserved ejection fraction. *Eur Heart J.* (2014) **35**:3452–62. doi: 10.1093/eurheartj/ehu193
15. Gerges C, Gerges M, Lang MB, Zhang Y, Jakowitsch J, Probst P, et al. Diastolic pulmonary vascular pressure gradient: a predictor of prognosis in "out-of-proportion" pulmonary hypertension. *Chest* (2013) **1433**:758–66. doi: 10.1378/chest.12-1653
16. Tampakakis E, Leary PJ, Selby VN, De Marco T, Cappola TP, Felker GM, et al. The diastolic pulmonary gradient does not predict survival in patients with pulmonary hypertension due to left heart disease. *JACC Heart Fail* (2015) **3**:9–16. doi: 10.1016/j.jchf.2014.07.010
17. Tedford RJ, Beaty CA, Mathai SC, Kolb TM, Damico R, Hassoun PM, et al. Prognostic value of the pre-transplant diastolic pulmonary artery pressure-to-pulmonary capillary wedge pressure gradient in cardiac transplant recipients with pulmonary hypertension. *J Heart Lung Transplant* (2014) **33**:289–197. doi: 10.1016/j.healun.2013.11.008
18. Robbins IM, Newman JH, Johnson RF, Hemnes AR, Fremont RD, Piana RN, et al. Association of the metabolic syndrome with pulmonary venous hypertension. *Chest* (2009) **136**:31–36. doi: 10.1378/chest.08-2008
19. Thenappan T, Shah SJ, Gomberg-Maitland M, Collander B, Vallakati A, Shroff P, et al. Clinical characteristics of pulmonary hypertension in patients with heart failure and preserved ejection fraction. *Circ Heart Fail* (2011) **4**:257–65. doi: 10.1161/CIRCHEARTFAILURE.110.958801
20. Charalampopoulos A, Howard LS, Tzoulaki I, Gin-Sing W, Grapsa J, Wilkins MR, et al. Response to pulmonary arterial hypertension drug therapies in patients with pulmonary arterial hypertension and cardiovascular risk factors. *Pulm Circ.* (2014) **4**:669–78. doi: 10.1086/678512
21. Milne EN. Forgotten gold in diagnosing pulmonary hypertension: the plain chest radiograph. *Radiographics* (2012) **32**:1085–7. doi: 10.1148/rg.324125021
22. McMurray JJ, Carson PE, Komajda M, McKelvie R, Zile MR, Ptaszynska A, et al. Heart failure with preserved ejection fraction: clinical characteristics of 4133 patients enrolled in the I-PRESERVE trial. *Eur J Heart Fail.* (2008) **10**:149–56. doi: 10.1016/j.ejheart.2007.12.010
23. Bonderman D, Wexberg P, Martischnig AM, Heinzl H, Lang MB, Sadushi R, et al. A noninvasive algorithm to exclude pre-capillary pulmonary hypertension. *Eur Respir J.* (2011) **37**:1096–103. doi: 10.1183/09031936.00089610
24. Jacobs W, Konings TC, Heymans MW, Boonstra A, Bogaard HJ, van Rossum AC, et al. Noninvasive identification of left-sided heart failure in a population suspected of pulmonary arterial hypertension. *Eur Respir J.* (2015) **46**:422–30. doi: 10.1183/09031936.00202814
25. Smith AA, Cowburn PJ, Parker ME, Denvir M, Puri S, Patel KR, et al. Impaired pulmonary diffusion during exercise in patients with chronic heart failure. *Circulation* (1999) **100**:1406–10.
26. Hurdman J, Condliffe R, Elliot CA, Davies C, Hill C, Wild JM, et al. ASPIRE registry: assessing the Spectrum of Pulmonary hypertension Identified at a referral centre. *Eur Respir J.* (2012) **39**:945–55. doi: 10.1183/09031936.00078411
27. Hemnes AR, Pugh ME, Newman AL, Robbins IM, Tolle J, Austin ED, et al. End tidal CO_2 tension: pulmonary arterial hypertension vs pulmonary venous hypertension and response to treatment. *Chest* (2011) **140**:1267–73. doi: 10.1378/chest.11-0155
28. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coates AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J.* (2016) **37**:2129–200. doi: 10.1093/eurheartj/ehw128
29. Rudski LG, Wyman WL, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the american society of echocardiography. endorsed by the european association of echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. *J Am Soc Echocardiogr.* (2010) **23**:685–713. doi: 10.1016/j.echo.2010.05.010
30. Fisher MR, Forfia PR, Chamera E, Housten-Harris T, Champion HC, Girgis RE, et al. Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. *Am J Respir Crit Care Med.* (2009) **179**:615–21. doi: 10.1164/rccm.200811-1691OC
31. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF III, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* (2016) **17**:1321–60. doi: 10.1016/j.echo.2016.01.011
32. Tonelli AR, Plana JC, Heresi GA, Dweik RA. Prevalence and prognostic value of left ventricular diastolic dysfunction in idiopathic and heritable pulmonary arterial hypertension. *Chest* (2012) **141**:1457–65. doi: 10.1378/chest.11-1903
33. Braunwald E, Awe W. The syndrome of severe mitral regurgitation with normal left atrial pressure. *Circulation* (1963) **27**:29–35.
34. Arkles JS, Opotowsky AR, Ojeda J, Rogers F, Liu T, Prassana V, et al. Shape of the right ventricular Doppler envelope predicts hemodynamics and right heart function in pulmonary hypertension. *Am J Respir Crit Care Med.* (2011) **183**:268–76. doi: 10.1164/rccm.201004-0601OC
35. Hussain N, Ramjug S, Hurdman J, Elliot C, Kiely D. Non-invasive testing can help differentiate idiopathic pulmonary arterial hypertension and pulmonary hypertension associated with heart failure with preserved ejection fraction. *Am J Respir Crit Care Med.* (2014) **189**:A1891. doi: 10.1164/ajrccm-conference. 2014.189.1_MeetingAbstracts.A1891
36. Afilalo J, Grapsa J, Nihoyannopoulos P, Beaudoin J, Gibbs JS, Channick RN, et al. Leaflet area as a determinant of tricuspid regurgitation severity in patients with pulmonary hypertension. *Circ Cardiovasc Imaging* (2015) **8**: e002714. doi: 10.1161/CIRCIMAGING.114.002714
37. Opotowsky AR, Ojeda J, Rogers F, Prasanna V, Clair M, Moko L, et al. A simple echocardiographic prediction rule for hemodynamics in pulmonary hypertension. *Circ Cardiovasc Imaging* (2012) **5**:765–75. doi: 10.1161/CIRCIMAGING.112.976654

38. D'Alto M, Romeo E, Argiento P, Pavelescu A, D'Andrea A, Di Marco GM, et al. A simple echocardiographic score for the diagnosis of pulmonary vascular disease in heart failure. *J Cardiovasc Med.* (2017) **18**:237–43. doi: 10.2459/JCM.0000000000000485
39. Mooij CF, de Wit CJ, Graham DA, Powell AJ, Geva T. Reproducibility of MRI measurements of right ventricular size and function in patients with normal and dilated ventricles. *J Magn Reson Imaging* (2008) **28**:67–73. doi: 10.1002/jmri.21407
40. Marcus JT, Vonk Noordegraaf A, Roeleveld RJ, Postmus PE, Heethaar RM, Van Rossum AC, et al. Impaired left ventricular filling due to right ventricular pressure overload in primary pulmonary hypertension: noninvasive monitoring using MRI. *Chest* (2001) **119**:1761–5
41. Sanz J, Kariisa M, Dellegrottaglie S, Prat-González S, Garcia MJ, Fuster V, et al. Evaluation of pulmonary artery stiffness in pulmonary hypertension with cardiac magnetic resonance. *JACC Cardiovasc Imaging* (2009) **2**:286–95. doi: 10.1016/j.jcmg.2008.08.007
42. Swift AJ, Rajaram S, Condliffe R, Capener D, Hurdman J, Elliot CA, et al. Diagnostic accuracy of cardiovascular magnetic resonance imaging of right ventricular morphology and function in the assessment of suspected pulmonary hypertension results from the ASPIRE registry. *J Cardiovasc Magn Reson.* (2012) **14**:40. doi: 10.1186/1532-429X-14-40
43. Richter SE, Roberts KE, Preston IR, Hill NS. A simple derived prediction score for the identification of an elevated pulmonary artery wedge pressure using precatheterization clinical data in patients referred to a Pulmonary Hypertension center. *Chest* (2016) **149**:1261–8. doi: 10.1378/chest.15-0819
44. Braunwald E, Frahm C. Studies on Starling's law of the heart. IV. Observations on the hemodynamic functions of the left atrium in man. *Circulation* (1961) **24**:633–42.
45. Walston A 2nd, Kendall ME. Comparison of pulmonary wedge and left atrial pressure in man. *Am Heart J.* (1973) **86**:159–64.
46. Robbins IM, Hemnes AR, Pugh ME, Brittain EL, Zhao DX, Piana RN, et al. High prevalence of occult pulmonary venous hypertension revealed by fluid challenge in pulmonary hypertension. *Circ Heart Fail* (2014) **7**:116–22. doi: 10.1161/CIRCHEARTFAILURE.113.000468
47. Fox BD, Shimony A, Langleben D, Hirsch A, Rudski L, Schlesinger R, et al. High prevalence of occult left heart disease in scleroderma-pulmonary hypertension. *Eur Respir J.* (2013) **42**:1083–91. doi: 10.1183/09031936.00091212
48. D'Alto M, Romeo E, Argiento P, Motoji Y, Correra A, Di Marco GM, et al. Clinical relevance of fluid challenge in patients evaluated for pulmonary hypertension. *Chest* (2017) **151**:119–26. doi: 10.1016/j.chest.2016.08.1439
49. Fujimoto N, Borlaug BA, Lewis GD, Hastings JL, Shafer KM, Bhella PS, et al. Hemodynamic responses to rapid saline loading: the impact of age, sex, and heart failure. *Circulation* (2013) **127**:55–62. doi: 10.1161/CIRCULATIONAHA.112.111302
50. Naeije R, Vanderpool R, Dhakal BP, Saggar R, Saggar R, Vachiery J-L, et al. Exercise-induced pulmonary hypertension. physiological basis and methodology concerns. *Am J Respir Crit Care Med.* (2013) **187**:576–83. doi: 10.1164/rccm.201211-2090CI
51. Maor E, Grossman Y, Balmor RG, Segel M, Fefer P, Ben-Zekry S, et al. Exercise haemodynamics may unmask the diagnosis of diastolic dysfunction among patients with pulmonary hypertension. *Eur J Heart Fail* (2015) **17**:151–8. doi: 10.1002/ejhf.198
52. Califf RM, Adams KF, McKenna WJ, Gheorghiade M, Uretsky BF, McNulty SE, et al.A randomized controlled trial of epoprostenol therapy for severe congestive heart failure: The Flolan International Randomized Survival Trial (FIRST). *Am Heart J.* (1997) **134**:44–54.
53. Kalra PR, Moon JC, Coats AJ. Do results of the ENABLE (Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure) spell the end for non-selective endothelin antagonism in heart failure? *Int J Cardiol.* (2002) **85**:195–97. doi: 10.1016/S0167-5273(02)00182-1
54. Bonderman D, Ghio S, Felix SB, Ghofrani HA, Michelakis E, Mitrovic V, et al. Riociguat for patients with pulmonary hypertension caused by systolic left ventricular dysfunction: a phase IIb double-blind, randomized, placebo-controlled, dose-ranging hemodynamic study. *Circulation* (2013) **128**:502–11. doi: 10.1161/CIRCULATIONAHA.113.001458
55. Bermejo J, Yotti R, García-Orta R, Sánchez-Fernández PL, Castaño M, Segovia-Cubero J, et al. Sildenafil for improving outcomes in patients with corrected valvalar heart disease and persistent pulmonary hypertension: a multicenter, double-blind, randomized, controlled trial. *Eur Heart J.* (2018) 39:1255–64. doi: 10.1093/eurheartj/ehx700

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 06 June 2018
doi: 10.3389/fmed.2018.00175

Circulating Protein Biomarkers in Systemic Sclerosis Related Pulmonary Arterial Hypertension: A Review of Published Data

Peter M. Hickey[1,2], *Allan Lawrie*[1] *and Robin Condliffe*[2]*

[1] Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, United Kingdom,
[2] Pulmonary Vascular Diseases Unit, Royal Hallamshire Hospital, Sheffield, United Kingdom

Pulmonary arterial hypertension (PAH) develops in 7–12% of patients with systemic sclerosis (SSc) and is associated with a 3 year survival of 52%. Early detection by screening is therefore recommended for all patients with SSc. Historically, screening has been performed using echocardiography and measurement of gas transfer. More recently the DETECT protocol, using a combination of biomarkers (including N-terminal pro-brain natriuretic peptide) and clinical parameters, has been developed. The optimal method of screening for PAH with high sensitivity and specificity is, however, not clear. Protein expression differences between different SSc disease phenotypes have been reported, and include alterations in concentration of NT-proBNP, endoglin, soluble vascular endothelial growth factor receptor 1, placenta growth factor, growth differentiation factor-15, vascular endothelial growth factor alpha, resistin-like molecule beta, and soluble thrombomodulin. This review summarizes the current knowledge of these protein changes in patients with SSc and PAH.

Keywords: systemic sclerosis, pulmonary arterial hypertension, protein, biomarkers, diagnosis

OPEN ACCESS

Edited by:
Anne Hilgendorff, Ludwig-Maximilians-Universität München, Germany

Reviewed by:
Peter Korsten, Universitätsmedizin Göttingen, Germany
Eleni Papakonstantinou, Aristotle University of Thessaloniki, Greece

***Correspondence:**
Robin Condliffe
robin.condliffe@sth.nhs.uk

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *26 January 2018*
Accepted: *21 May 2018*
Published: *06 June 2018*

Citation:
Hickey PM, Lawrie A and Condliffe R (2018) Circulating Protein Biomarkers in Systemic Sclerosis Related Pulmonary Arterial Hypertension: A Review of Published Data. Front. Med. 5:175. doi: 10.3389/fmed.2018.00175

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare disease characterized by a progressive vasculopathy of the small pulmonary arteries leading to increased pulmonary vascular resistance and right ventricular afterload. This results in right ventricular failure and premature death. PAH may be idiopathic, heritable, related to various drugs and toxins or may be associated with a number of medical conditions including congenital systemic to pulmonary shunts, portal hypertension, HIV and connective tissue disease, most notably systemic sclerosis (SSc). Although these forms of PAH have similarities in underlying pathophysiology and treatment regime, it is increasingly recognized that there are differences in pathobiology, response to treatment and prognosis.

Between 7 and 12% of patients with SSc develop PAH (SSc-PAH) at some point in their disease course (1–4). Compared to idiopathic pulmonary arterial hypertension (IPAH), histological patterns in SSc-PAH show greater intra-individual variability, a relative absence of plexiform lesions, more prominence of intimal fibrosis and a much greater involvement of pulmonary venules (5). Prognosis is significantly worse than in other forms of PAH with median survival of 3 and 7.8 years in SSc-PAH and IPAH cohorts respectively (6). Response to treatment is generally felt to be worse in SSc-PAH than in IPAH (7).

The diagnosis of PAH can be challenging, due in part to the relatively low incidence of this condition, and in part due to the non-specific nature of the early symptoms. As a result, the diagnosis of PAH is often made at an advanced stage after a large proportion of the pulmonary vascular bed has been irreversibly obliterated by pathological vascular remodeling.

Given the progressive nature of vascular remodeling in PAH, interest in the outcome of patients detected early has led to investigation of alternative screening methods in patients with systemic sclerosis. It is thought that early diagnosis and treatment leads to a favorable prognosis (8, 9). Historically, screening for PAH in SSc has involved estimating systolic pulmonary arterial pressure at annual echocardiography ± the measurement of diffusion capacity of the lung for carbon monoxide (DL_{CO}). The DETECT protocol was developed to provide an evidence-based multi-modality approach to screening patients with SSc for the presence of PAH. It combines variables from clinical examination, echocardiography, electrocardiography, pulmonary function tests and blood tests [uric acid and N-terminal pro-brain type natriuretic peptide (NT-proBNP)] to produce a score with high sensitivity (95%), but relatively low specificity (48%) for the presence of PAH (10). The DETECT protocol involves several steps which add complexity and in real-life clinical practice, may prove significantly more expensive and can lead to delay and even failure to refer for definitive investigations (11).

Abnormal concentrations of many candidate proteins have been shown in both the tissue and circulating compartments in patients with PAH. These proteins may reflect the underlying pulmonary vascular disease as well as the response of the right ventricle to the increased afterload. They may also reflect the underlying SSc rather than PAH. It is conceivable that a panel of protein biomarkers may have utility in identifying PAH in still asymptomatic patients with systemic sclerosis. This paper reviews the current published evidence base for altered protein biomarker concentrations in SSc-PAH.

METHODS

To identify suitable primary research articles on this topic, a literature search was conducted using Ovid Medline and PubMed. Keywords used were "Systemic sclerosis," "Scleroderma," "Pulmonary hypertension," "PAH," "Protein," and "Biomarker." Date of publication was limited from 1990 to present day. One hundred and forty eight publications were returned from this search.

We included studies identifying a cohort of patients diagnosed with systemic sclerosis with PAH with comparator groups including healthy volunteers (HV), systemic sclerosis without pulmonary hypertension (SSc-no PAH) and/or idiopathic PAH.

Studies were included if they reported data on differential protein expression between subgroups which were related to objective measurements of pulmonary hypertension.

Protein Biomarkers in SSc-PAH

The circulating protein biomarkers identified by the literature search are summarized in **Table 1** and **Figure 1**.

N-Terminal Pro-Brain Natriuretic Peptide (NT-proBNP)

NT-proBNP is a marker of myocardial stress and therefore a non-specific marker for pulmonary hypertension (PH). Brain type natriuretic peptide (BNP) and NT-proBNP remain the only blood-based biomarkers suggested by guidelines for routine clinical use (21). NT-proBNP is an inactive cleavage product released during the activation of BNP from its prohormone. BNP is released in response to ventricular stretch and stimulates natriuresis and diuresis via the kidney in order to reduce ventricular preload. NT-proBNP is elevated in PH of any cause (22) and correlates with echocardiographic, hemodynamic and functional measurements (23–25).

NT-proBNP may be elevated in systemic sclerosis in the absence of pulmonary hypertension as a result of left ventricular disease and primary myocardial involvement (26).

In a prospective observational study of 109 patients with systemic sclerosis, including 68 with PH and 41 without PH at right heart catheter, Williams et al. set out to evaluate the utility of NT-proBNP concentrations as a screening tool for PAH. NT-proBNP concentration was significantly higher in patients with PAH than without (1474 pg/ml vs. 139 pg/ml respectively, $p = 0.0002$). The authors also reported a significant correlation between NT-proBNP concentration and mean pulmonary arterial pressure (mPAP) ($r = 0.62, p < 0.0001$), pulmonary vascular resistance (PVR) ($r = 0.81$, $p < 0.0001$) and right atrial pressure (RAP) ($r = 0.53$, $p < 0.0001$) at right heart catheterization (RHC). For the ability to accurately diagnose PAH a threshold of 395 pg/ml was selected, returning a sensitivity 55.9%, specificity 95.1%, PPV 95.1% and NPV 56.5%. Longitudinal analysis of baseline and change in serial NT-proBNP measurements both demonstrated significant prognostic utility (27). More recent work has provided validation, with Chung et al. reporting a sensitivity and specificity of 73 and 78% respectively for NT-proBNP at a threshold of 210 pg/ml, slightly superior to that of BNP at 71 and 59% respectively at a threshold concentration of 64 pg/ml (13).

Comparing between PAH phenotypes in a study of 98 prevalent PAH patients (SSc-PAH $n = 55$; IPAH $n = 38$; Anorexigen $n = 5$), Mathai et al. found that NT-proBNP levels were significantly higher in the SSc-PAH group vs. IPAH group (1846 pg/ml vs. 808.5 pg/ml respectively, $p < 0.01$), and this was despite a significantly higher mPAP in the patients with IPAH (41 mmHg vs. 48 mmHg, SSc vs. IPAH respectively, $p < 0.01$). The authors also noted stronger correlations between NT-proBNP concentrations and hemodynamic measures of PAH for patients with SSc-PAH than for those with IPAH; cardiac index (CI) ($r = -0.58, p < 0.01$ vs. $r = -0.46, p < 0.01$ respectively); PVR ($r = 0.54$, $p < 0.01$ vs. $r = 0.41$, $p < 0.01$ respectively). When serial protein measurements were analyzed in each subgroup, the prognostic value of NT-proBNP for predicting death remained only in the group with SSc-PAH (SSc-PAH: hazard ratio (HR) 3.07, $p < 0.01$; IPAH: HR 2.02, $p = 0.29$) (14).

The DETECT study investigated a population of SSc patients who were enriched for the presence of PAH by the inclusion of patients with a DL_{CO} <60% predicted (10). NT-proBNP

TABLE 1 | Summary of potential protein biomarkers in SSc-PAH.

Protein	Comparison Groups	Number of Patients	Outcome	Correlations in SSc-PAH	Reference
NT-proBNP	SSc-PAH vs. SSc SSc-PAH vs. SSc SSc-PAH vs. IPAH	109 329 98	Significantly higher in SSc-PAH vs. SSc. Sens 55.9%, Spec 95.1%. Correlated with invasive hemodynamics NT-proBNP superior to BNP for detection of PAH in SSc Significantly higher in SSc-PAH, correlated with hemodynamics and predicted survival in SSc-PAH group.	mPAP ($r = 0.62$; $p < 0.0001$) PVR ($r = 0.81$; $p < 0.0001$) CI ($r = -0.58$; $p < 0.01$) PVR ($r = 0.54$; $p < 0.01$)	(12) (13) (14)
Endoglin	SSc-PAH vs. SSc vs. HV	60	Serum levels significantly higher in SSc-PAH than control		(15)
sFLT-1	SSc-PAH vs. SSc	77	Plasma levels significantly higher in SSc-PAH and correlate with RVSP and inversely with DL_{CO}. Possible predictor of PH progression.	RVSP ($r = 0.32$; $p = 0.01$) DL_{CO} (−0.29; $p = 0.01$)	(16)
PlGF	SSc-PAH vs. SSc	77	Plasma levels significantly higher in SSc-PAH. Correlates with severity of Raynaud's phenomenon and inversely with DL_{CO}.	DL_{CO} ($r = -0.031$; $p = 0.01$)	(16)
VEGF-A	SSC-PAH vs. SSc vs. HV	53	Serum levels significantly higher in SSc-PAH than either SSc or HV. Levels correlate with echocardiographic sPAP, dyspnoea score and DL_{CO}.	sPAP ($r = 0.58$; $p < 0.01$) DL_{CO} ($r = -0.47$; $p < 0.01$)	(17)
GDF-15	SSc-PAH vs. SSc	54	Plasma levels significantly higher in SSc-PAH, correlate with echocardiographic RVSP and circulating NT-proBNP. Discriminates between PH and non-PH.	RVSP ($r = 0.56$; $p < 0.001$)	(18)
RELM-ß	SSc-PAH vs. IPAH vs. HV	26	Tissue concentrations significantly higher in SSc-PAH than in IPAH or HV.		(19)
sThrombomodulin	SSc-PAH vs. SSc vs. HV	92	Significantly higher plasma levels in SSc-PAH compared to either SSc or HV.		(20)

NT-proBNP, N-terminal pro-brain type natriuretic protein; sFLT-1, soluble vascular endothelial growth factor receptor 1; PlGF, placenta growth factor; VEGF-A, vascular endothelial growth factor A; GDF-15, growth differentiation factor-15; RELM-ß, resistin like molecule-ß; sThrombomodulin, soluble thrombomodulin; SSc-PAH, systemic sclerosis related pulmonary arterial hypertension; SSc, systemic sclerosis; IPAH, idiopathic pulmonary arterial hypertension; HV, healthy volunteer; Sens, sensitivity; PH, pulmonary hypertension; Spec, specificity; mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; CI, cardiac index; RVSP, right ventricular systolic pressure; DL_{CO}, diffusing capacity for carbon monoxide; sPAP, systolic pulmonary artery pressure; EC, endothelial cells.

was included in a final 2-step algorithm which also included electrocardiography and echocardiography to select patients to proceed to RHC. Sensitivity for the detection of PAH was high (96%) but specificity was only 48%.

Both BNP and NT-proBNP levels have been demonstrated to be important prognostic predictors at baseline in PAH (28, 29). Subsequently, the change in NT-proBNP level after therapy was shown to be a powerful independent predictor of survival (30). More recently three large studies have confirmed the importance of changes in NT-proBNP in the risk stratification of patients with PAH during follow-up. (12, 31, 32).

Endoglin

Transforming growth factor beta (TGF-ß) signaling has been strongly implicated in the pathogenesis of PAH, and extensively studied, particularly with regard to bone morphogenetic protein receptor type-2 mutations (26). TGF-ß signaling regulates several processes including cellular proliferation and angiogenesis. Endoglin (Eng) is a transmembrane protein expressed in endothelial cells which acts as a TGF-ß signaling complex component (33).

Both TGF-ß serum concentration and Eng level are raised in IPAH patients, with Eng localized to endothelial cells in tissue samples (34). Germline Eng mutation have shown a protective effect against the development of pulmonary hypertension in heterozygous models exposed to chronic hypoxia (34).

Coral-Alvarado et al. investigated circulating Eng concentration in 60 patients (20 SSc-PAH; 20 SSc-no PAH; 20 HV). PH was diagnosed by estimation of systolic pulmonary artery pressure >35 mmHg, or tricuspid regurgitant jet velocity >3 m/s. The authors report higher Eng concentrations in the SSc-PAH group, however possibly due to small study numbers, the difference is only statistically significant between SSc-PAH vs. healthy volunteer (HV) groups (SSc-PAH: 6.89 ng/ml, SSc-no PAH: 6.2 ng/ml, HV: 5.42 ng/ml; SSc-PAH vs. SSc-no PAH $p = 0.2447$, SSc-PAH vs. HV $p = 0.0006$, SSc-no PAH vs. HV $p = 0.057$). There was no correlation noted between Eng concentration and echocardiographic measurements of PH (15).

There is some evidence for altered Eng expression in PAH, however in SSc specifically, this evidence is weak in part due to small study sizes and study design. Given the potential role of Eng in TGF-ß signaling, a role in the pathogenesis of PAH

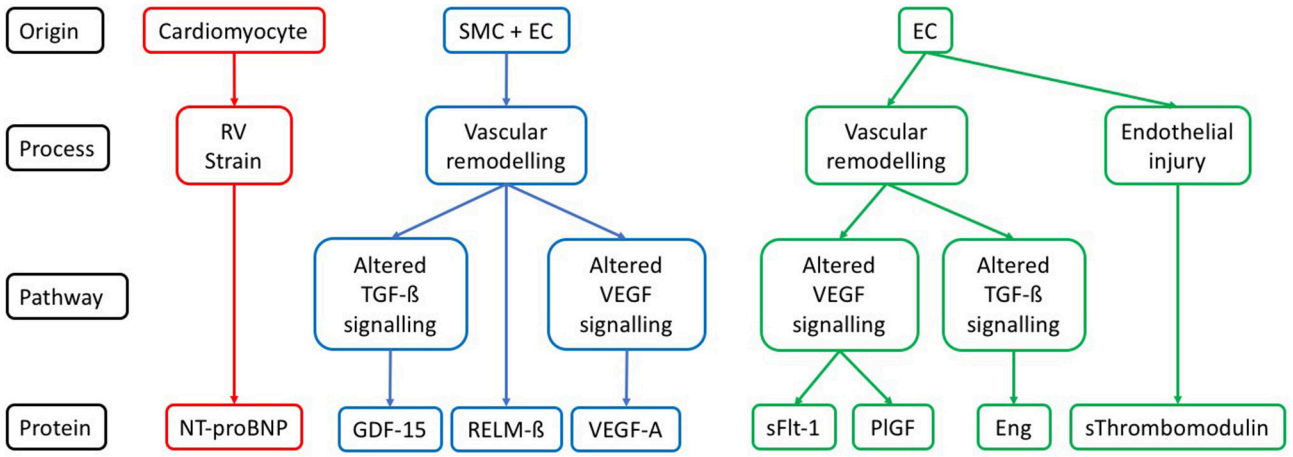


FIGURE 1 | Cellular origin and pathways for each protein described in the context of SSc-PAH**.** Description of the likely origin of each protein, along with the pathophysiological process it has a role in. If a component of one of the pathways known to be relevant to pathogenesis of PAH then this is also given. SMC, vascular smooth muscle cell; EC, vascular endothelial cell; RV, right ventricle; TGF-ß, transforming growth factor beta; VEGF, vascular endothelial growth factor; NT-proBNP, N-terminal pro-brain natriuretic peptide; GDF-15, growth differentiation factor-15; RELM-ß, resistin-like molecule beta; VEGF-A, vascular endothelial growth factor A; sFLT-1, soluble vascular endothelial growth factor receptor 1; PlGF, placenta growth factor; Eng, endoglin; sThrombomodulin, soluble thrombomodulin.

remains reasonable, however further work in this area is needed to establish its role.

VEGF-A

Vascular Endothelial Growth Factor-A (VEGF-A) is a member of the PDGF superfamily of growth factors. It is one of the most potent regulators of angiogenesis, and acts on vascular endothelial cells through stimulation of KDR (VEGF receptor 2) and FLT-1 (VEGF receptor 1) to promote angiogenesis, increase vascular permeability and stimulate endothelial cell migration (35, 36).

Serum VEGF-A concentrations are known to be elevated in patients with PAH and have been demonstrated within plexiform lesions of remodeled vasculature (17, 37).

In a study including 53 participants (SSc-PAH $n = 20$, SSc-no PAH $n = 20$, HV $n = 13$) Papaioannou et al. examined the relationship of serum VEGF-A concentration to echocardiographic markers of pulmonary hypertension. In this study, participants were treatment naive, and any patients with pulmonary fibrosis were excluded. Estimated sPAP >35 mmHg was used to define patients with SSc-PAH. The authors found significantly higher VEGF-A concentrations in all patients with SSc as compared to HV (267 pg/ml vs. 192 pg/ml respectively, $p < 0.01$), and further found that those with SSc-PAH had higher levels than those with SSc-no PAH (352 pg/ml vs. 240 pg/ml respectively, $p < 0.01$). In patients with SSc, significant correlations were found between serum VEGF-A concentration and systolic pulmonary arterial pressure (sPAP) ($r = 0.58$, $p < 0.01$); MRC dyspnoea score ($r = 0.34$, $p = 0.031$); and DLco ($r = -0.47$, $p < 0.01$). In multivariate modeling of sPAP as the dependent variable, VEGF-A concentration remained a significant predictor when adjusted for age and gender (17).

VEGF-A expression is known to be upregulated in both patients PAH, and with systemic sclerosis, both conditions characterized by pathologically excessive endothelial activation. In patients with SSc-PAH the VEGF pathway is upregulated, however baseline levels have not been assessed for utility as diagnostic biomarkers.

Placenta Growth Factor (PlGF) and Soluble Vascular Endothelial Growth Factor (VEGF) Receptor 1 (sFLT-1)

Placenta growth factor is a member of the vascular endothelial growth factor family of proteins which binds with high affinity for VEGF receptor 1 (FLT-1/ VEGF-R1), but not for VEGF receptor 2 (KDR/ VEGFR2)—regarded as the main effector protein of VEGF signaling (38). PlGF alone does not stimulate tyrosine kinase phosphorylation or proliferation in human endothelial cell lines, however the addition of PlGF potentiates the effect of VEGF-A in stimulating proliferation of cultured endothelial cells (38).

sFLT-1 is a variant of VEGF receptor 1 (FLT-1) which can bind VEGF-A, VEGF-B, and Placenta growth factor (PlGF). It functions as a decoy receptor, downregulating free ligand and therefore thought to control excessive endothelial activity (39).

Recognizing the need for further study into diagnostic biomarkers for patients with SSc-PAH, McMahan et al. designed a case-control study of 77 patients with SSc (37 with PH, 40 without PH). The groups were unbalanced for age (64.9 vs. 55.9 respectively, $p < 0.01$) and lung volumes (FVC% 67.5 vs. 88.1 respectively, $p < 0.01$). Diagnosis of PH was based on mPAP $\geq$ 25 mmHg at right heart catheterization. The authors report that both PlGF (24.8 pg/ml vs. 19.1 pg/ml, $p = 0.02$) and sFLT-1 (101.8 pg/ml vs. 89.7 pg/ml, $p = 0.02$) are significantly upregulated in patients with PH that in those without. Both proteins were significantly inversely correlated to DLco (PlGF: $r = -0.31$,

$p = 0.01$ and sFLT-1: $r = -0.29$, $p = 0.01$). sFLT-1 was also correlated to RVSP ($r = 0.32$, $p = 0.01$) (16).

This study was designed to evaluate potential biomarkers of pulmonary hypertension in systemic sclerosis. No comment is made about extent of pulmonary fibrosis, so it remains conceivable that these are imbalanced given the difference in baseline pulmonary function tests, and it is not clear why no comparisons were given for protein concentration and invasive right heart catheter measurements. Although protein concentration changes are noted, no statistics have been given for the performance of these proteins as diagnostic markers.

GDF-15

Growth Differentiation Factor-15 (GDF-15) is a member of the TGF-ß superfamily of cytokines playing an important role in cell growth and differentiation. It is a stress responsive cytokine associated with tissue damage and inflammation. Increased levels have been reported in heart failure, atherosclerosis, endothelial dysfunction and diabetes and have been linked to disease progression and prognosis (40).

In treatment naïve IPAH, serum GDF-15 is increased and is a significant predictor of survival (41). In a mixed cohort of PAH patients, tissue levels of GDF-15 are increased—localizing to the pulmonary endothelium, and in remodeled vessels strong signals are identified in plexiform lesions (42). *In-vitro* studies using pulmonary endothelial cells and varying concentrations of GDF-15 resulted in reduction in hypoxia induced apoptosis suggesting a potential pathological mechanism in PAH (42).

Meadows et al. studied a cohort of 111 patients (SSc-PAH $n = 30$, SSc-no PAH $n = 24$, IPAH $n = 44$, HV $n = 13$) for circulating GDF-15 concentrations. PH was defined at right heart catheterization. Patients with PAH were already established on PH specific therapy at the time of entry to study. Both plasma and tissue levels of GDF-15 were elevated in SSc-PAH (442 pg/ml), and differentiated it from SSc without PAH (108 pg/ml, $p = 0.0004$), IPAH (173 pg/ml, $p = 0.0003$) and HV (66 pg/ml, $p = 0.0013$). Within the SSc subgroup, GDF-15 levels correlated with echocardiographic RVSP ($r = 0.556$, $p < 0.001$), and with NT-proBNP concentration ($r = 0.484$, $p < 0.001$), but not with other invasive hemodynamics. On diagnostic ROC analysis, GDF-15 has been shown to have good discriminative power with area under curve (AUC) 0.91 for differentiation of SSc-PAH from SSc without PH with an optimal threshold for GDF-15 of 125 pg/ml demonstrating 93% sensitivity and 88% specificity for the presence of SSc-PAH. Furthermore, patients below this threshold were found to have significantly improved survival (18).

Resistin-Like Molecule-ß (RELM-ß)

RELM-ß is a member of a relatively newly described resistin family. Largely studied through their effects on animal models, these proteins have been shown to induce angiogenesis and vascular remodeling (19).

Following the identification that hypoxia induced mitogenic factor(HIMF) is upregulated in animal models of PH, Angelini et al. sought to evaluate this in human tissues. In a small study involving 26 prevalent patients (SSc-PAH $n = 9$, IPAH $n = 11$, HV $n = 6$), the authors found that in human lung tissue samples, RELM-ß (a close human homolog to HIMF) is upregulated in patients with SSc-PAH as compared to healthy control ($p < 0.01$, measured by relative intensity on western blot) and localizes to remodeled vasculature. In comparison, although some expression of RELM-ß was noted in remodeled vessels of patients with IPAH, this was inconsistent, and relative quantification showed no difference between IPAH and HV concentrations. Additional *in-vitro* study showed mitogenic activity of RELM-ß on both human lung microvascular endothelial cells and human pulmonary artery smooth muscle cells (19).

This is a relatively novel candidate protein, which appears to show higher expression in SSc-PAH, however more work is needed to assess its concentration in the circulating compartment if it is to be considered further as a biomarker as lung tissue samples are not practical for this purpose.

Soluble Thrombomodulin (sThrombomodulin)

Thrombomodulin is a glycoprotein expressed on endothelial cells. Its physiological function is to bind thrombin and alter its activity, to subsequently activate protein C (15). The pathogenesis of both systemic sclerosis and PAH involves and injury to and activation of the vascular endothelium. Soluble thrombomodulin is increased in conditions associated with endothelial damage (43).

Stratton et al. studied 92 patients (SSc-PAH $n = 34$, SSc-no PAH $n = 38$, HV $n = 20$) and found that sThrombomodulin was increased plasma of patients with SSc-PAH (65.4 ng/ml) compared to SSc without PH (43.3 ng/ml, $p < 0.05$), and healthy controls (38.1 ng/ml, $p < 0.05$). There was no difference in circulating concentration between SSc without PH and healthy control (20). This is in contrast to previous studies which have shown a significant decrease in circulating sThrombomodulin concentration in patients with PAH (IPAH and PAH due to Eisenmenger's' syndrome) compared to healthy controls (26 vs. 44 ng/ml respectively, $p = 0.0001$) (44).

DISCUSSION

A biomarker has been defined by the NIH as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." Strimbu, et al. (45) NT-proBNP is the most widely studied circulating biomarker in clinical use in patients with suspected or known PAH. Elevations in NT-proBNP result from right ventricular (RV) strain as a result of increased RV afterload. As it does not reflect the underlying pathophysiology of the pulmonary arterial vasculopathy resulting in increased RV afterload in PAH, NT-proBNP levels can be elevated due to other pathophysiological processes including increased RV afterload due to PH arising from left heart disease and from disease processes directly affecting the myocardium. As such, the specificity of NT-proBNP in the diagnosis of SSc-PAH tends to be rather low resulting in a significant number of RHCs being performed in patients who do not in the end

have PAH (10). Furthermore, given the dismal prognosis in SSc-PAH, identifying patients early in their disease process before the development of RV strain is desirable (46, 47). The identification of a biomarker or panel of biomarkers which more reflect the underlying pulmonary vasculopathy in SSc-PAH prior to the development of RV strain is therefore of interest.

The data described in the current manuscript summaries the current evidence for various candidate circulating diagnostic biomarkers for SSc-PAH, several of which do relate known pathways known to be important in PAH pathogenesis, especially the TGF-ß and VEGF pathways. Further study within well phenotyped cohorts of patients to compare the performance of these candidate circulating biomarkers against NT-proBNP and the DETECT protocol are clearly warranted.

AUTHOR CONTRIBUTIONS

PH wrote and reviewed manuscript. AL supervised, and reviewed manuscript making comments and alterations. RC supervised writing and manuscript, reviewed and made significant alterations and changes.

FUNDING

PH is a Donald Heath Clinical Research Training Fellow funded jointly by The University of Sheffield, Sheffield Teaching Hospitals Foundation Trust and Actelion Pharmaceuticals UK Ltd. AL is a British Heart Foundation Senior Basic Science Research Fellow (FS/13/48/30453).

REFERENCES

1. Tyndall AJ, Bannert B, Vonk M, Airò P, Cozzi F, Carreira PE, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis.* (2010) **69**:1809–15. doi: 10.1136/ard.2009.114264
2. Hachulla E, Gressin V, Guillevin L, Carpentier P, Diot E, Sibilia J, et al. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthritis Rheum.* (2005) **52**:3792–800. doi: 10.1002/art.21433
3. Mukerjee D, St George D, Coleiro B, Knight C, Denton CP, Davar J, et al. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hypertension: application of a registry approach. *Ann Rheum Dis.* (2003) **62**:1088–93. doi: 10.1136/ard.62.11.1088
4. Lefèvre G, Dauchet L, Hachulla E, Montani D, Sobanski V, Lambert M, et al. Survival and prognostic factors in systemic sclerosis–associated pulmonary hypertension: a systematic review and meta–analysis. *Arthritis Rheum.* (2013) **65**:2412–23.doi: 10.1002/art.38029
5. 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) **34**:371–9. doi: 10.1183/09031936.00106008
6. Ramjug S, Hussain N, Hurdman J, Billings C, Charalampopoulos A, Elliot CA, et al. Idiopathic and systemic sclerosis-associated pulmonary arterial hypertension: a comparison of demographic, hemodynamic, and mri characteristics and outcomes. *Chest* (2017) **152**:92–102. doi: 10.1016/j.chest.2017.02.010
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) **192**:1111–7. doi: 10.1164/rccm.201507-1456OC
8. Humbert M, Gerry Coghlan J, Khanna D. Early detection and management of pulmonary arterial hypertension. *Eur Respir J.* (2012) **21**:306–12. doi: 10.1183/09059180.00005112
9. Lau EMT, Humbert M, Celermajer DS. Early detection of pulmonary arterial hypertension. *Nat Rev Cardiol.* (2015) **12**:143–55. doi: 10.1038/nrcardio.2014.191
10. Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D, et al. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis.* (2014) **73**:1340–9. doi: 10.1136/annrheumdis-2013-203301
11. Vandecasteele E, Drieghe B, Melsens K, Thevissen K, De Pauw M, Deschepper E, et al. Screening for pulmonary arterial hypertension in an unselected prospective systemic sclerosis cohort. *Eur Respir J.* (2017) **49**:1602275. doi: 10.1183/13993003.02275-2016
12. Hoeper MM, Kramer T, Pan Z, Eichstaedt CA, Spiesshoefer J, Benjamin N, et al. Mortality in pulmonary arterial hypertension: prediction by the 2015 European pulmonary hypertension guidelines risk stratification model. *Eur Respir J.* (2017) **50**:1700740. doi: 10.1183/13993003.00740-2017
13. Chung L, Fairchild RM, Furst DE, Li S, Alkassab F, Bolster MB, et al., (2017). Utility of B-type natriuretic peptides in the assessment of patients with systemic sclerosis-associated pulmonary hypertension in the PHAROS registry. *Clin Exp Rheumatol.* **35**(Suppl. 106):106–13.
14. Mathai SC, Bueso M, Hummers LK, Boyce D, Lechtzin N, Le Pavec J, et al. Disproportionate elevation of N-terminal pro-brain natriuretic peptide in scleroderma-related pulmonary hypertension. *Eur Respir J.* (2010) **35**:95–104. doi: 10.1183/09031936.00074309
15. Coral-Alvarado PX, Garces MF, Caminos JE, Iglesias-Gamarra A, Restrepo JF, Quintana G. Serum endoglin levels in patients suffering from systemic sclerosis and elevated systolic pulmonary arterial pressure. *Int J Rheumatol.* (2010) **2010**:969383. doi: 10.1155/2010/969383
16. McMahan Z, Schoenhoff F, Van Eyk J. E, Wigley F. M, Hummers L. K (2015). Biomarkers of pulmonary hypertension in patients with scleroderma: a case-control study. *Arthritis Res Ther.* **17**:201. doi: 10.1186/s13075-015-0712-4
17. 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) **9**:18. doi: 10.1186/1471-2466-9-18
18. Meadows CA, Risbano MG, Zhang L, Geraci MW, Tuder RM, Collier DH, et al. Increased expression of growth differentiation factor-15 in systemic sclerosis-associated pulmonary arterial hypertension. *Chest* (2011) **139**:994–1002. doi: 10.1378/chest.10-0302
19. Angelini DJ, Su Q, Yamaji-Kegan K, Fan C, Teng X, Hassoun PM, et al. Resistin-like molecule-beta in scleroderma-associated pulmonary hypertension. *Am J Respir Cell Mol. Biol.* (2009) **41**:553–61. doi: 10.1165/rcmb.2008-0271OC
20. Stratton RJ, Pompon L, Coghlan JG, Pearson JD, Black CM. (2000). Soluble thrombomodulin concentration is raised in scleroderma associated pulmonary hypertension. *Ann Rheum Dis.* (1997) **59**:132–4. doi: 10.1136/ard.59.2.132
21. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A et al. *2015 ESC/ERS Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension: the Joint Task Force for the Diagnosis and Treatment of Pulmonary.* (2015). Available online at: https://academic.oup.com/eurheartj/article-abstract/37/1/67/2887599
22. Warwick G, Thomas PS, Yates DH. Biomarkers in pulmonary hypertension. *Eur Respir J.* (2008) **32**:503–12. doi: 10.1183/09031936.00160307
23. Leuchte HH, Holzapfel M, Baumgartner RA, Ding I, Neurohr C, Vogeser M, et al. Clinical significance of brain natriuretic peptide in primary pulmonary hypertension. *J Am Coll Cardiol* (2004) **43**:764–70. doi: 10.1016/j.jacc.2003.09.051
24. Fijalkowska A, Kurzyna M, Torbicki A, Szewczyk G, Florczyk M, Pruszczyk P, et al. Serum N-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. *Chest* (2006) **129**:1313–21. doi: 10.1378/chest.129.5.1313

25. Avouac J, Meune C, Chenevier-Gobeaux C, Borderie D, Lefevre G, Kahan A, et al. Cardiac biomarkers in systemic sclerosis: contribution of high-sensitivity cardiac troponin in addition to N-terminal pro-brain natriuretic peptide. *Arthritis Care Res.* (2015) **67**:1022–30. doi: 10.1002/acr.22547
26. Machado RD, Southgate L, Eichstaedt CA, Aldred MA, Austin ED, Best DH, et al. Pulmonary arterial hypertension: a current perspective on established and emerging molecular genetic defects. *Hum Mutat.* (2015) **36**:1113–27. doi: 10.1002/humu.22904
27. Williams MH, Handler CE, Akram R, Smith CJ, Das C, Smee J, et al. Role of N-terminal brain natriuretic peptide (N-TproBNP) in scleroderma-associated pulmonary arterial hypertension. *Eur Heart J.* (2006) **27**:1485–94. doi: 10.1093/eurheartj/ehi891
28. Nagaya N, Nishikimi T, Uematsu M, Satoh T, Kyotani S, Sakamaki F, et al. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation* (2000) **102**:865–70. doi: 10.1161/01.CIR.102.8.865
29. Andreassen AK, Wergeland R, Simonsen S, Geiran O, Guevara C, Ueland T. N-terminal pro-B-type natriuretic peptide as an indicator of disease severity in a heterogeneous group of patients with chronic precapillary pulmonary hypertension. *Am J Cardiol.* (2006) **98**:525–9. doi: 10.1016/j.amjcard.2006.02.061
30. Nickel N, Golpon H, Greer M, Knudsen L, Olsson K, Westerkamp V, et al. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. *Eur Respir J.* (2012) **39**:589–96. doi: 10.1183/09031936.00092311
31. Kylhammar D, Kjellström B, Hjalmarsson C, Jansson K, Nisell M, Söderberg S, et al. A comprehensive risk stratification at early follow-up determines prognosis in pulmonary arterial hypertension. *Eur Heart J.* (2017). doi: 10.1093/eurheartj/ehx257
32. Boucly A, Weatherald J, Savale L, Jaïs X, Cottin V, Prevot G, et al. Risk assessment, prognosis and guideline implementation in pulmonary arterial hypertension. *Eur Respir J.* (2017) **50**:1700889. doi: 10.1183/13993003.00889-2017
33. Conley BA, Smith JD, Guerrero-Esteo M, Bernabeu C, Vary CP. Endoglin, a TGF-β receptor-associated protein, is expressed by smooth muscle cells in human atherosclerotic plaques. *Atherosclerosis* (2000) **153**:323–35. doi: 10.1016/S0021-9150(00)00422-6
34. Gore B, Izikki M, Mercier O, Dewachter L, Fadel E, Humbert M et al. Key role of the endothelial TGF-β/ALK1/endoglin signaling pathway in humans and rodents pulmonary hypertension. *PLoS ONE* (2014) **9**:e100310. doi: 10.1371/journal.pone.0100310
35. Shibuya M. (2011). Vascular Endothelial Growth Factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. *Genes Cancer* (2010) **2**:1097–105. doi: 10.1177/1947601911423031
36. Voelkel NF, Gomez-Arroyo J. The role of vascular endothelial growth factor in pulmonary arterial hypertension. *Am J Respir Cell Mol Biol.* (2014) **51**:474–84. doi: 10.1165/rcmb.2014-0045TR
37. Eddahibi S, Humbert M, Sediame S, Chouaid C, Partovian C, Maître B et al., (2000). Imbalance between platelet vascular endothelial growth factor and platelet-derived growth factor in pulmonary hypertension. Effect of prostacyclin therapy. *Am J Respir Crit Care Med.* **162**(4 Pt 1):1493–9. doi: 10.1164/ajrccm.162.4.2003124
38. Park JE, Chen HH, Winer J, Houck KA, Ferrara N. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, *in vitro* and *in vivo*, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J Biol Chem.* (1994) **269**:25646–54.
39. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol.* (2006) **7**:359–71. doi: 10.1038/nrm1911
40. Adela R, Banerjee SK. GDF-15 as a target and biomarker for diabetes and cardiovascular diseases: a translational prospective. *J Diabetes Res.* (2015) **2015**:490842. doi: 10.1155/2015/490842
41. Nickel N, Kempf T, Tapken H, Tongers J, Laenger F, Lehmann U, et al. Growth differentiation factor-15 in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2008) **178**:534–41. doi: 10.1164/rccm.200802-235OC
42. Nickel N, Jonigk D, Kempf T, Bockmeyer CL, Maegel L, Rische J, et al. GDF-15 is abundantly expressed in plexiform lesions in patients with pulmonary arterial hypertension and affects proliferation and apoptosis of pulmonary endothelial cells. *Respir Res.* (2011) **12**:62. doi: 10.1186/1465-9921-12-62
43. Mercié, P., Seigneur M, Constans J, Boisseau M, Conri C. Assay of plasma thrombomodulin in systemic diseases. *La Revue de*. (1997) **18**:126–31.
44. Cacoub P, Karmochkine M, Dorent R, Nataf P, Piette JC, Godeau P, et al. Plasma levels of thrombomodulin in pulmonary hypertension. *Am J Med.* (1996) **101**:160–4. doi: 10.1016/S0002-9343(96)80070-2
45. Strimbu K, Tavel JA. What are biomarkers? *Curr Opin HIV AIDS* (2010) **5**:463–6. doi: 10.1097/COH.0b013e32833ed177
46. Coghlan JG, Wolf M, Distler O, Denton CP, Doelberg M, Harutyunova S, et al. Incidence of pulmonary hypertension and determining factors in patients with systemic sclerosis. *Eur Respir J.* (2018) **51**:1701197. doi: 10.1183/13993003.01197-2017
47. Condliffe R, Kovacs G. Identifying early pulmonary arterial hypertension in patients with systemic sclerosis. *Eur Respir J.* (2018) **51**:1800495. doi: 10.1183/13993003.00495-2018

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 08 June 2018
doi: 10.3389/fcvm.2018.00053

Pulmonary Artery Size in Interstitial Lung Disease and Pulmonary Hypertension: Association with Interstitial Lung Disease Severity and Diagnostic Utility

*Matthew Chin [1], Christopher Johns [1], Benjamin J. Currie [1], Nicholas Weatherley [1], Catherine Hill [2], Charlie Elliot [2], Smitha Rajaram [2], Jim M. Wild [1], Robin Condliffe [2], Stephen Bianchi [2], David G. Kiely [2] and Andrew J. Swift [1,3]**

[1] Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Royal Hallamshire Hospital, Sheffield, United Kingdom, [2] Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom, [3] Institute for in silico Medicine, Sheffield, United Kingdom

OPEN ACCESS

Edited by:
Sebastian Kelle,
Deutsches Herzzentrum Berlin,
Germany

Reviewed by:
John Hoe,
MediRad Associates ltd, Singapore
Maria Aurora Morales,
Consiglio Nazionale Delle Ricerche
(CNR), Italy

***Correspondence:**
Andrew J. Swift
a.j.swift@sheffield.ac.uk

Specialty section:
This article was submitted to
Cardiovascular Imaging,
a section of the journal
Frontiers in Cardiovascular Medicine

Received: 31 January 2018
Accepted: 09 May 2018
Published: 08 June 2018

Citation:
Chin M, Johns C, Currie BJ,
Weatherley N, Hill C, Elliot C,
Rajaram S, Wild JM, Condliffe R,
Bianchi S, Kiely DG and Swift AJ
(2018) Pulmonary Artery Size in
Interstitial Lung Disease and
Pulmonary Hypertension: Association
with Interstitial Lung Disease Severity
and Diagnostic Utility.
Front. Cardiovasc. Med. 5:53.
doi: 10.3389/fcvm.2018.00053

Purpose: It is postulated that ILD causes PA dilatation independent of the presence of pulmonary hypertension (PH), so the use of PA size to screen for PH is not recommended. The aims of this study were to investigate the association of PA size with the presence and severity of ILD and to assess the diagnostic accuracy of PA size for detecting PH.

Methods: Incident patients referred to a tertiary PH centre underwent baseline thoracic CT, MRI and right heart catheterisation (RHC). Pulmonary artery diameter was measured on CT pulmonary angiography and pulmonary arterial areas on MRI. A thoracic radiologist scored the severity of ILD on CT from 0 to 4, 0 = absent, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% extent of involvement. Receiver operating characteristic analysis and linear regression were employed to assess diagnostic accuracy and independent associations of PA size.

Results: 110 had suspected PH due to ILD (age 65 years (SD 13), M:F 37:73) and 379 had suspected PH without ILD (age 64 years (SD 13), M:F 161:218). CT derived main PA diameter was accurate for detection of PH in patients both with and without ILD - AUC 0.873, $p =< 0.001$, and AUC 0.835, $p =< 0.001$, respectively, as was MRI diastolic PA area, AUC 0.897, $p =< 0.001$, and AUC 0.857, $p =< 0.001$, respectively Significant correlations were identified between mean pulmonary arterial pressure (mPAP) and PA diameter in ILD ($r = 0.608$, $p < 0.001$), and non-ILD cohort ($r = 0.426$, $p < 0.001$). PA size was independently associated with mPAP ($p < 0.001$) and BSA ($p = 0.001$), but not with forced vital capacity % predicted ($p = 0.597$), Transfer factor of the lungs for carbon monoxide (T_{LCO}) % predicted ($p = 0.321$) or the presence of ILD on CT ($p = 0.905$). The severity of ILD was not associated with pulmonary artery dilatation ($r = 0.071$, $p = 0.459$).

Conclusions: Pulmonary arterial pressure elevation leads to pulmonary arterial dilation, which is not independently influenced by the presence or severity of ILD measured by FVC, T_{LCO},

Abbreviations: AA, Ascending Aorta; ILD, Interstitial Lung Disease; mPAP, mean Pulmonary Artery Pressure; NSIP, Non-specific Interstitial Pneumonia; PA, Pulmonary Artery; UIP, Usual Interstitial Pneumonia.

or disease severity on CT. Pulmonary arterial diameter has diagnostic value in patients with or without ILD and suspected PH.

Keywords: interstitial lung disease, computed tomography (CT) scanning, right heart catheterisation, pulmonary artery diameter, pulmonary hypertension

INTRODUCTION

Pulmonary hypertension (PH) is defined on right heart catheterisation (RHC), as a resting mean pulmonary artery pressure (mPAP) greater than or equal to 25 mmHg (1, 2). PH commonly complicates lung disease and chronic hypoxia, such as interstitial lung disease (ILD). When present in lung disease, PH is associated with a poor outcome (3).

CT is used to diagnose and phenotype suspected ILD, and is often part of the workup of patients with unexplained breathlessness and suspected PH (4). Dilatation of the main pulmonary artery (PA) or major branch vessels has been identified as markers of the presence of PH and is often the first imaging finding to suggest the diagnosis (5–9). As CT is commonly used in the investigation of patients with ILD, it would be useful to use the pulmonary arterial size to screen for the presence of pulmonary hypertension. Routine CT pulmonary angiography is performed without ECG gating. Pulmonary arterial size changes during the cardiac cycle. MRI is typically gated to the cardiac cycle and allows assessment of pulmonary arterial size at both systole and diastole. Some authors have suggested that in the presence of established lung fibrosis, the main PA diameter is not accurate for estimation of mean pulmonary arterial pressure as dilatation of the main PA develops in patients with pulmonary fibrosis in the absence of PH (5, 10).

The aim of this study was to investigate the role of main pulmonary arterial size in patients with ILD and suspected pulmonary hypertension. Firstly, by investigating the factors associated with main pulmonary arterial dilatation, including markers of disease severity in interstitial lung disease. Secondly, to compare the diagnostic accuracy of PA size in patients with suspected pulmonary hypertension with and without ILD.

METHODS

Patients

Consecutive patients who were referred to a pulmonary hypertension centre from 24 April 2012 to 30 March 2016 were identified from the ASPIRE registry (3). Patients with a CT scan within 90 days of MRI and RHC were included. In order to meet inclusion criteria, a diagnostic quality CT pulmonary angiogram (CTPA) with a slice thickness of less than 5 mm was required. Patients underwent systemic evaluation as part of their routine clinical workup, which included clinical review, multi-modality imaging and lung function testing.

The aetiology of pulmonary hypertension was decided at a multi-disciplinary team meeting, based upon review of radiological, RHC and clinical information. The North Sheffield Ethics Committee approved this study and institutional review board approval was attained.

All patients with ILD were assessed for radiological disease pattern on CT. The most common pathological patterns of fibrosis referred to our PH centre are usual interstitial pneumonia (UIP) and non-specific interstitial pneumonia (NSIP). These groups were prospectively separated to examine for differences between these groups.

CT Acquisition

The majority of CTPA scans (76.8%) were conducted at Sheffield's Pulmonary Vascular Disease Unit with a further 114 cases performed at the referring hospitals in Wales or the North of England. All Sheffield CTPA cases were performed on a 64-slice MDCT scanner (light-speed General Electric Medical Systems, Milwaukee, WI), with standard acquisition parameters: 100 mA with automated dose reduction, 120 kV, pitch 1, rotation time 0.5 s and 0.625 mm slice thickness. A 400 × 400 mm field of view was used with an acquisition matrix of 512 × 512. 100 ml of intravenous contrast agent (Ultravist, Bayer, Berlin, Germany) was administered at a rate of 5 ml/sec through a wide bore cannula, into a large central vein, typically in the ante-cubital fossa. The scan was then timed using a bolus tracking technique. HRCT was reconstructed using the contrast-enhanced acquisitions with 1.25 mm collimation from the apex of the lung to the diaphragm. In order to be included, any CTPA from an outside Trust had to be of diagnostic quality, as decided by a radiologist and slice thickness ≤5 mm. In the 23.2% (114) CTPAs performed outside of Sheffield, CT scanner and detailed acquisition parameters were not available for reporting.

MR Acquisition

Cardiac magnetic resonance (CMR) imaging was performed on a 1.5T whole body scanner GE HDx (GE Healthcare, Milwaukee, USA), using an 8-channel cardiac coil. Patients were in the supine position with a surface coil and with retrospective ECG gating. A retrospective cardiac gated multi-slice balanced steady state free precession (bSSFP) sequence was performed orthogonal to the main pulmonary artery. The bSSFP sequence parameters were: TR 2.8 ms, TE 1.0 ms, Flip angle of 50°, FOV = 48 × 43.2, 256 × 256 matrix, 125 kHz bandwidth and slice thickness of 10 mm (11).

Pulmonary Function Testing

All patients underwent lung function testing. Percent of predicted values for forced vital capacity (FVC), and transfer factor of the lungs for carbon monoxide (T_{LCO}) were calculated. The gender, age and physiology (GAP) score is a simple tool for estimating mortality in ILD from demographic and pulmonary function metrics and was calculated as previously described (12).

CT Image Analysis

Image analysis was carried out on picture archive and communication system (PACS) imaging on CE stamped Barco

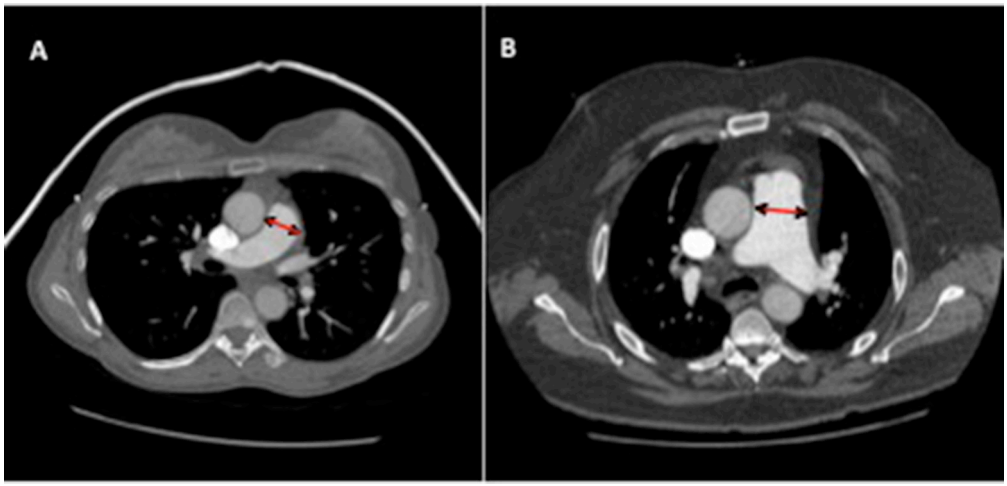


FIGURE 1 | CT images of pulmonary artery diameter measurements in patients with **(A)** a patient without PH (mean pulmonary arterial pressure of 20 mmHg), **(B)** a patient with PH with moderate elevation in pulmonary arterial pressure (mPAP 54 mmHg). Diameter measured where largest and most consistent - proximal to bifurcation, perpendicular to direction of vessel.

diagnostic monitors. The observer was blinded to the all other clinical and imaging data.

The main pulmonary artery was measured proximal to bifurcation, perpendicular to the direction of the vessel at the point where largest diameter is most consistent. The ascending aorta diameter was also measured on the same CT slice, see **Figure 1**. Right and left pulmonary artery diameters were recorded 1 cm from the bifurcation, at their most consistent value. A thoracic radiologist scored the severity of ILD on CT, using the Likert semi-quantitative score, ranging from 0 to 4, 0 = absent, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% involvement (13). The imaging subtype of ILD was also recorded (UIP or NSIP).

MR Image Analysis

Image analysis was performed on a GE Advantage Workstation 4.4 and GE Advantage Workstation Report Card. Scans were defined as non-diagnostic when image quality significantly affected cardiac measurements or volumetric analysis could not be accurately performed. From the magnitude phase imaging images, the maximal (systolic) and minimal (diastolic) PA areas were measured, and relative area change (RAC) was defined by the following equation: RAC = (maximum area-minimum area)/ minimum area (14, 15).

Statistics

The differences in CT and MRI pulmonary arterial size between the patients with and without ILD were analysed using an independent *t*-test for continuous data and the chi-square for categorical data. ANOVA with Bonferroni correction was used for multiple group comparison. Pearson's correlation coefficient was used to assess the correlation between CT and MRI pulmonary arterial measurements and mPAP. Pearson correlations were conducted for cohorts of ILD, non-ILD and radiological subtypes against CT and MRI derived metric variables. Receiver operating characteristic (ROC) analysis was performed to determine diagnostic accuracy of CT and MRI pulmonary arterial size measurements with area under the ROC curve (AUC) results presented. Multiple t-tests were used to assess intergroup variance of PA diameter, PA to Aorta (PA:AA) ratio and PA relative to body mass index (PA index). The relationship between PA diameter with candidate predictors mPAP, ILD severity of CT, FVC T_{LCO}, and age, sex and body surface area (BSA) was assessed using multivariate linear regression analysis.

Statistical analysis was performed in IBM SPSS Statistics 22 (SPSS, Chicago) and graphed in GraphPad Prism (GraphPad, San Diego). A two-tailed *p*-value of <0.05 was considered statically significant.

RESULTS

Patients

From the ASPIRE database 489 patients with suspected PH were identified, including 198 males and 291 females. 420 patients had pulmonary hypertension. The mean age for the whole cohort was 65 (SD - SD 13). One hundred and ten patients had CT features of ILD (101 patients with pulmonary hypertension and 9 patents without), of which 46 patients had UIP and 43 had NSIP radiological subtypes. Chronic EAA (n = 1), asbestosis (n = 1), desquamative interstitial pneumonia (n = 1), chronic extrinsic allergic, alveolitis (n = 1), silicosis (n = 1), Langerhans cell histiocytosis (n = 1), radiotherapy (n = 1) and post infective interstitial disease and scarring (n = 4). 379 patients with suspected PH had no CT evidence of ILD (319 patients with pulmonary hypertension and 60 patients without) as shown in **Figure 2. Table 1** provides the baseline demographic data, lung function, CT and MRI derived variables for the study cohort as a whole and split into the ILD and not ILD cohorts. The mean (SD) interval between CT and RHC was 17.6 days (46.1), the interval between MRI and RHC was 0.2 days (4.2).

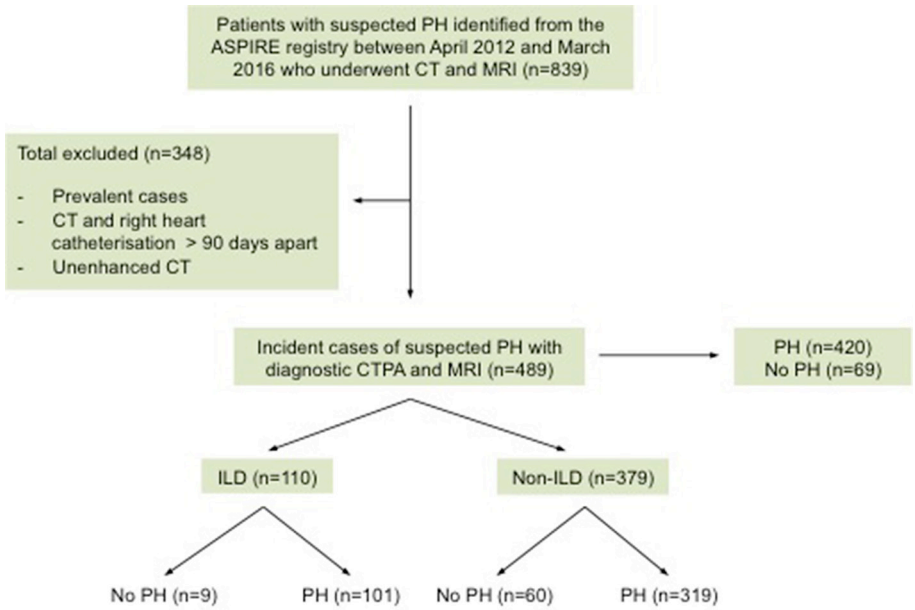


FIGURE 2 | The Study Cohort. PH, pulmonary hypertension; CT, computed tomography scan; MRI, magnetic resonance imaging; MPA, main pulmonary artery; CTPA, computed tomography pulmonary angiogram; ILD, interstitial.

TABLE 1 | Demographic table.

		Whole Cohort	ILD	Non-ILD	*p* Value*
		Mean (SD) *N* = 489	**Mean (SD) *N* = 110**	**Mean (SD) *N* = 379**	
Demographics					
Age		65 (13)	66 (11)	64 (13)	0.656
Sex	M/F	198/291	37/73	161/218	
	PH/No PH	420/69	101/9	319/60	0.003
WHO Functional Class	I/II/III/IV	1/59/378/42	0/9/80/18	1/50/298/24	<0.001
ILD Sub-Types	UIP/NSIP/Sarcoid/Chronic EAA, Other		46/43/11/1/20		
Right Heart Catheter					
	mRAP (mmHg)	10 (6)	9 (6)	10 (5)	0.017
	mPAP (mmHg)	41 (15)	41 (13)	42 (15)	0.856
	PAWP (mmHg)	13 (5)	12 (5)	13 (5)	0.014
	CI (L/min/m2)	2.7 (0.8)	2.7 (0.8)	2.7 (0.8)	0.981
	PVR (dyn.s.m-5)	529.2 (394.3)	539.7 (348.7)	526.1 (408.1)	0.267
Pulmonary function tests					
	Predicted FVC %	81.6	72.7	85.0	<0.001
	FVC (l)	2.9 (1.1)	2.4 (0.9)	3.1 (1.1)	<0.001
	Predicted T_{LCO} %	47.9	33.7	52.3	<0.001
	T_{LCO} (l)	3.9 (2.0)	2.5 (1.6)	4.3 (2.0)	<0.001
CT					
	Main PA diameter (mm)	32 (6)	31 (4.9)	32 (5.8)	0.092
	PA/AA	1 (0.2)	1 (0.2)	1 (0.2)	0.453
	PA/BSA	17.6 (3.5)	18 (3.0)	18 (3.6)	0.434
	Right PA diameter (mm)	26 (5)	25 (4.4)	26 (4.7)	0.234
	Left PA diameter (mm)	24 (4)	24 (3.4)	25 (4.0)	0.309
MRI					
	Systolic PA area (mm^2)	920 (306)	887 (269)	930 (316)	0.607
	Diastolic PA area (mm^2)	833 (276)	807 (255)	814 (281)	0.809
	PA relative area (mm^2)	10.9 (9.5)	10.6 (7.2)	10.9 (9.3)	0.394

WHO, World Health Organisation; mRAP, mean Right Arterial Pressure; mPAP, mean Pulmonary Arterial Pressure; PAWP, Pulmonary Artery Wedge Pressure; CI, Cardiac Index; PVR, Pulmonary Vascular Resistance; TLCO, Transfer Factor of the lungs for Carbon Monoxide; FVC, Forced Volume Capacity.

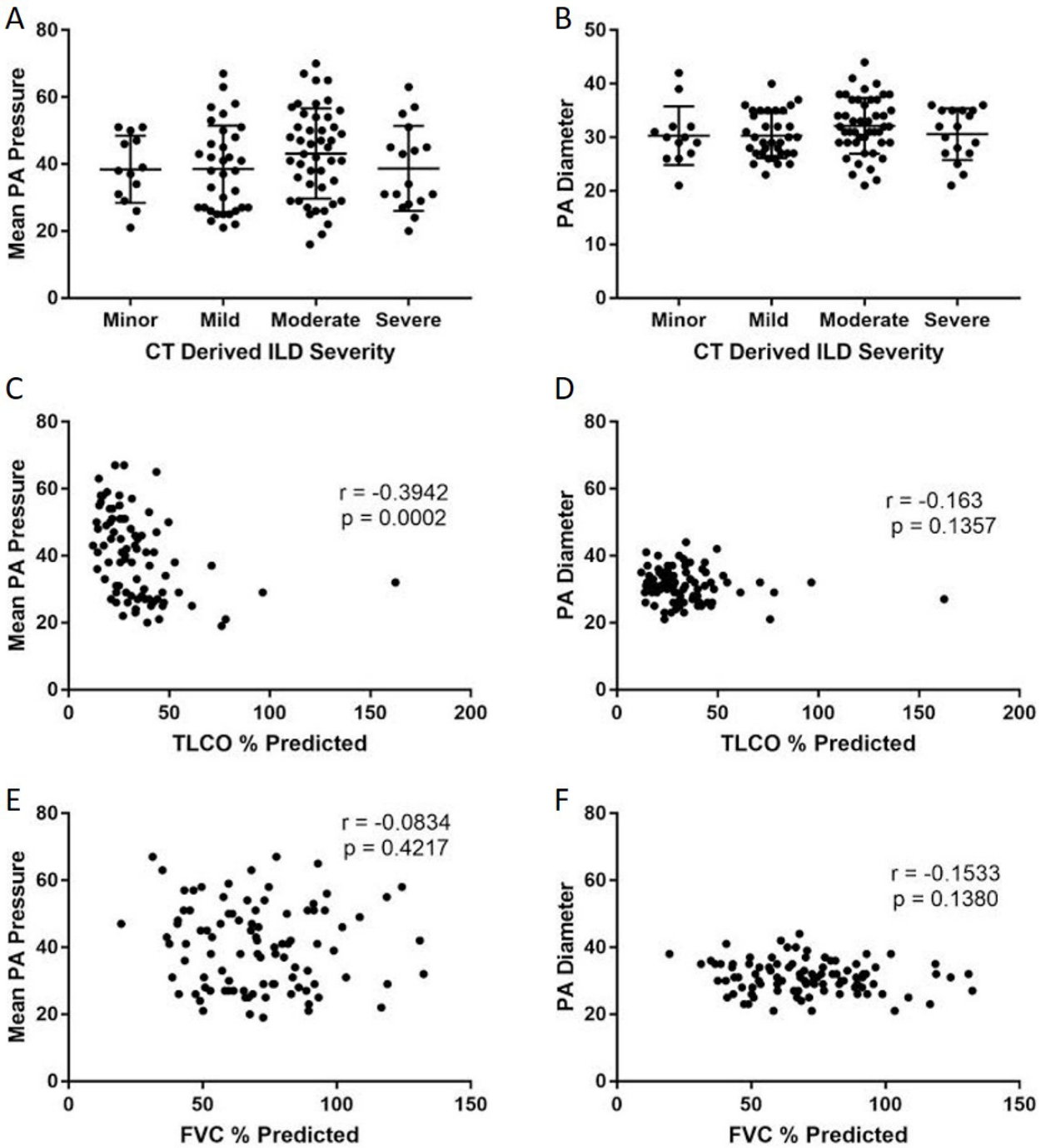


FIGURE 3 | Correlations between mean PA pressure and PA diameter with pulmonary function tests and CT-derived severity score. Graphs **(A)** and **(B)** represent the association with ILD severity on CT in ILD cohort. Graphs **(C)** and **(D)** scatter plot showing the association of PA pressure and diameter with TLCO %predicted and graphs **(E)** and **(F)** scatter plots showing the association of PA pressure and diameter with FVC %predicted.

Group Comparisons

Patients with PH had larger pulmonary arteries (33 mm) than patients without PH (27 mm) ($p < 0.001$) In the ILD cohort, there was no significance between PA size diameters in different semi-quantitative radiological severity scores of ILD, see (**Figure 3A**, mPAP and ILD severity). There was no

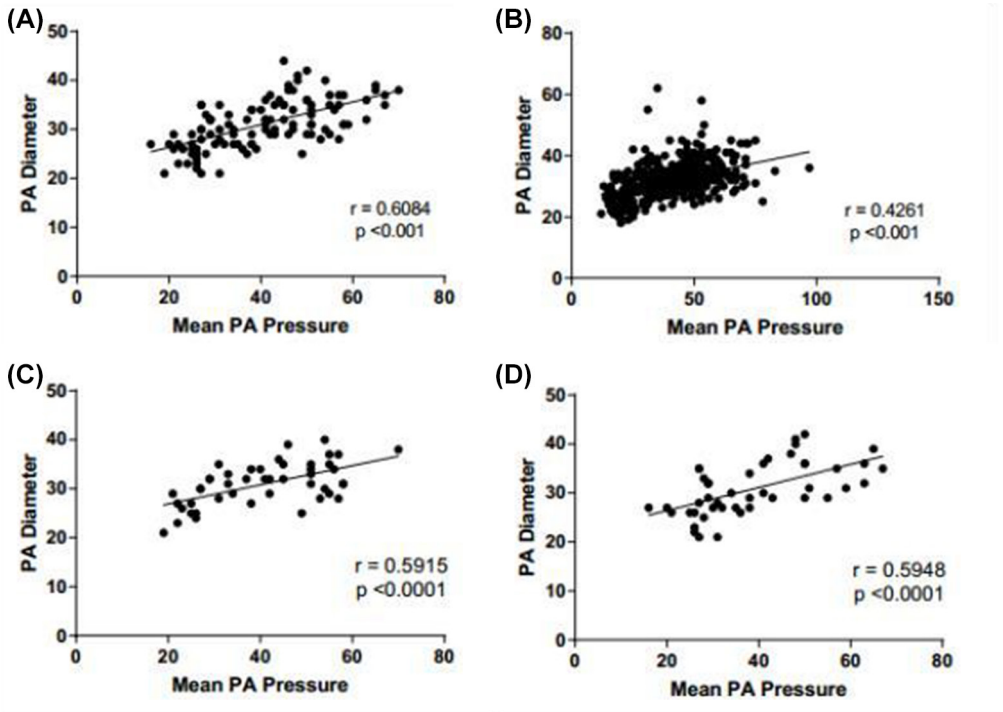


FIGURE 4 | Scatter distribution showing the relationship between mean PA pressure and PA diameter. Subgroups include ILD cohort **(A)**, non-ILD **(B)**, ILD with UIP pattern of disease **(C)** and ILD with NSIP pattern of disease **(D)**.

difference in mPAP between radiological severities of ILD scores (**Figure 3B**, PA diameter and ILD severity). Furthermore there were no significant differences in PA size or diameter between CT derived ILD severity within the pathological subgroups, UIP $p = 0.630$ and NSIP $p = 1.000$.

Correlations

CT derived main PA diameter correlated positively with mPAP in the ILD cohort (r = 0.608; $p < 0.001$), which improved when the pulmonary artery diameter was indexed to body surface area (r = 0.674 ; $p < 0.001$). MRI derived systolic and diastolic pulmonary artery area also correlated significantly with mPAP in the ILD cohort, (r = 0.423 and 0.479 respectively, $p < 0.001$). In the pathological subtypes, PA diameter correlated with mPAP for both UIP (r = 0.592; $p < 0.001$) and NSIP (r = 0.621; $p < 0.001$). Each are shown in **Figure 4.** In the non-ILD cohort, the correlation between mPAP and PA diameter was weaker but still significant, r = 0.426 ($p < 0.001$). **Table 2** presents Pearson correlations for ILD and non-ILD cohorts, with pathological UIP and NSIP subtype analysis. In the ILD cohort, there was

TABLE 2 | Correlations of CT and MRI measurements with mean pulmonary arterial pressure.

Pearson Correlations to mPAP	ILD		UIP		NSIP		Non ILD	
	R Value	*p Value*	*R Value*	*p Value*	*R Value*	*p Value*	*R Value*	*p Value*
CT								
PA Diameter	0.608	<0.001	0.592	<0.001	0.621	<0.001	0.426	<0.001
PA/AA Ratio	0.478	<0.001	0.515	<0.001	0.631	<0.001	0.431	<0.001
PA Index	0.498	<0.001	0.374	0.01	0.674	<0.001	0.402	<0.001
Right PA Diameter	0.466	<0.001	0.396	0.006	0.472	0.002	0.262	<0.001
Left PA Diameter	0.393	<0.001	0.406	0.005	0.656	0.029	0.332	<0.001
MRI								
Systolic PA area	0.423	<0.001	0.461	0.001	0.510	<0.001	0.353	<0.001
Diastolic PA area	0.479	<0.001	0.563	<0.001	0.558	<0.001	0.426	<0.001
PA relative area change	–0.397	<0.001	–0.457	0.001	–0.387	0.010	–0.310	<0.001

AA , Ascending Aorta; ILD , Interstitial Lung Disease; mPAP , mean Pulmonary Artery Pressure; NSIP , Non-specific Interstitial Pneumonia; PA , Pulmonary Artery; UIP , Usual Interstitial Pneumonia.

TABLE 3 | Area under the receiver operating characteristic curve for CT and MRI pulmonary arterial measurements in ILD and non-ILD cohorts.

CT and MRI Metrics	ILD		Non ILD	
	AUC	*p* value	AUC	*p* value
CT				
Main PA Diameter	0.873	<0.001	0.835	<0.001
PA/AA	0.799	0.003	0.794	<0.001
PA Index	0.595	0.347	0.836	<0.001
Right PA Diameter	0.753	0.012	0.757	<0.001
Left PA Diameter	0.729	0.023	0.754	<0.001
MRI				
Systolic PA area	0.887	<0.001	0.824	<0.001
Diastolic PA area	0.897	<0.001	0.857	<0.001
PA relative area change	0.388	0.266	0.365	<0.001

AA , Ascending Aorta; PA , Pulmonary Artery.

TABLE 4 | Linear regression analysis, identifying covariates with independent association with pulmonary arterial diameter.

Model	Regression Co-efficient	SE	R	T Statistic	*p* value
(Constant)	11.060	3.471		3.187	0.002
PA Mean	0.205	0.033	0.525	6.168	<0.001
BSA	6.738	1.914	0.300	3.520	0.001

BSA, Body Surface Area; PA, Pulmonary Artery.

no significant correlation between PA diameter and FVC (r = −0.113; $p = 0.301$) or T_{LCO} (r = −0.041; $p = 0.692$) respectively, as shown in **Figure 3** shows the correlations between mean PA pressure and PA diameter for ILD (r = 0.608; $p < 0.001$), non-ILD (r = 0.461; $p < 0.001$), UIP (r = 0.592; $p < 0.001$), NSIP (r = 0.595; $p < 0.001$).

Diagnostic Accuracy

Table 3 provides the ROC area under the curve (AUC) values for CT and MRI metrics in both ILD and non-ILD cohorts for the diagnosis of PH. In the ILD cohort, main PA diameter on CT had strong diagnostic accuracy, AUC = 0.873 as did systolic and diastolic PA area on MRI (AUC = 0.887 and AUC = 0.897, respectively). In addition, high diagnostic accuracy of PA diameter on CT, AUC 0.835 and PA systolic and diastolic areas (AUC 0.824 and 0.857, respectively) on MRI were found in the non-ILD cohort. The relative area change of the pulmonary artery during the cardiac cycle was a weaker diagnostic marker than pulmonary arterial size.

A previous threshold of 29mm tested in the ILD cohort resulted in 75.2% sensitivity, 88.9% specificity, 98.7% positive predictive values and 24.0% negative predictive value. In the non-ILD cohort, pulmonary arterial diameter was also accurate for the diagnosis of PH, with AUC=0.835 (p<0.001). The optimal threshold identified was 30mm, providing 76.3% sensitivity and 73.3% specificity. **Figure 5** shows the ROC curves for the CTPA measured pulmonary arterial diameter metrics (PA diameter, PA:AA ratio and PA index) in both ILD and non-ILD cohorts.

The highest AUC values for MRI derived variables in the ILD cohort (**Table 2**) were diastolic pulmonary arterial area (0.897) and systolic pulmonary arterial area (0.887). MRI variables have shown a similar diagnostic accuracy within this study cohort compared to CT derived parameters of PA size.

Factors Associated with Pulmonary Arterial Size in ILD

At multivariate linear regression analysis, mPAP and body surface area were independent predictors of pulmonary arterial size, [11.1 + (0.2 × mean pulmonary arterial pressure) + (6.7 × BSA)]. Age, sex, GAP score, ILD severity on CT, FVC %predicted and T_{LCO} %predicted were not multivariate predictors of pulmonary artery diameter (10). **Table 4** shows linear regression analysis.

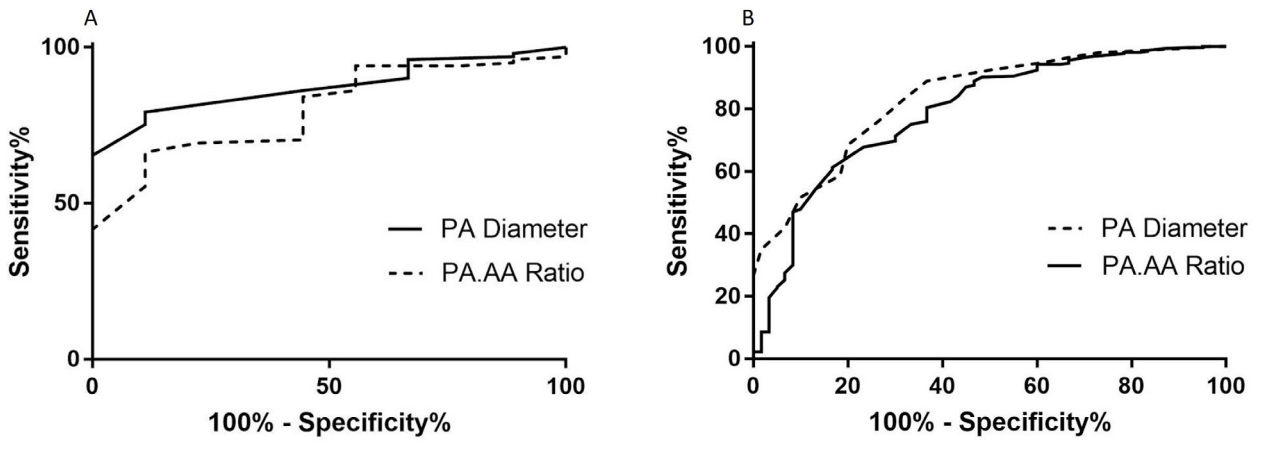


FIGURE 5 | ROC analysis showing the area under the curve for both ILD **(A)** and non-ILD **(B)** cohorts for the CT measured main pulmonary artery size (PA diameter and PA:AA Ratio)

Mean pulmonary arterial diameter had an adjusted correlation coefficient of 0.575 ($p < 0.001$) and BSA of 0.249 ($p = 0.001$).

DISCUSSION

In this retrospective study of 491 patients with suspected pulmonary hypertension referred to a tertiary referral centre, we show that main pulmonary arterial diameter has similar diagnostic utility in patients with and without ILD. In our cohort, pulmonary arterial diameter is independently associated with mean pulmonary arterial pressure and body size. The size of the pulmonary artery was not independently influenced by semi-quantitative radiological severity score of ILD, FVC, T_{LCO}, or GAP score.

It has been postulated that in patients with pulmonary fibrosis the pulmonary artery dilates in the absence of elevated pulmonary arterial pressure, as consequence of architectural distortion of the lung parenchyma. In contrast, in this cohort of patients, we have found that pulmonary arterial diameter is diagnostic of pulmonary hypertension in ILD, to a similar accuracy as seen in patients with pulmonary hypertension who do not have ILD (AUC 0.874 for ILD cohort and AUC 0.835 for non-ILD cohort), irrespective of the underlying radiological ILD pattern. UIP is typified by significant parenchymal distortion, including honeycombing, traction bronchiectasis and volume loss (16), therefore if PA size is driven by architectural destruction in ILD, it should be apparent in this group. However, PA size remains more closely associated with PH metrics than quantitative metrics of ILD severity in patients with UIP. In addition, the optimal pulmonary arterial diameter threshold for prediction of elevated mean pulmonary artery pressure was relatively low in patients with ILD at 29 mm, similar to 30 mm for patients in the non-ILD cohort. Mean pulmonary arterial diameter in our ILD cohort with pulmonary hypertension was 32 mm, compared to a mean of 25 mm in patients with ILD without pulmonary hypertension.

As expected, T_{LCO} and FVC were lower in the patients with ILD than those without. However, the ILD and non-ILD cohorts were well-matched in terms of PH severity, as defined by their RHC metrics. The clinical and radiological markers of ILD severity ($T_{LCO,}$ FVC and CT semi-quantitative severity score) had weak correlations with pulmonary arterial diameter ($r = -0.148$, $p = 0.174$/$r = -0.153$, $p = 0.138$/$r = 0.082$, $p = 0.393$ respectively). Furthermore, there is a strong correlation between PA diameter and mean PA pressure within the ILD cohort ($r = 0.608$). On multivariate linear regression, the clinical and radiological markers of disease severity were not independent predictors of pulmonary arterial size. In fact, the independent predictors were body surface area and pulmonary arterial pressure.

Both CT and MRI derived pulmonary arterial size metrics were assessed. MRI variables showed high diagnostic accuracy with both systolic and diastolic pulmonary arterial area having diagnostic utility in both ILD and non-ILD cohorts. MRI derived diastolic pulmonary arterial area, was the most accurate diagnostic variable across the whole study cohort, regardless of the presence of ILD. Diastolic pulmonary arterial size was of systematically marginally higher diagnostic accuracy than systolic. We postulate that this is because of greater pulsatility of the pulmonary artery of a patient without pulmonary hypertension, allowing the pulmonary artery to reduce in size in diastole to a greater extent relative to a patient with pulmonary hypertension.

PA size measured on MRI also correlated well with RHC derived mean pulmonary arterial pressure and showed similar diagnostic accuracy to CT derived PA diameter. This trend was similar in both patients with and without interstitial lung disease. A limitation of routine CT pulmonary angiography is lack of cardiac gating; MRI with cardiac gating has provided additional proof of the equivalent utility of PA diameter in interstitial lung disease and non- interstitial lung disease

The pulmonary artery can dilate over time in a patient with elevated pulmonary arterial pressure (17). Hence the absolute instantaneous measure of pulmonary arterial pressure in a patient with pulmonary hypertension will be dependent not only on the pressure but also the duration of the disease, which explain the variable correlation observed between pulmonary arterial size and pressure in patients with suspected PH (5–7, 11, 18–20). All patients were referred with suspected pulmonary hypertension, however it is unknown how long the pressure has been elevated, given the insidious onset of the disease, absolute quantification of duration of disease is challenging.

LIMITATIONS

This study is limited by its retrospective design at a single tertiary referral centre, although in order to reduce bias from the retrospective analysis, the cohort is made up of consecutive patients. As this was performed in a pulmonary hypertension referral centre, there is a bias towards the presence of PH and as such there are only a few patients with no PH, so these results are only valid in the setting of a PH referral centre. It is expected, however that the strong correlation between mPAP and PA size in this cohort of patients would be similar in a non-selected patient cohort. A study in a non-PH centre, in a cohort with more even distribution of patients with and without pulmonary hypertension would be of benefit.

Interstitial lung diseases are a heterogeneous group of diseases with idiopathic and known-cause aetiologies. We have grouped the largest cohorts of fibrotic ILDs here to explore if the architectural destruction of UIP had a greater influence on PA dilatation than NSIP, but in practice these are not clinical diagnoses. However, in practice these are the patterns most likely to lead to fibrotic-driven architectural distortion and so we have taken a pragmatic approach in separating these. Further work evaluating the impact of varying forms of ILD, such as scleroderma related and drug induced ILD on the pulmonary vasculature, in terms of PA size and haemodynamic changes would be of value.

CONCLUSION

Pulmonary arterial pressure elevation leads to pulmonary arterial dilation, which is not independently influenced by the presence or severity of ILD measured by FVC, T_{LCO}, or disease severity on CT.

Pulmonary arterial diameter has diagnostic value in patients with or without ILD, key for the screening of suspected PH.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of "North Sheffield Ethics Committee". The protocol was approved by the "Sheffield Hospitals institutional review board".

AUTHOR CONTRIBUTIONS

AS and DK conceived the idea for the study. AS, MC, NW, SB, BC and CJ participated in the study design. AS, DC acquired the MRI data. Image analysis was performed by AS, MC, BC, CJ. AS, MC, SR, CE, CJ, RC, DK, NW, JMW analysed and interpreted the MR data. AS, MC, SR, CE, CJ, NW, RC, DK, JMW, SB drafted the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by NIHR grant NIHR-RP-R3-12-027, Wellcome grant 205188/Z/16/Z and MRC grant MR/M008894/1. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health. D. Capener was part funded by an unrestricted research grant from Bayer.

REFERENCES

1. Kiely DG, Elliot CA, Sabroe I, Condliffe R. Pulmonary hypertension: diagnosis and management. *BMJ* (2013) 346:f2028. doi: 10.1136/bmj.f2028
2. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J* (2016) 37(1):67–119. doi: 10.1093/eurheartj/ehv317
3. Hurdman J, Condliffe R, Elliot CA, Davies C, Hill C, Wild JM, et al. ASPIRE registry: assessing the Spectrum of Pulmonary hypertension Identified at a REferral centre. *Eur Respir J* (2012) 39(4):945–55. doi: 10.1183/09031936.00078411
4. Rajaram S, Swift AJ, Condliffe R, Johns C, Elliot CA, Hill C, et al. CT features of pulmonary arterial hypertension and its major subtypes: a systematic CT evaluation of 292 patients from the ASPIRE Registry. *Thorax* (2015) 70(4):382–7. doi: 10.1136/thoraxjnl-2014-206088
5. Devaraj A, Wells AU, Meister MG, Corte TJ, Hansell DM. The effect of diffuse pulmonary fibrosis on the reliability of CT signs of pulmonary hypertension. *Radiology* (2008) 249(3):1042–9. doi: 10.1148/radiol.2492080269
6. Edwards PD, Bull RK, Coulden R. CT measurement of main pulmonary artery diameter. *Br J Radiol* (1998) 71(850):1018–20. doi: 10.1259/bjr.71.850.10211060
7. Shen Y, Wan C, Tian P, Wu Y, Li X, Yang T, et al. CT-base pulmonary artery measurement in the detection of pulmonary hypertension: a meta-analysis and systematic review. *Medicine* (2014) 93(27):e256. doi: 10.1097/MD.0000000000000256
8. Ng CS, Wells AU, Padley SP. A CT sign of chronic pulmonary arterial hypertension: the ratio of main pulmonary artery to aortic diameter. *J Thorac Imaging* (1999) 14(4):270–8.
9. Kuriyama K, Gamsu G, Stern RG, Cann CE, Herfkens RJ, Brundage BH. CT-determined pulmonary artery diameters in predicting pulmonary hypertension. *Invest Radiol* (1984) 19(1):16–22. doi: 10.1097/00004424-198401000-00005
10. Alhamad EH, Al-Boukai AA, Al-Kassimi FA, Alfaleh HF, Alshamiri MQ, Alzeer AH, et al. Prediction of pulmonary hypertension in patients with or without interstitial lung disease: reliability of CT findings. *Radiology* (2011) 260(3):875–83. doi: 10.1148/radiol.11103532
11. Swift AJ, Rajaram S, Condliffe R, Capener D, Hurdman J, Elliot CA, et al. Diagnostic accuracy of cardiovascular magnetic resonance imaging of right ventricular morphology and function in the assessment of suspected pulmonary hypertension results from the ASPIRE registry. *J Cardiovasc Magn Reson* (2012) 14:40. doi: 10.1186/1532-429X-14-40
12. Ley B, Ryerson CJ, Vittinghoff E, Ryu JH, Tomassetti S, Lee JS, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Ann Intern Med* (2012) 156(10):684–91. doi: 10.7326/0003-4819-156-10-201205150-00004
13. Kazerooni EA, Martinez FJ, Flint A, Jamadar DA, Gross BH, Spizarny DL, et al. Thin-section CT obtained at 10-mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. *AJR Am J Roentgenol* (1997) 169(4):977–83. doi: 10.2214/ajr.169.4.9308447
14. Sanz J, Kariisa M, Dellegrottaglie S, Prat-González S, Garcia MJ, Fuster V, et al. Evaluation of pulmonary artery stiffness in pulmonary hypertension with cardiac magnetic resonance. *JACC Cardiovasc Imaging* (2009) 2(3):286–95. doi: 10.1016/j.jcmg.2008.08.007
15. Toshner MR, Gopalan D, Suntharalingam J, Treacy C, Soon E, Sheares KK, et al. Pulmonary arterial size and response to sildenafil in chronic thromboembolic pulmonary hypertension. *J Heart Lung Transplant* (2010) 29(6):610–5. doi: 10.1016/j.healun.2009.12.014
16. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* (2011) 183(6):788–824. doi: 10.1164/rccm.2009-040GL
17. Boerrigter B, Mauritz GJ, Marcus JT, Helderman F, Postmus PE, Westerhof N, et al. Progressive dilatation of the main pulmonary artery is a characteristic of pulmonary arterial hypertension and is not related to changes in pressure. *Chest* (2010) 138(6):1395–401. doi: 10.1378/chest.10-0363
18. Devaraj A, Hansell DM. Computed tomography signs of pulmonary hypertension: old and new observations. *Clin Radiol* (2009) 64(8):751–60. doi: 10.1016/j.crad.2008.12.005
19. Zisman DA, Karlamangla AS, Ross DJ, Keane MP, Belperio JA, Saggar R, et al. High-resolution chest CT findings do not predict the presence of pulmonary hypertension in advanced idiopathic pulmonary fibrosis. *Chest* (2007) 132(3):773–9. doi: 10.1378/chest.07-0116
20. Devaraj A, Wells AU, Meister MG, Corte TJ, Wort SJ, Hansell DM. Detection of pulmonary hypertension with multidetector CT and echocardiography alone and in combination. *Radiology* (2010) 254(2):609–16. doi: 10.1148/radiol.09090548

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 21 June 2018
doi: 10.3389/fmed.2018.00172

Incremental Shuttle Walking Test Distance Is Reduced in Patients With Pulmonary Hypertension in World Health Organisation Functional Class I

*Catherine G. Billings[1], Robert Lewis[1], Iain J. Armstrong[1], Judith A. Hurdman[1], Ian A. Smith[1], Matthew Austin[1], Charlie A. Elliot[1], Athanasios Charalampopoulos[1], Ian Sabroe[2], Allan Lawrie[2], A. A. Roger Thompson[2], Robin Condliffe[1] and David G. Kiely[1,2,3]**

[1] Sheffield Pulmonary Vascular Disease Unit, Sheffield Teaching Hospitals NHS Foundation Trust, Royal Hallamshire Hospital, Sheffield, United Kingdom, [2] Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Medical School, Sheffield, United Kingdom, [3] Insigneo Institute for in Silico Medicine, University of Sheffield, Sheffield, United Kingdom

OPEN ACCESS

Edited by:
Argyrios Tzouvelekis, Alexander Fleming Biomedical Sciences Research Center, Greece

Reviewed by:
Iraklis M. Tsangaris, National and Kapodistrian University of Athens, Greece
Michael Furian, Klinik Für Pneumologie, Universitätsspital Zürich, Switzerland

***Correspondence:**
David G. Kiely
david.kiely@sth.nhs.uk

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: 01 February 2018
Accepted: 17 May 2018
Published: 21 June 2018

Citation:
Billings CG, Lewis R, Armstrong IJ, Hurdman JA, Smith IA, Austin M, Elliot CA, Charalampopoulos A, Sabroe I, Lawrie A, Thompson AAR, Condliffe R and Kiely DG (2018) Incremental Shuttle Walking Test Distance Is Reduced in Patients With Pulmonary Hypertension in World Health Organisation Functional Class I. Front. Med. 5:172. doi: 10.3389/fmed.2018.00172

Background: There is increasing interest in screening for and diagnosing pulmonary hypertension earlier in the course of disease. However, there is limited data on cardiopulmonary abnormalities in patients with pulmonary hypertension newly diagnosed in World Health Organization Function Class (WHO FC) I.

Methods: Data were retrieved from the ASPIRE registry (Assessing the Spectrum of Pulmonary hypertension Identified at a REferral center) for consecutive treatment naïve patients diagnosed with pulmonary hypertension by cardiac catheterization between 2001 and 2010 who underwent incremental shuttle walk exercise testing.

Results: Eight hundred and ninety-five patients were diagnosed with Group 1-5 pulmonary hypertension. Despite the absence of symptoms, patients in WHO FC I ($n = 9$) had a significant reduction in exercise capacity (Incremental shuttle walk distance percent predicted (ISWD%pred) 65 ± 13%, Z score −1.77 ± 1.05), and modest pulmonary hypertension with a median (interquartile range) pulmonary artery pressure 31(20) mmHg and pulmonary vascular resistance 2.1(8.2) Wood Units, despite a normal diffusion of carbon monoxide adjusted for age and sex (DLco)%pred 99 ± 40%. Compared to patients in WHO FC I, patients in WHO FC II ($n = 162$) had a lower ISWD%pred 43 ± 22 and lower DLco%pred 65 ± 21%.

Conclusion: Our results demonstrate that patients with newly diagnosed pulmonary hypertension with no or minimal symptomatic limitation have a significant reduction of exercise capacity.

Keywords: incremental shuttle walk test, pulmonary hypertension, WHO functional class, screening, early diagnosis, hemodynamics, exercise testing

INTRODUCTION

Despite advances in treatment, pulmonary hypertension (PH) remains a progressive life-limiting disease (1). Studies have suggested that earlier intervention results in better outcomes (2, 3). However, patients are usually diagnosed when the disease is advanced (4, 5) and consequently there is interest in developing strategies to enable earlier diagnosis (6). To diagnose pulmonary hypertension prior to the development of significant disease, it is recommended that people at increased risk of developing pulmonary hypertension such as patients with systemic sclerosis should be screened (7). These international guidelines emphasize the importance of echocardiography in the screening process. In systemic sclerosis where the prevalence of pulmonary arterial hypertension is particularly high, investigators have also recommended the use of diffusion capacity of the lung for carbon monoxide percent predicted (DLco%pred) which is frequently reduced in patients with systemic sclerosis and pulmonary arterial hypertension (8–10). More recently a cross-sectional, international study looked at a large number of candidate biomarkers in systemic sclerosis to construct a model to aid decisions to proceed to cardiac catheterisation (the DETECT study) (11).

At rest the pulmonary circulation has large microcirculatory reserves. These are recruited during exercise, increasing the capillary surface area available for gas exchange and maintaining a low pulmonary artery pressure despite increased flow (12). Any reduction in pulmonary vasculature reserves may therefore be first detected during exercise. Although exercise testing using the 6-min walk test was included as a candidate marker in the DETECT study, and may have been expected to contribute to an early diagnostic model, it had no utility in the model constructed to diagnose pulmonary arterial hypertension. This may reflect the inability of the 6-min walk test to identify the presence of early pulmonary vascular disease given its ceiling effect where in mild disease 6 min walking test distance no longer reflects maximal oxygen aerobic capacity (13–15) or disease severity (16) or be due to an inability of patients with systemic sclerosis to exercise as a consequence of musculoskeletal disease. In addition, a systematic review of studies looking at the correlation of the New York Heart Association (NYHA) Classification and the 6-min walk distance (6MWD) in patients with heart failure without musculoskeletal disease (17) also found no significant difference between asymptomatic/mildly symptomatic patients (NYHA I and II).

The incremental shuttle walking test (ISWT) has no ceiling effect (18) and correlates better with peak exercise capacity than the 6-min walking test (19). We have hypothesized that the incremental shuttle walking test will be reduced in patients with pulmonary hypertension in World Health Organization functional class I (WHO FC I) when the patients have either no or minor symptoms of breathlessness.

METHODS

Data were retrieved from the ASPIRE registry (Assessing the Spectrum of Pulmonary hypertension Identified at a REferral center) for consecutive, treatment naïve patients diagnosed with pulmonary hypertension between 2001 and 2010 (4). The systematic assessment of the patients has previously described in detail (4). Patients included in this retrospective study were diagnosed as Group 1–5 PH and were required to have mean pulmonary artery pressure at right heart catheterisation of at least 25 mmHg and had a baseline ISWT within 3 months of cardiac catheterization.

Incremental Shuttle Walk Test

The ISWT was performed according to the method of Singh et al. (20). Patients were asked to walk as far as possible around the 10 m course keeping in time to an audio signal until they were too breathless or could no longer keep up with the speed. The initial walking speed was 0.50 m/s and this increased incrementally every minute to a maximum of 2.37 m/s. Breathlessness was measured at rest and at the end of the test using the modified Borg scale. Heart rate was measured throughout the test. ISWT distance (ISWD) percent predicted (ISWD%pred) and z score were calculated for each patient based on sex, age and BMI using the equation derived by Probst et al. (21). No supplemental oxygen was used during testing. If patients could not walk or could not walk without oxygen the distance was recorded as 0 m.

Lung Function Tests

Lung function tests were performed in accordance to the European Respiratory Society guidelines (22–25). Predicted DLco (DLco%pred) and z score were calculated for each patient

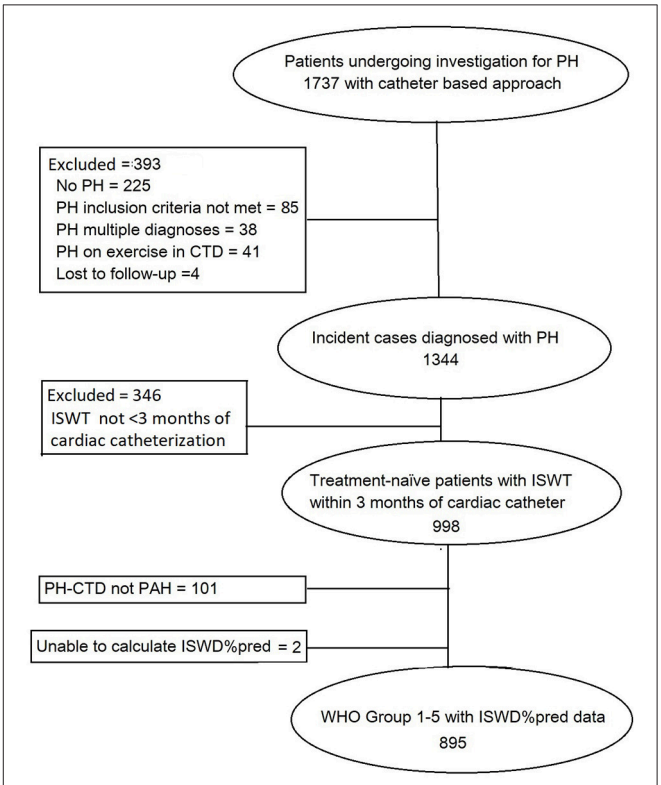


FIGURE 1 | Patient flow diagram.

TABLE 1 | Patient characteristics and hemodynamic parameters.

	WHO functional class			
	I	**II**	**III**	**IV**
n	9	162	592	132
Sex male/female %	44/56	46/54	38/62	37/63
Age years	51 ± 13	56 ± 16	61 ± 16*	64 ± 16
BMI kg/m2	28.2 ± 4.7	28.0 ± 6.1	27.7 ± 6.2	27.1 ± 6.6
Smoking % Never/Ex/Current	43/57/0	48/44/8	38/51/12	36/57/7
Pack years	10 ± 16	12 ± 19	17 ± 20	25 ± 30†
Oxygen therapy % None/LTOT/Other	100/0/0	89/6/5	62/32/7	26/68/6
HEMODYNAMICS				
mRAP mmHg	8(8)	8(7)	9(8)	12(8)†
mPAP mmHg	31(20)	37(16)	45(17)	47(12)
sPAP mmHg	51(38)	58(25)	74(29)	75(24)
Wedge mmHg	16 ± 9	13 ± 6	12 ± 6	11 ± 5
CI L/min/m^2	3.4 ± 1.8	3.1 ± 0.8	2.7 ± 0.8*	2.1 ± 0.6†
PVR Wood Unit	2.1(8.2)	3.6(3.8)	7.0(6.9)*	11.5(6.8)†
SmvO2 %	67 ± 6	67 ± 7	63 ± 9*	58 ± 9†
ISWT				
ISWD m	450(150)	280(258)▲	120(140)*	10(58)†
ISWD%pred	65(20)	42(28)▲	20(21)*	3(9) †
ISWD Z score	−1.77 ± 1.05	−2.36 ± 1.36▲	−3.11 ± 1.14*	−3.48 ± 1.10†
Starting SaO2 %	96.7 ± 2.1	93.8 ± 4.2▲	91.0 ± 6.3*	85.0†
Lowest SaO2 %	84.2 ± 10.5	83.4 ± 11.0	82.6 ± 11.2	79.1 ± 11.6†
Starting HR bpm	85. ± 14	82 ± 17	83 ± 17	85 ± 18
Highest HR bpm	144 ± 37	125 ± 30	114 ± 23*	110 ± 23
Resting SBP mmHg	125 ± 25	131 ± 22	127 ± 20*	117 ± 19†
Highest SBP mmHg	159 ± 11	158 ± 25	145 ± 27*	128 ± 24†
Borg dyspnea score				
Pre-test	0.6 ± 0.7	0.7 ± 1.0	1.1 ± 1.3*	1.8 ± 1.4†
Post-test	3.8 ± 1.6	4.2 ± 1.9	4.3 ± 1.9	4.7 ± 2.0†
LUNG FUNCTION				
FEV1%pred	97 ± 14	75 ± 23▲	74 ± 21	67 ± 23†
FVC%pred	103 ± 17	87 ± 23	87 ± 23	81 ± 27
DLco mmol/min/kPa	7.6 ± 3.2	5.6 ± 2.2▲	4.3 ± 2.0*	2.9 ± 1.7†
DLco%pred	99 ± 40	72 ± 24▲	59 ± 22*	41 ± 21†
DLco Z score	−0.57 ± 3.43	−2.22 ± 1.96▲	−3.45 ± 2.37*	−5.69 ± 3.10†

*Presented as mean ± SD for parametric data and median(interquartile range) for nonparametric data. Categorical variables were presented as %. ISWT, incremental shuttle walk test; WHO FC, World Health Organisation functional class; BMI, body mass index; mRAP, mean right atrial pressure; mPAP, mean pulmonary arterial pressure; sPAP, systolic pulmonary arterial pressure; CI, cardiac index; PVR, pulmonary vascular resistance; SmvO2, mixed venous oxygen saturation; ISWD, incremental shuttle walk test distance; %pred: percent predicted; SaO2, oxygen saturation; HR, heart rate; SBP, systolic blood pressure; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; DLco, diffusion factor across the lung for carbon monoxide; z score, standardized score; ▲$p < 0.05$ WHO FC I vs. WHO FC II; *$p < 0.05$ WHO FC II vs. WHO FC III; †$p < 0.05$ WHO FC III vs. WHO FC IV.*

using the Global Lung Function Initiative (GLI) reference equations (26).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics v19 (SPSS, Chicago, IL, USA). Data is presented as mean ± SD for parametric data and median (interquartile range) for nonparametric data. Categorical variables were presented as %. To evaluate our hypothesis that the incremental shuttle walking test will be reduced in WHO FC I, the main outcome variable was ISWD%pred. DLco%pred was a secondary outcome to be used in comparison to the ISWD. Pearson's correlation test was used to assess correlations between these parameters and WHO FC and hemodynamic parameters. The student *t*-test and Mann Whitney U-test and Kruskal-Wallis test were used to compare groups. Event (death or transplantation) free survival from date of diagnosis was estimated using the Kaplan–Meier method with comparison between groups performed by the log-rank test. Cox proportional hazards regression analysis was used to assess the effect of ISWD%pred, age, sex, BMI, mPAP and DLco on survival time. As left to right shunt is known to result in high DLco%pred (27),

TABLE 2 | Correlation of ISWD%pred with WHO FC, hemodynamics, dyspnea score and DLco%pred.

	N	*r*	95% CI		*p*
WHO FC	895	−0.576	−0.598	−0.553	< 0.001
mRAP	778	−0.238	−0.301	−0.175	< 0.001
sPAP	762	−0.145	−0.214	−0.075	< 0.001
mPAP	777	−0.171	−0.239	−0.103	< 0.001
PVR	755	−0.263	−0.327	−0.195	< 0.001
CI	754	0.271	0.139	0.336	< 0.001
SmvO2	762	0.284	0.217	0.348	< 0.001
Wedge	724	0.014	−0.059	0.087	0.697
Borg Pre	631	−0.366	−0.432	−0.297	< 0.001
Borg Post	617	0.032	0.111	−0.047	0.433
DLco%pred	815	0.371	0.311	0.413	< 0.001

ISWD%pred, Incremental Shuttle Walk Test distance percent predicted; WHO FC, World Health Organization functional class; mRAP, mean right atrial pressure; sPAP, systolic pulmonary arterial pressure; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; CI, cardiac index; SmvO2, mixed venous oxygen saturation; ISWD, Incremental Shuttle Walk Test distance; %pred, percent predicted; DLco%pred, diffusion factor across the lung for carbon monoxide percent predicted.

separate analyses were also performed omitting patients with congenital heart disease. Post-hoc analyses were also performed on the group of patients in WHO FC I and II with pulmonary hypertension related to systemic sclerosis who had undergone screening for PH. A p-value of <0.05 was deemed statistically significant. Ethical approval was granted by the North Sheffield Research Ethics Committee (Reference No. 06/Q2308/8).

RESULTS

During the duration of the study 895 patients were diagnosed with pulmonary hypertension and met the entry criteria (**Figure 1**). At diagnosis 9 patients (1%) were in WHO functional class 1, and 162 (18%), 592 (66%), and 132 (15%) in WHO Functional Class II, III, and IV, respectively. Of those in WHO FC I, 3 had systemic sclerosis (one of whom had a PAWP $>$ 15 mmHg), 3 had congenital heart disease with left to right shunts, 1 left heart disease, 1 chronic thromboembolic pulmonary hypertension and 1 hereditary pulmonary arterial hypertension.

Demographics and Pulmonary Hemodynamics

Patients' characteristics are shown in **Table 1**. Patients in WHO FC I were not significantly different to patients in the other WHO FC's with respect to age, sex distribution or smoking history. No patients in WHO FC I were on oxygen therapy compared with 6% in WHO FC II. Patients in WHO FC I had modest pulmonary hypertension: mPAP median(Interquartile range) 31(20) mmHg, preserved cardiac index (CI): mean ± standard deviation 3.4 ± 1.8 l/min/m^2 with no significant difference compared to WHO FC II.

Incremental Shuttle Walking Test and Lung Function Testing

Incremental shuttle walking distances (absolute, %pred and standardized (z) score) were all significantly higher in WHO FC I compared to WHO FC II. In addition, resting oxygen saturation pre-test was significantly higher in WHO FC I compared to WHO FC II. There was no significant difference in Borg dyspnea score between these 2 groups either pre- or post-ISWT and no significant difference in highest heart rate or highest systolic blood pressure measured. ISWD%pred correlated significantly (p all <0.05) with WHO FC, mRAP, mPAP, sPAP, CI, PVR, and $SmVO_2$ (**Table 2**).

There were also significant differences in lung function between WHO FC I and II. DLco, DLco%pred, DLco z score and FVC were all significantly higher in WHO FC I. DLco%pred correlated with WHO FC, CI, PVR, and $SmVO_2$ but not mRAP, mPAP, or sPAP. There was a stronger correlation of WHO FC with ISWD%pred compared with correlation to DLco%pred, −0.576 and −0.382, respectively.

Although there were significant correlations between WHO FC and ISWD%pred and DLco%pred, there was heterogeneity in ISWD%pred and particularly DLco%pred within each WHO FC (**Figure 2**). **Figures 3A,B** show the median and interquartile range of results for ISWD%pred and DLco%pred, respectively, for all patients by WHO FC. A large interquartile range was noted for DLco%pred in WHO FC I. When patients with congenital heart disease were omitted from analysis (**Figures 3C,D**) the interquartile range of DLco%pred in WHO FC I was reduced. However, although ISWD%pred still discriminated between patients in WHO FC I and II (63 ± 15 vs. 45 ± 23 $p = 0.02$), DLco%pred (74 ± 14 vs. 69 ± 17) and FVC%pred (96 ± 12 vs. 89 ± 23) were no longer significantly different ($p > 0.05$). Similar patterns of heterogeneity was seen in the frequency of standardized scores.

To assess the suitability of the ISWT as a screening tool, cut-off points using %pred and z-score were investigated. **Figure 4** shows the percentage of patients with an ISWD%pred of <80%. A greater percentage of patients were identified using a cut-off of <80 ISWD%pred than by using the DLco%pred <80 cut-off. **Figure 2** shows that for any given %pred cut-off point, ISWD%pred will positively identify more of the PH patients than DLco%pred. Using z scores, ISWD is again a better discriminator than DLco (**Figure 5**). In WHO FC 1 44% of patients had an ISWD below the 5th percentile and 78% were below the 10th percentile. Overall 73% of patients in WHO FC I and II are below the 5th percentile for ISWD compared to only 58% below the 5th percentile for DLco. Eighty four percent vs. 66% respectively were below the 10th percentile.

Survival Analysis

Kaplan-Meier survival analysis demonstrated that WHO FC was a significant predictor of outcome with decreasing survival with increasing WHO FC ($p < 0.0001$) (**Figure 6**). Multivariate Cox survival analysis including the parameters ISWD%pred, age, sex, BMI, mPAP and DLco showed that ISWD%pred remained a significant predictor ($p < 0.001$) (**Table 3**).

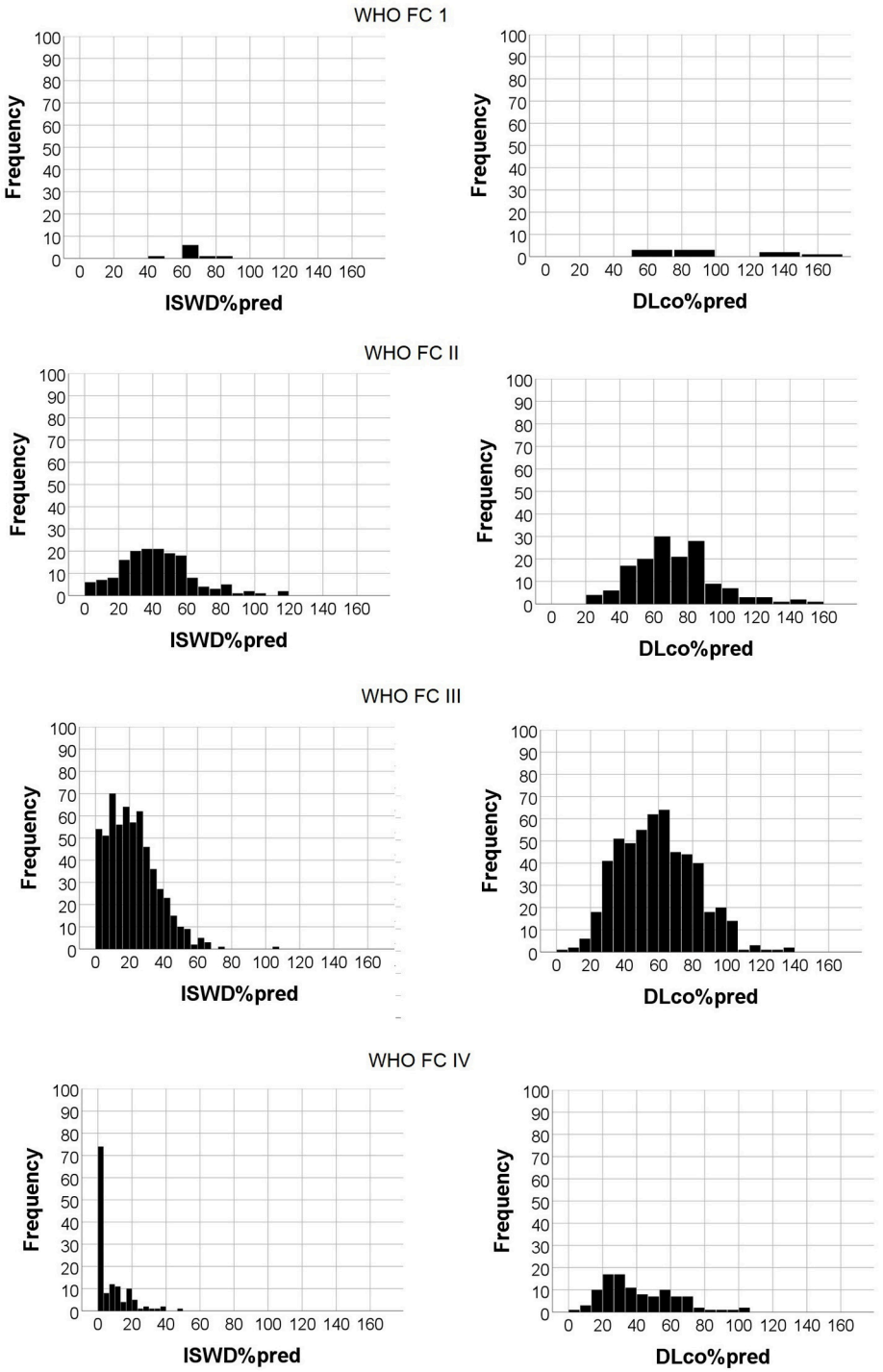


FIGURE 2 | Histogram of Incremental shuttle walk distance percent predicted (ISWD%pred) and Diffusing capacity across the lung for carbon monoxide percent predicted (DLco%pred) frequencies by World Health Organization functional class.

DISCUSSION

To our knowledge we have shown for the first time that patients with pulmonary hypertension in WHO FC I have a significant reduction in exercise capacity compared to predicted values. We have also demonstrated that exercise capacity is more sensitive than measurements of gas transfer made at rest in identifying patients with pulmonary hypertension in WHO FC I and WHO FC II. A single cut-off point is often suggested for screening as it is easy to remember and apply. We evaluated the use of a cut-off

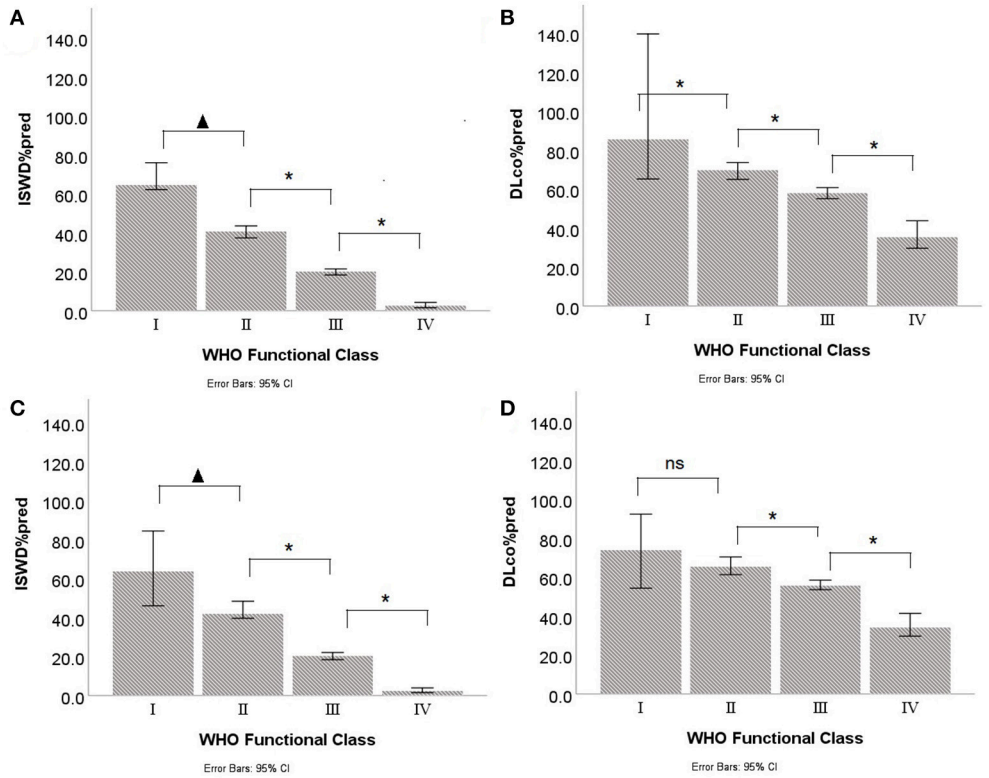


FIGURE 3 | Median and interquartile ranges for Incremental shuttle walk distance percent predicted (ISWD%pred) and Diffusing capacity across the lung for carbon monoxide percent predicted (DLco%pred) by World Health Organization Functional Class (WHO FC) in **(A,B)** all patients and **(C,D)** with congenital heart disease patients excluded. $p < 0.05$; $^{*}p < 0.005$; ns, not significant.

point of 80%pred looking for early changes and found that 89% of patients in WHO FCI were below this cut-off point. Whilst traditionally the normal range for a lung function parameters was considered as being between 80 and 120%pred it is known now that this could lead to misdiagnosis (28). We therefore also examined percentile scores as cut-off points and again found that the ISWT was a sensitive test with 73% of patients in WHO FC I and II below the 5th percentile for ISWD.

In addition to confirming the findings of previous studies (4, 29, 30) that WHO FC has a significant impact on survival, we have also demonstrated that in the absence of symptoms of breathlessness or in the presence of mild symptoms (i.e. patients in WHO FC 1/II) patients have a modest elevation of pulmonary artery pressure at initial diagnosis. In contrast patients with more severe symptoms of breathlessness (WHO FC III and IV) had significantly higher mean pulmonary artery pressure elevation. Therefore strategies to diagnose patients earlier when they have less symptomatic limitation is likely to identify patient with less severe pulmonary haemodynamic disease. Patients in WHO FC I included 3 patients with systemic sclerosis who had been identified from screening regimens and these patients had only mild elevation of mean pulmonary artery pressure, median 27 mmHg. Patients not undergoing regular screening, may also have been referred on the basis of echocardiograms performed for the assessment of incidental murmurs or on the basis of morphological changes consistent with pulmonary hypertension seen on cross sectional imaging.

It could be possible that there is some misclassification of the patients as classification into WHO FC is limited by patient and physician subjectivity and agreement between observers is often poor (31). There are however significant differences in ISWD%pred and DLco%predicted between FC I and II and there is a trend for increased survival. Breathlessness post ISWT is no greater in WHO FC I suggesting that the increased distance walked is not due to increased effort. This study, therefore, does emphasize that if we are to rely on self-reported symptoms of breathlessness to diagnose pulmonary hypertension then patients will have established hemodynamic changes of pulmonary hypertension at the time of diagnosis.

Current ESC/ERS guidelines recommend the use of Doppler echocardiography for screening for pulmonary hypertension in at risk patients. Like all screening tools echocardiography has limitations. In 10–20% of patients it is not possible to obtain interpretable results and the precision of echocardiography estimation of systolic pulmonary artery pressure can be poor (32). To overcome these difficulties other data, using lung function tests and utilizing gas transfer (which is reduced as a consequence of vascular involvement), have been used inscreening algorithms.

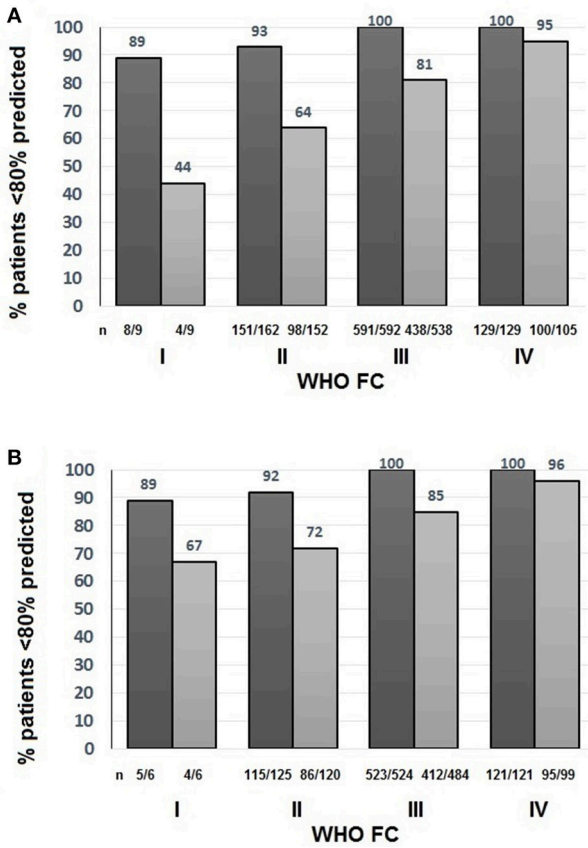


FIGURE 4 | Percentage of patients with Incremental shuttle walk distance percent predicted (ISWD%pred) and Diffusing capacity across the lung for carbon monoxide percent predicted (DLco%pred) less than 80% predicted in **(A)** All patients **(B)** Patients with congenital heart disease excluded by World Health Organization Functional Class (WHO FC).

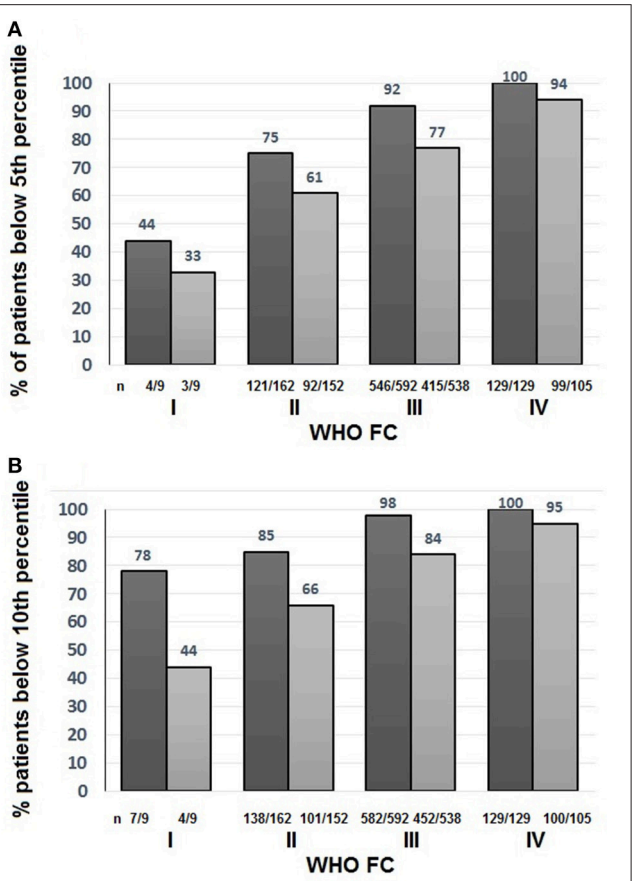


FIGURE 5 | Percentage of all patients with Incremental shuttle walk distance percent predicted (ISWD%pred) and Diffusing capacity across the lung for carbon monoxide percent predicted (DLco%pred) less than **(A)** the 5th percentile and **(B)** the 10th percentile World Health Organization Functional Class (WHO FC).

Most work has been done in systemic sclerosis using DLco%pred. Hanchulla et al. (9) in a French multi-center trial found that a low DLco of <60% predicted was associated with a higher probability of PAH with only 30% of the newly diagnosed patients having a DLco > 60%pred. A UK study among 243 systemic sclerosis-associated PH patients found that <10% had a DLco >60%pred (10). Guidelines now suggest using a cut-off of DLco%pred <60% as part of the screening algorithm indicative of possible pulmonary hypertension in systemic sclerosis (33). This strategy has been shown to successfully enrich this population of patients undergoing right heart catheter to investigate possible pulmonary hypertension (11). However, whilst this algorithm has been used with some success in systemic sclerosis it is not applicable in patients with pulmonary hypertension associated with other aetiologies (34). Our study highlights the large range of DLco%pred found in WHO FC I, even when omitting patients with congenital heart disease. In contrast ISWD%pred correlated well with WHO FC with <10% of patients in WHO FC I and II having an ISWD >80%pred.

A number of studies have looked at the use of exercise testing including cardiopulmonary exercise testing, exercise Doppler echocardiography or diffusion capacity during exercise (35–39) to try to detect loss of compliance in the cardiopulmonary circulation earlier than parameters measured at rest but the tests used are complex. The advantage of the ISWT for screening is that it is a very simple test to perform and has been shown to reflect disease severity without a ceiling effect (8). Data from this study suggest it might be suited to detecting early disease. Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension, for which specific therapies exist, are rare and therefore early identification relies on having a high degree of awareness in patients at risk and deploying appropriate disease specific strategies. Reducing the time to diagnosis and institution of treatment for patients with pulmonary hypertension requires a number of complimentary approaches and screening for pulmonary hypertension in at risk groups is only one approach. In addition to considering pulmonary hypertension in high risk groups (e.g., systemic sclerosis, portal hypertension, HIV, family history of PAH), there needs to be an increased awareness amongst patients to seek advice when they have exercise limitation and for physicians

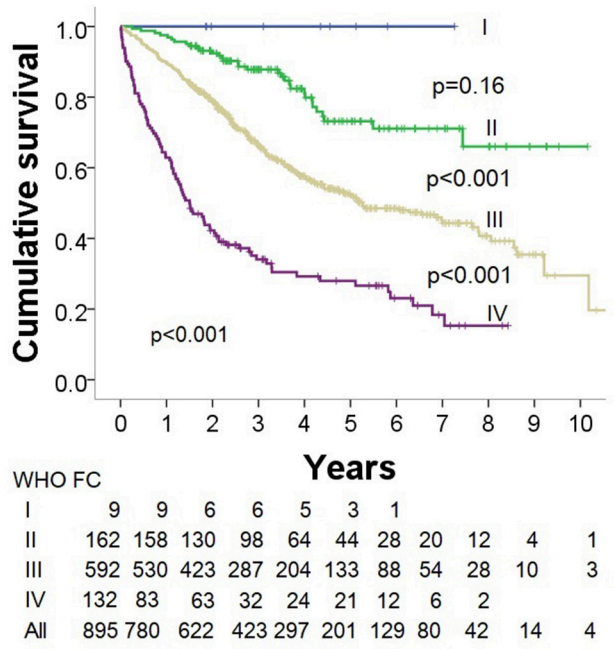


FIGURE 6 | Kaplan Meier survival analysis by World Health Organisation functional class.

TABLE 3 | Multivariate Cox survival analysis.

	HR	95% CI	*p*
ISWD%pred	0.983	0.975 – 0.990	<0.0001
Age	1.017	1.007 – 1.026	<0.0001
BMI	0.980	0.961 – 0.999	0.044
mPAP	1.022	1.011 – 1.032	<0.0001
DLco%pred	0.974	0.967 – 0.980	<0.0001

ISWD%pred, Incremental shuttle walk test percent predicted; BMI, body mass index; mPAP, mean pulmonary arterial pressure; DLco%pred, diffusion factor across the lung for carbon monoxide percent predicted; HR, hazard ratio; CI, confidence interval.

to more systematically assess the breathless patient. Our results suggest that exercise limitation (identified using a maximal exercise test) is an almost universal finding in patients with pulmonary hypertension even in patients who are asymptomatic. Further study is required to assess whether the incremental shuttle walking test could be used as a first line investigation or as part of a battery of tests to screen at risk patients, proceeding to more complex testing if abnormal.

LIMITATIONS

This was a retrospective, single center study. Within the studied population only 1% of patients were diagnosed in WHO FC I and 18% in WHO FC II. These percentages are small (but are very similar to those found in the Geissen Pulmonary Hypertension Registry, which has 1.0% patients in WHO FC I, and confirm the findings in other registries (4, 26) that the majority of patients are diagnosed with PH in WHO FC III when symptomatic and haemodynamic severity are advanced. Given the small number of patients in WHO FC I, interpretation of negative results must be viewed with caution Classification of WHO FC may be limited by patient and physician subjectivity and agreement between observers is often poor (31). However in this study there appears to be a trend to increased survival in WHO FC 1 patients and ISWD is significantly higher in patients in WHO FC I. Although patient motivation could affect distance walked, the ISWT is externally paced which limits the effect of motivation and patients in WHO FC I did not report a higher dyspnoea score post-test suggesting that the difference in distance walked was not due to greater patient effort.

CONCLUSION

Our results demonstrate that patients with newly diagnosed pulmonary hypertension with no or minimal symptomatic limitation have a significant reduction of exercise capacity.

AUTHOR CONTRIBUTIONS

CB, IA, RC, and DK: study design; JH, RL, CB, IAS, MA, AC, CE, RC, and DK: performance of the research; CB, AT, CE, IS, AC, AL, and DK: data analysis; CB and DK: writing of the paper; All authors: revision of manuscript.

FUNDING

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript. JH 's research fellowship during the period of the study was part-funded by an unrestricted educational grant from Actelion Pharmaceuticals Ltd. The funding bodies had no involvement in the development of this research or manuscript.

Financial Relationships to commercial entities not related to submitted manuscript. In the past 3 years AC, CE, DK, and RC have received have received honoraria for participation in advisory boards and for giving lectures and have received funding to attend educational meetings from personal fees and non-financial support to attend educational meetings from Actelion Pharmaceuticals Ltd and GSK. CE, DK, and RC also received honoraria for participation in advisory boards and for giving lectures and non-financial support from Bayer. In addition, RC received fees and support from United Therapeutics. IAS has received an unrestricted educational grant from GSK to support a clinical teaching meeting, an unrestricted educational grant from Triniti Chiesi Ltd. to support medical humanities research and projects, and funding to attend educational meetings from Boehringer Ingelheim and Actelion Pharmaceuticals Ltd. JH received funding from GSK and Pfizer to attend educational meetings. AT received funding from Actelion Pharmaceuticals Ltd. to attend educational meetings. IAS and CB received support from the Pulmonary Hypertension Association UK to attend educational meetings.

REFERENCES

1. Kiely DG, Elliot CA, Sabroe I, Condliffe R. Pulmonary hypertension: diagnosis and management. *BMJ* (2013) 346:f2028. doi: 10.1136/bmj.f2028
2. Humbert M, Yaici A, de Groote P, Montani D, Sitbon O, Launay D, et al. Screening for pulmonary arterial hypertension in patients with systemic sclerosis: clinical characteristics at diagnosis and long-term survival. *Arthr Rheumatism* (2011) 63:3522–30. doi: 10.1002/art.30541
3. Vizza CD, Badagliacca R, Messick CR, Rao Y, Nelsen AC, Benza RL. The impact of delayed treatment on 6-minute walk distance test in patients with pulmonary arterial hypertension: a meta-analysis. *Int J Cardiol.* (2018) 254:299–301. doi: 10.1016/j.ijcard.2017.12.016
4. Hurdman J, Condliffe R, Elliot CA, Davies C, Hill C, Wild JM, et al. ASPIRE registry: assessing the Spectrum of Pulmonary hypertension Identified at a REferral centre. *Eur Respir J.* (2012) 39:945–55. doi: 10.1183/09031936.00078411
5. Gall H, Felix JF, Schneck FK, Milger K, Sommer N, Voswinckel R, et al. The Giessen Pulmonary Hypertension Registry: Survival in pulmonary hypertension subgroups. *J Heart Lung Transp.* (2017) 36:957–67. doi: 10.1016/j.healun.2017.02.016
6. Lau EM, Manes A, Celermajer DS, Galiè N. Early detection of pulmonary vascular disease in pulmonary arterial hypertension: time to move forward. *Eur Heart J.* (2011) 32:2489–98. doi: 10.1093/eurheartj/ehr160
7. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J.* (2016) 37:67–119. doi: 10.1093/eurheartj/ehv317
8. Elliot CA, KielyDG. Pulmonary hypertension: diagnosis and treatment. *Clin Med.* (2004) 4:211–5. doi: 10.7861/clinmedicine.4-3-211
9. Hachulla E, Gressin V, Guillevin L, Carpentier P, Diot E, Sibilia J, et al. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthr Rheum* (2005) 52:3792–3800. doi: 10.1002/art.21433
10. Schwaiger JP, Khanna D, Gerry Coghlan J. Screening patients with scleroderma for pulmonary arterial hypertension and implications for other at-risk populations. *Eur Respir Rev.* (2013) 22:515–25. doi: 10.1183/09059180.00006013
11. Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D, et al. DETECT study group. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis.* (2014) 73:1340–9. doi: 10.1136/annrheumdis-2013-203301
12. Lee G, DuBois AB. Pulmonary capillary blood flow in man. *J Clin Invest.* (1955) 34:1380–90. doi: 10.1172/JCI103187
13. Lipkin DP, Scriven AJ, Crake T, Poole-Wilson PA. Six minute walking test for assessing exercise capacity in chronic heart failure. *Br Med J.* (1986) 292:653–5.
14. Deboeck G, Taboada D, Hagan G, Treacy C, Page K, Sheares K, et al. Maximal cardiac output determines 6 minutes walking distance in pulmonary hypertension. *PLoS ONE* (2014) 9:e92324. doi: 10.1371/journal.pone.0092324
15. van der Plas MN, Duffels MGJ, Ponse D, Mulder BJM, Bresser P. Bosentan in mild pulmonary hypertension. *Lancet* (2008) 372:1730. doi: 10.1016/S0140-6736(08)61725-0
16. Degano B, Sithon O, Savale L, Garcia G, O'Callaghan DS, Jaïs X, et al. Characterization of pulmonary arterial hypertension patients walking more than 450 m in 6 min at diagnosis. *Chest* (2010) 137:1297–303. doi: 10.1378/chest.09-2060
17. Yap J1, Lim FY, Gao F, Teo LL, Lam CS, Yeo KK. Correlation of the New York Heart association classification and the 6-minute walk distance: a systematic review. *Clin Cardiol.* (2015) 38:621–8. doi: 10.1002/clc.22468
18. Billings CG, Hurdman JA, Condliffe R, Elliot CA, Smith IA, Austin M, et al. Incremental shuttle walk test distance and autonomic dysfunction predict survival in pulmonary arterial hypertension. *J Heart Lung Trans.* (2017) 36:871–9. doi: 10.1016/j.healun.2017.04.008
19. Irisawa H, Takeuchi K, Inui N, Miyakawa S, Morishima Y, Mizushima T, et al. Incremental shuttle walk test as a valuable assessment of exercise performance in patients with pulmonary hypertension. *Circ J.* (2014) 78:215–21. doi: 10.1253/circj.CJ-13-0238
20. Singh SJ, Morgan MDL, Scott S, Walters D, Hardman AE. Development of a shuttle walking test of disability in patients with chronic airways obstruction. *Thorax* (1992) 47:1019–24.
21. Probst VS, Hernandes NA, Teixeira DC, Felcar JM, Mesquita RB, Gonçalves CG, et al. Reference values for the incremental shuttle walking test. *Respir Med.* (2012) 106:243–8. doi: 10.1016/j.rmed.2011.07.023
22. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J.* (1993) 6(Suppl. 16):S5–S40.
23. Cotes JE, Chinn DJ, Quanjer PH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity). Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statem ent of the European Respiratory Society. *Eur Respir J.* (1993) 6(Suppl. 16), S41–S52.
24. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J.* (2005) 26:319–38. doi: 10.1183/09031936.05.00034805
25. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J.* (2005) 26:720–35. doi: 10.1183/09031936.05.00034905
26. Stanojevic S, Graham BL, Cooper BG, Thompson BR, Carter KW, Francis RW, et al. On behalf of the Global Lung Function Initiative TLCO working group. Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians. *Eur Respir J.* (2017) 50:170001. doi: 10.1183/13993003.00010-2017
27. Burgess JH. Pulmonary diffusing capacity in disorders of the pulmonary circulation. *Circulation* (1974) 49:541–50.
28. Miller MR, Quanjer PH, Swanney MP, Ruppel G, Enright PL. Interpreting lung function data using 80% predicted and fixed thresholds misclassifies more than 20% of patients. *Chest* (2011) 139:52–9. doi: 10.1378/chest.10-0189
29. Farber HW, Miller DP, Poms AD, Badesch DB, Frost AE, Muros-Le Rouzic E, et al. Five-Year outcomes of patients enrolled in the REVEAL Registry. *Chest* (2015) 148:1043–54. doi: 10.1378/chest.15-030
30. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* (2010) 122:156–63. doi: 10.1161/CIRCULATIONAHA.109.911818
31. Taichman DB, McGoon MD, Harhay MO, Archer-Chicko C, Sager JS, Murugappan M, et al. Wide variation in clinicians' assessment of New York Heart Association/World Health Organization functional class in patients with pulmonary arterial hypertension. *Mayo Clin Proc.* (2009) 84:586–92. doi: 10.1016/S0025-6196(11)60747-7
32. Fisher MR, Forfia PR, Chamera E, Housten-Harris T, Champion HC, Girgis RE, et al. Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. *Am J Respir Crit Care Med.* (2009) 179:615–21. doi: 10.1164/rccm.200811-1691OC
33. Khanna D, Gladue H, Channick R, Chung L, Distler O, Furst DE, et al. Scleroderma Foundation and Pulmonary Hypertension Association. Recommendations for screening and detection of connective-tissue disease associated pulmonary arterial hypertension. *Arthritis Rheum.* (2013) 65:3194–201. doi: 10.1002/art.38172
34. Lau EMT, Humbert M, Celermajer DS. Early detection of pulmonary arterial hypertension. *Nat Rev. Cardiol.* (2015) 12:143–155. doi: 10.1038/nrcardio.2014.191
35. Dumitrescu D, Nagel C, Kovacs G, Bollmann T, Halank M, Winkler J, et al. Cardiopulmonary exercise testing for detecting pulmonary arterial hypertension in systemic sclerosis. *Heart* (2017) 103:774–82. doi: 10.1136/heartjnl-2016-309981

36. Mueller J, Heck PB, Ewert P, HagerA. Noninvasive screening for pulmonary hypertension by exercise testing in congenital heart disease. *Ann Thorac Surg*. (2017) 103:1544–49. doi: 10.1016/j.athoracsur.2016.09.038
37. Trip P, Vonk-Noordegraaf A, Bogaard HJ. Cardiopulmonary exercise testing reveals onset of disease and response to treatment in a case of heritable pulmonary arterial hypertension. *Pulm Circ*. (2012) 2:387–9. doi: 10.4103/2045-8932.101658
38. Baptista R, Serra S, Martins R, Teixeira R, Castro G, Salvador MJ, et al. Exercise echocardiography for the assessment of pulmonary hypertension in systemic sclerosis: a systematic review. *Arthr Res Ther*. (2016) 18:153. doi: 10.1186/s13075-016-1051-9
39. Legnani D, Rizzi M, Sarzi-Puttini P, Cristiano A, La Spina T, Frassanito F, et al. Diffusing pulmonary capacity measured during effort: a possible early marker of pulmonary involvement in Systemic Sclerosis. *Isr Med Assoc J*. (2015) 17:739–43.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

REVIEW
published: 18 July 2018
doi: 10.3389/fmed.2018.00204

Pulmonary Arterial Stiffness: An Early and Pervasive Driver of Pulmonary Arterial Hypertension

*Wei Sun and Stephen Y. Chan**

Division of Cardiology, Department of Medicine, Center for Pulmonary Vascular Biology and Medicine, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, University of Pittsburgh School of Medicine and University of Pittsburgh Medical Center, Pittsburgh, PA, United States

Pulmonary arterial hypertension (PAH) is a historically neglected and highly morbid vascular disease that leads to right heart failure and, in some cases, death. The molecular origins of this disease have been poorly defined, and as such, current pulmonary vasodilator therapies do not cure or reverse this disease. Although extracellular matrix (ECM) remodeling and pulmonary arterial stiffening have long been associated with end-stage PAH, recent studies have reported that such vascular stiffening can occur early in pathogenesis. Furthermore, there is emerging evidence that ECM stiffening may represent a key first step in pathogenic reprogramming and molecular crosstalk among endothelial, smooth muscle, and fibroblast cells in the remodeled pulmonary vessel. Such processes represent the convergence of activation of a number of specific mechanoactivated signaling pathways, microRNAs, and metabolic pathways in pulmonary vasculature. In this review, we summarize the contemporary understanding of vascular stiffening as a driver of PAH, its mechanisms, potential therapeutic targets and clinical perspectives. Of note, early intervention targeting arterial stiffness may break the vicious cycle of PAH progression, leading to outcome improvement which has not been demonstrated by current vasodilator therapy.

Keywords: pulmonary arterial hypertension, arterial stiffness, endothelium, extracellular matrix, vascular metabolism

OPEN ACCESS

Edited by:
Anne Hilgendorff, Ludwig-Maximilians-Universität München, Germany

Reviewed by:
Michael Adam O'Reilly, University of Rochester, United States
Tsogyal Daniela Latshang, Cantonal Hospital Graubuenden, Switzerland

***Correspondence:**
Stephen Y. Chan
chansy@pitt.edu

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *02 January 2018*
Accepted: *28 June 2018*
Published: *18 July 2018*

Citation:
Sun W and Chan SY (2018) Pulmonary Arterial Stiffness: An Early and Pervasive Driver of Pulmonary Arterial Hypertension. Front. Med. 5:204. doi: 10.3389/fmed.2018.00204

INTRODUCTION

Pulmonary hypertension (PH) is defined as an elevation of the mean pulmonary artery pressure above 25 mmHg at rest. It can be divided into 5 clinical groups according to the World Health Organization (WHO) Classification of PH, last updated in 2013 (1) and scheduled to be revised later in 2018. Briefly, Group 1 PH is pulmonary arterial hypertension (PAH), often familial or heritable, related to connective tissue disease or toxins, with unknown underlying etiology (idiopathic PH). Group 2 refers to PH caused by left heart disease (pulmonary vein hypertension). Group 3 includes pulmonary hypertension resulting from lung diseases or hypoxia, such as chronic obstructive lung disease or sleep apnea. Group 4 disease is related to chronic thromboembolic obstruction in pulmonary arteries. Group 5 includes other less-common causes such as hematological or metabolic disorders that do not fit into any of the other four groups. The different groups of PH differ not only in fundamental pathogenesis but also patients' prognosis and corresponding treatment strategies. As a severe form of PH, PAH (WHO clinical Group 1) is a devastating

condition characterized by progressive pulmonary vascular remodeling and gradual obstruction of pulmonary arterioles (at times, from pathognomonic plexiform lesions), resulting in increased pulmonary vascular resistance and pressure. The increase in pulmonary arterial pressure can lead to right ventricular heart hypertrophy, failure, and premature death.

PAH has idiopathic, heritable, and comorbid etiologies, associated with other chronic diseases. Although PAH is a highly morbid disease, it has been historically neglected since symptoms such as dyspnea and right heart failure often present late in disease. The onset of PH symptoms is unfortunately associated with a markedly impaired prognosis, and the historical survival rate of PAH without treatment is only 34% at 5 years (2). The current treatment options for PAH—including calcium-channel blockers, prostanoids, endothelin receptor antagonists, and phosphodiesterase inhibitors/soluble guanylyl cyclase agonists—primarily target pulmonary vascular dilation, thus lowering pulmonary arterial pressure, providing symptom relief and prolonging of the time to clinical deterioration. These treatments, however, are not curative and do not stop or reverse this disease. This may stem from the fact that these medications largely do not target to molecular origins of PAH (3), many of which still remain poorly defined. As such, there are obvious and critical needs, both scientifically and clinically, for advanced insights into the mechanisms of PAH pathogenesis in order to identify novel biomarkers for the early detection of PAH, to offer valid prognosis estimation, and to define novel targets for specific molecular therapeutics.

In this article, we will focus on the emerging appreciation of pulmonary vascular and extracellular matrix (ECM) stiffening as a distinct and early initiating cause of PAH. We will summarize the current understanding of pulmonary vascular stiffening, which serves not only as a consequence of PAH but also a critical driver of disease. We will review the progress in the studies on the mechanisms of pulmonary vascular stiffening, the potential therapeutic targets, and relevant clinical perspectives.

PULMONARY ARTERIAL STIFFENING AND ECM REMODELING IN PAH

Pulmonary Arterial Remodeling and Stiffening

Pulmonary arterial stiffening is a key component in the pathogenesis of PAH. Stiffness occurs in the proximal and distal pulmonary arteries in multiple subtypes of PAH (4, 5), and stiffness can serve as an index of disease progression (6). At the histologic level, pulmonary vessel stiffening in PAH is characterized by the activation and proliferation of all cellular subtypes in the three layers of the pulmonary arterial wall, including endothelial cells of the tunica intima, vascular smooth muscle cells of the tunica media, as well as adventitial fibroblasts. Spatially and functionally associated with the endothelial cell layer in the intima, the fibroblasts and myofibroblasts in the media and adventitia are activated, resulting in the synthesis and stromal deposition of ECM components. Within the tunica media, activated smooth muscle cells together with myofibroblasts derived from the adventitia can also migrate into the endothelial cell layer. As a result of this complex interplay between cells and ECM components, a concentric hyperplasia of the tunica intima develops (7). Of note, there is convincing evidence to suggest the active interaction between inflammatory cells especially macrophages with pulmonary arterial cells which plays important roles in the pathogenesis of PAH (8). Interestingly, the histopathological feature of this remodeling can resemble allograft vasculopathy as found after heart transplantation (9). The expression of some fetal variants of certain cell adhesion modulating proteins such as fibronectin or tenascin-C, as well as the impact of fibroblast to myofibroblast trans-differentiation and vascular smooth muscle cell activation have been extensively described in both settings of allograft vasculopathy and PAH. This remodeling process in the pulmonary vasculature results in a progressive decrease in arterial compliance and an increase in vascular stiffening.

Clinically, pulmonary arterial stiffening can be assessed by pulse wave velocity measurement via non-invasive ultrasound or magnetic resonance imaging, based on the pulsatile characteristics of the pulmonary arteries. Pulse wave velocity is the speed of flow waves propagate along the lumen, which depend on the stiffness and dimensions of the blood vessels. Concomitant measurement of pulmonary arterial stiffness indices could allow for more precise prediction for risk of developing severe PAH and mortality, especially in the early stage of the disease (10). For example, in children with congenital heart disease, increased pulmonary arterial stiffening predicts the progression into advanced PAH even in the patients with low pulmonary vascular resistance, suggesting intrinsic pulmonary arterial stiffness may serve as an independent index that may enhance predictions of disease progression and survival (11).

Pulmonary arterial compliance (PACa), as measured invasively or more recently via non-invasive means, is another pulmonary arterial stiffening index (6, 12). PACa refers the change in pulmonary arterial cross-sectional area or volume over change in pressure. Unlike pulmonary arterial resistance, which is calculated by the pressure difference across the pulmonary vasculature under a certain cardiac output, PACa reflects the pulmonary arterial stiffness more directly. PACa is increasingly appreciated as a parameter of prognostic relevance in patients with various subtypes and severities of PH (13, 14). In chronic thromboembolic PH (WHO Group 4), reduced PACa is a determinant of poor functional capacity and is a marker of poor prognosis in the patients. Reduced PACa may even serve as the most prominent hemodynamic feature found in early stages of PAH. Furthermore, in PH due to left heart disease (WHO Group 2), reduced PACa has been found to offer prognostic value, both in those with elevated and normal pulmonary vascular resistance (15).

ECM Stiffening

At the histologic level, ECM remodeling plays a central role in pulmonary arterial stiffening. The ECM network provides biophysical support for various cells in the vessel wall, thus maintaining the mechanical stability and elastic recoil of the arteries. Biochemical and biophysical signals induced by

the ECM direct vascular cellular function, differentiation, migration and apoptosis, enable intracellular communication, playing a decisive role in vascular development, remodeling processes and maintenance of vascular homeostasis, as previously reviewed (16). The pulmonary arterial ECM is highly dynamic, consisting mostly of collagens, elastins, and laminins as well as other components such as fibronectin, tenascin C, and glucosaminoglycans. Dynamic balance of the ECM is dictated by bidirectional alterations of proteolytic enzymes such as a disintegrin and metalloproteases (ADAMs), matrix metalloproteinases (MMPs), and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). In PAH, buildup of collagen, neutrophil-elastase and MMPs, along with decrease of their modulating counterparts TIMPs causes an imbalance of ECM turnover and subsequently a pathogenic alteration of the structure and stiffness of the pulmonary vessel wall. A landmark of this remodeling process, endothelial-to-mesenchymal transition, is controlled by a complex upstream intracellular signaling network including TGF-β, Wnt, and Notch pathways (17). The restoration of ECM kinetics has been promoted as a strategy to prevent or ameliorate vascular remodeling processes but only recently have the molecular control points of this balance begun to be clarified.

ECM remodeling and stiffening can be triggered by various PAH pathogenic factors, such as vascular injury, pro-inflammation factors, abnormal growth factor expression, and/or hypoxia exposure. It has been shown that accumulation of ECM is a key pathologic change notable across the vascular wall in PH (18). Vascular-specific serine elastase activity in the ECM has been implicated in PAH (19). In parallel with elastase activity, MMPs exhibit an elevated activity, especially MMP-2/9, leading to accelerated turnover of ECM and play an important role in the initial remodeling of ECM in pulmonary vessels in both patients and animal models of PAH (20–22). Since the function of MMPs is tightly controlled by endogenous inhibitors TIMPs, the loss of balance between MMPs and TIMPs has been shown to induce ECM remodeling in patients with idiopathic PAH. As an example, Wang et al. found that vascular collagen buildup promotes hypoxia-induced pulmonary vascular remodeling in both small and large arteries (23). ECM remodeling could also delay the recovery of pulmonary hemodynamics and subsequently worsen right ventricular function (24). In regard of pulmonary vascular adventitial remodeling, the expression of type I collagen in cultured fibroblasts is upregulated by hypoxia (25). In rodent hypoxia-induced PH models, the expression of ECM proteins, especially the collagens that contribute to arterial stiffening, has been found to be elevated, even after hypoxic stress has been removed. This process is thought to be mediated by impaired degradation of type I collagen (26).

Approaches to target ECM remodeling in experimental animal models have shown efficacy in reducing PAH. Zhang et al. demonstrated that the extension of arterial stiffening is alleviated by inhibiting 15-lipoxygenase (15-LO) and consequent collagen accumulation in pulmonary arteries in hypoxia mouse models (25). Alternatively, in monocrotaline (MCT)-induced PAH in rats, the activation of AMP activated protein kinase (AMPK) by the AMPK agonist metformin inhibited ECM remodeling in pulmonary arteries, thus reducing the elevated right ventricle systolic pressure and right ventricle hypertrophy (27). The mechanisms underlying AMPK-induced suppression of ECM remodeling in pulmonary arteries were coupled to decreased MMP-2/9 activity and TIMP-1 expression. Importantly, however, the known pleiotropy of AMPK agonism in PAH likely extends far beyond the direct effects on pulmonary vascular stiffness (28, 29). Thus, while these results suggest the potential therapeutic value of AMPK activation in PAH, these studies did not fully prove the causative role of matrix stiffening *per se* in this disease. Phosphodiesterase type 5 enzyme (PDE-5) inhibitors are widely used in the clinical management of PH through the mechanism of NO mediated vasodilatation. As mentioned above, it is generally thought that the vasodilators present no significant effect in stopping or reversing the progression of PAH. However, data exist that show the PDE-5 inhibitor sildenafil restores BMP signaling in cultured BMPR2 deficient smooth muscle cells, and also reduces pulmonary vascular remodeling in the MCT-induced PAH rat model (30).

ARTERIAL AND ECM STIFFENING AS EARLY DRIVER OF PAH

Although the connection of pulmonary vascular stiffening/ECM remodeling to PAH has been well established, until recently, the question remained unanswered as to whether ECM remodeling represented simply an end-stage consequence of PAH. Contemporary studies, including work from our group, have advanced the concept that vascular stiffening and ECM remodeling are early and potent pathogenic triggers in PAH, occurring at time points prior to typical hemodynamic and histologic evidence of PAH or medial thickening (31–33). Several major mechanisms have been identified in this process, especially the activation of mechanosensitive metabolic pathways, the pro-inflammatory phenotype switch of activated fibroblasts and macrophages, and the involvement of certain groups of mechanosensitive microRNAs.

Metabolic Mechanisms Involved in Pulmonary Vascular Stiffening in PAH

Metabolic reprogramming in both the diseased pulmonary vasculature and right ventricle is increasingly appreciated as a crucial mediator of the pro-proliferative condition in PAH. Not only hypoxia but also other PAH-inducing factors, such as genetic mutations, congenital heart disease, scleroderma, and HIV infection, are all related to profound metabolic dysregulation in the pulmonary vasculature. For instance, broad metabolic reprogramming, beyond aerobic glycolysis and independent of hypoxia, has been identified through metabolomic screening in pulmonary endothelial cells carrying a bone morphogenetic protein receptor type 2 (BMPR2) mutation, a known genetic driver of PAH (34). The utilization and metabolism of glutamine have also been found to be increased in the course of rat right ventricle remodeling and human PAH (35).

The shift of energy production from mitochondrial oxidative phosphorylation to glycolysis chronically (Warburg-like effect),

represents a central feature of the extensive reprogramming found in pulmonary arterial endothelial cells and smooth muscle cells in PAH, as reviewed previously (36). Furthermore, such metabolic reprogramming has also been linked to a pathophenotypic switch of both pulmonary vascular macrophages and fibroblasts which then can contribute extensively to pulmonary arterial stiffening and matrix remodeling in PAH. In both experimental hypoxic PH in rodents and human PAH, a model of cellular crosstalk has emerged whereby adventitial fibroblasts can recruit, retain, and activate naive macrophages into a hyper-proliferative, apoptosis-resistant, and proinflammatory phenotype (37–39). To drive this process in adventitial fibroblasts, pathogenic suppression of mitochondrial bioenergetics was found to be accompanied by increased mitochondrial fragmentation, resulting in less-efficient ATP synthesis, hyperpolarized mitochondria, and increased mitochondrial superoxide production. In contexts both dependent and independent of hypoxia, these changes resulted in a pro-oxidative and pro-inflammatory status in the pulmonary vascular micro-environment (38, 40), thus driving matrix alterations and resulting in pulmonary vascular stiffening. Metabolic reprogramming in activated macrophages has also been described in PAH, highly dependent on increased aerobic glycolysis, altered TCA cycle activity, and reduced mitochondrial respiration (Warburg-like effect). Such reprogramming has been linked to a number of distinct and complicated mechanisms involving a cohort of metabolic enzymes and accumulated metabolites, as well as transcriptional regulatory factors such as the hypoxia inducible factor (HIF)-1α and downstream signaling molecules such as STAT3 [see reviews by (41, 42)].

Of note, the energy production from the increased glycolysis alone is not sufficient to fulfill the metabolic demands for the cells that are actively proliferating during the process of vascular remodeling in PAH. The energy generated from the classic tricarboxylic acid (TCA) cycle is necessary to maintain the cellular reprogramming process. Additionally, large amounts of various precursor substrates are produced via TCA cycle activity. These substrates are critical for amino acid, carbohydrate and lipid biosynthesis which supports rapid cell growth and proliferation. In doing so, a large quantity of carbon intermediates are consumed continuously and requires replenishment. This replenishment of TCA carbon intermediates, also termed anaplerosis, depends on two major mechanisms. One is glutaminolysis, which deamidates glutamine via the enzyme glutaminase 1 (GLS1). The other pathway is the carboxylation of pyruvate to oxaloacetate, which is mediated by ATP-dependent pyruvate carboxylase. Particularly, GLS1-mediated glutaminolysis in the proliferating cells during pulmonary vascular remodeling serves as a critical mechanism to support not only the mobilization of cellular energy, carbon and nitrogen to maintain cellular biomass but also the metabolic switch from oxidative phosphorylation to glycolysis.

Recently, emerging evidence, including findings from our group, has demonstrated a link between matrix mechanotransduction (i.e., mechanisms by which cells sense and react to external mechanical forces and convert extracellular mechanical cues into intracellular signaling) and pulmonary vascular metabolic reprogramming, thus triggering the initiation and development of PAH (31, 33). We previously defined the pulmonary vascular function of YAP (Yes Associated Protein 1) and TAZ (Transcriptional Coactivator with PDZ-Binding Motif, or WWRT1), which serve as transcriptional coactivators in the Hippo signaling pathway. YAP/TAZ are mechanoactivated by stiff ECM in pulmonary vascular cell types and function as central regulators of cellular proliferation. They do so by activating the enzyme GLS1 which promotes glutaminolysis and anaplerosis and downstream effects on cellular proliferation, migration, and apoptosis among multiple vascular cell types in a timed and stage-specific manner. As such, YAP/TAZ-dependent control of glutaminolysis may act as a central mechanism of the pulmonary vascular dysfunction induced by the change of the extracellular environment in PAH (**Figure 1**) (33).

Besides YAP/TAZ–GLS1 activation, some additional mechanisms may also be involved in the metabolic switch in the setting of pulmonary arterial stiffening. The enzymes lactate dehydrogenase A (LDHA) and the ATP-dependent pyruvate carboxylase (PC) have both been identified as associated checkpoints in stiffness-induced alterations of glycolysis and anaplerosis, respectively (33). Furthermore, YAP/TAZ signaling has been linked to AMPK activation, resulting in the induction of aerobic glycolysis (43). These findings suggest broader control over metabolic reprogramming in PAH by YAP/TAZ and vascular stiffness. Future work will be important to delineate more completely the connections of pulmonary vascular stiffness and glutaminolysis with cellular reprogramming, crosstalk, and also the timed evolution of cellular function and identity in PAH. These findings may also endorse the application of novel pharmacologic agents targeting the metabolic effects of vascular stiffness to prevent or even reverse PAH, which will be further discussed below.

MicroRNA-Related Mechanisms in Pulmonary Arterial Stiffening

MicroRNAs (miRNAs) are non-coding RNAs that vary in length between 19 and 25 nucleotides. They regulate gene expression by affecting mRNA stability and translation into protein, and they serve as essential mediators of multiple cellular processes involving cell-cell and cell-matrix interactions (44). Although many crucial roles of miRNAs in the cardiovascular system have been well established, studies of miRNAs in PAH are still advancing (3, 45). Until recently, the interaction between miRNAs and pulmonary arterial stiffening/ECM remodeling had not been well established, and the targets and downstream mechanisms of miRNAs were largely unexplored.

Numerous miRNAs have been found to function in the pathogenesis and progression of PAH, as previously reviewed (46). Among them, the miR-130/301 family has been shown to regulate the systems-wide, proliferative and vasoconstrictive actions in pulmonary vasculature. The involvement of miRNAs in pulmonary ECM biology, their targets and mechanisms, have been recently studied as an early driver of PAH progression. Specifically, mechanosensitive YAP/TAZ signaling has been found to activate miR-130/301 to form a feedback loop, thus

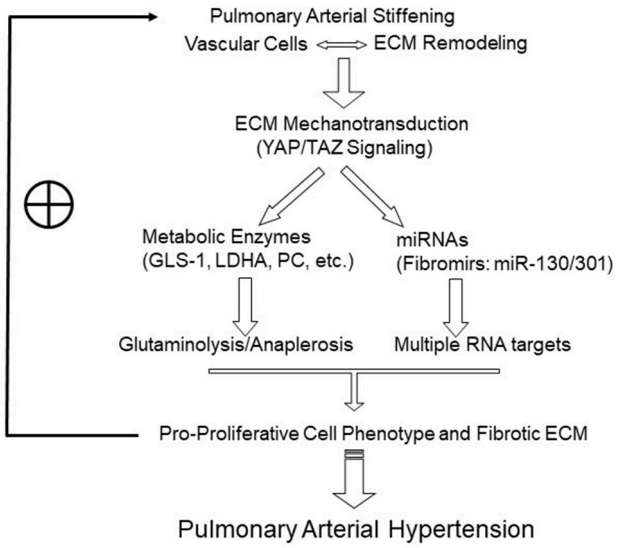


FIGURE 1 | Model of the positive feedback loop and vicious cycle triggered by pulmonary arterial and matrix stiffening in PAH inception and development. Pulmonary arterial stiffening and matrix remodeling activate mechanosensitive signaling. A prominent example includes YAP/TAZ-dependent reprogramming, which induces the key metabolic enzymes to promote glutaminolysis and glycolysis and sustains the metabolic needs of hyperproliferative vascular cells. In the other hand, mechanoactive YAP/TAZ signaling also induces miRNAs that are related to tissue fibrosis and remodeling ("fibromirs"), through interacting with multiple, and potentially synergistic, target mRNAs. These mechanisms drive pro-proliferative vascular cell phenotypes and ECM remodeling and in turn further enhance mechanotransduction, forming a vicious cycle underlying the progression of PAH. ECM, extracellular matrix. The symbol ⊕ indicates a positive feedback effect to maintain the vicious cycle.

promoting PAH via ECM remodeling and vascular stiffening (31). The downstream mediators of the YAP/TAZ-miR-130/301 circuit relevant to ECM remodeling were found to involve predominantly the pro-proliferative PPARγ-APOE-LRP8-LOX pathway. Overexpression or knockdown of PPARγ or its direct target ApoE, disrupted miR130/301 mediated vascular collagen disposition and remodeling (31). Other secreted factors, such as fibroblast growth factor 2 (FGF2), interleukin-6 (IL-6), and endothelin 1 (EDN1), as well as miRNAs such as miR-21 and miR-27a, have further defined an overlapping molecular hierarchy important to the remodeled ECM in PAH [as reviewed in Negi and Chan (3)]. The early development of ECM remodeling in PAH and the positive feedback loop connecting the ECM to the YAP/TAZ-miR-130/301 circuit suggest the pathogenic relevance of this axis both early and late in disease as well as present a model of self-sustained propagation of ECM remodeling throughout the pulmonary vascular tree as PAH progresses.

Much future work remains in studying the roles of miRNAs in pulmonary arterial stiffening. It is likely that an even more complex and broad interactome is active among YAP/TAZ, a cohort of fibrosis-related miRNAs including miR-130/301, and their targets. For example, independent of YAP/TAZ, pulmonary vessel stiffening has also been found to be regulated by a miRNA-dependent process involving the Runt-related transcription factor 2 (Runx2) to promote vascular calcification (47). Moreover, mechanoactivation of YAP/TAZ independent of miR-130/301 can promote increased matrix deposition and stiffening, thus propagating vessel stiffness throughout the pulmonary vascular tree in PAH (48). Recent studies have implicated the control of metabolic, inflammatory, and proliferative regulatory programs in diseased adventitial fibroblasts in PAH to miR-124 (49, 50) and transcriptional regulators, such as the C-terminal binding protein-1 (CtBP1) (51) and the polypyrimidine tract binding protein 1 (PTBP1)-pyruvate kinase muscle (PKM) axis (49).

Thus, a set of mechanosensitive and ECM-related miRNAs ("fibromirs") may comprise an important link in the complex regulation of ECM stiffening in PAH (**Figure 1**). Future work will be required to define additional mechanisms of RNA-dependent control of this process in PAH, specifically in regard to other aspects of ECM remodeling beyond biogenesis, such as matrix degradation and turnover.

NOVEL TRANSLATIONAL APPROACHES TARGETING PULMONARY ARTERIAL STIFFENING IN PAH

Diagnostic and Therapeutic Targets in the ECM for PAH

Given the pathogenic importance of vascular ECM reorganization in PAH, investment is ongoing to identify matrix components that may serve either as biomarkers for diagnosis and prognostic estimation in PAH or as true therapeutic targets. The translational value of measuring fetal ECM components, such as tenascin-C variants, as biomarkers, both in tissue and in circulating blood, has been proposed (52, 53). Due to the stable extracellular deposition, these matrix components could also be considered as target molecules for specific therapeutic modulation in PAH. For example, fibronectin and tenascin-C have been considered as feasible molecular targets for antibody-based delivery of diagnostic agents (e.g., radionuclides) or direct therapeutics (i.e., bioactive payloads such as cytokines or small molecule inhibitors). Such agents, in particular immunocytokines or antibody-drug conjugates, have been successfully administered in a variety of animal models of neoplastic and non-neoplastic chronic-inflammatory diseases (54–56). Applications of these approaches in PAH could advance considerably in the near future.

Therapeutic Strategy Targeting miRNA-Dependent or Metabolic Mechanisms of Pulmonary Arterial Stiffening

Multiple novel therapeutic gene targets in adventitial fibroblasts have shown promise recently in experimental rodent models of PH, including the CtBP1 inhibitor 4-methylthio-2-oxobutyric acid (MTOB) (51) and the PKM2 inhibitors TEPP-46 and shikonin (49) among others. Importantly, however, similar to targeting AMPK activity, the therapeutic effects of these pleiotropic agents may not solely be dependent on their effects

on pulmonary vascular stiffness alone. On the other hand, the description of a true mechanosensitive YAP/TAZ-GLS1 circuit regulating glutaminolysis and cellular proliferation sets the stage for developing novel clinical management strategies in PAH that target pulmonary arterial stiffening more directly. As a historical example, the lysyl oxidase (Lox) inhibitor β-aminopropionitrile (BAPN) has been used to improve both hemodynamic and histologic indices of PAH in experimental rodent models, thus reinforcing the therapeutic feasibility of targeting collagen cross-linking and ECM reprogramming in PH (33, 57–59). Consistent with more recent evidence supporting the important roles of YAP and GLS1 in the pathogenesis of PAH, a YAP inhibitor such as verteporfin (33, 60), an oligonucleotide inhibitor of the miR-130/301 family (61), as well as GLS1 inhibitors such as CB-839 and C968 (33) have all been shown to mediate robust improvement of rodent PAH. Downstream of YAP, a liver X receptor (LXR) agonist GW3965, which upregulates ApoE level when administered orally to hypoxia treated mice, was shown to ameliorate ECM remodeling and PAH through interaction with YAP/TAZ-miR-130/301 circuit *in vivo* (31, 62). Importantly, verteporfin is already approved for use intravenously in treatment of age-related macular degeneration (63). Cyclic YAP-like peptides that interrupt YAP-TEAD interactions in oncogenesis (64) could also be applied to PAH. Moreover, CB-839, acting as oral GLS1 inhibitor, is developed for cancer therapy and currently under evaluation in an early human clinical trial (Clinical Trial NCT02071862) (65). Notably, since LXR along with the sterol regulatory element-binding protein (SREBP) also act together to mediate the actions of AMPK (66), there may exist an even greater interdependence and convergence of YAP with AMPK than already appreciated. Therefore, a strategy of repurposing these inhibitors for YAP, GLS1, or miR-130/301, potentially along with a LXR agonist and/or AMPK modulator, may provide a rare opportunity to offer novel "matrix" therapeutics for PAH without the delay of needing to develop new inhibitors *de novo*.

SUMMARY

ECM remodeling and pulmonary arterial stiffening have long been associated with PAH. However, only recently have we begun to appreciate that pulmonary vascular and ECM stiffening act as crucial early triggers in pathogenesis of this mysterious disease. Emerging evidence has demonstrated that pulmonary vascular stiffening activates mechanosensitive signaling such as YAP/TAZ signaling which in turn regulates downstream miRNAs and metabolic targets essential for PAH development (**Figure 1**). Further understanding of this and other novel mechanisms related to pulmonary arterial stiffening will strengthen the development of diagnostic, therapeutic, and potentially preventative, approaches targeting the early initiation of arterial stiffness. As such, it is hoped that the vicious cycle of PAH progression may be broken or entirely avoided, thus leading to disease prevention or reversal, which has not been possible thus far with current therapies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This work was supported by NIH grants R01 HL124021, HL 122596, HL 138437, and UH2 TR002073 as well as AHA Established Investigator Award 18EIA339600027 (SYC).

REFERENCES

1. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* (2013) 62:D34–41. doi: 10.1016/j.jacc.2013.10.029
2. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med.* (1991) 115:343–9. doi: 10.7326/0003-4819-115-5-343
3. Negi V, Chan SY. Discerning functional hierarchies of microRNAs in pulmonary hypertension. *JCI Insight* (2017) 2:e91327. doi: 10.1172/jci.insight.91327
4. Wang Z, Chesler NC. Pulmonary vascular wall stiffness: an important contributor to the increased right ventricular afterload with pulmonary hypertension. *Pulm Circ.* (2011) 1:212–23. doi: 10.4103/2045-8932.83453
5. Lammers S, Scott D, Hunter K, Tan W, Shandas R, Stenmark KR. Mechanics and function of the pulmonary vasculature: implications for pulmonary vascular disease and right ventricular function. *Compr Physiol.* (2012) 2:295–319. doi: 10.1002/cphy.c100070
6. Gan CT, Lankhaar JW, Westerhof N, Marcus JT, Becker A, Twisk JW, et al. Noninvasively assessed pulmonary artery stiffness predicts mortality in pulmonary arterial hypertension. *Chest* (2007) 132:1906–12. doi: 10.1378/chest.07-1246
7. Tuder RM, Archer SL, Dorfmuller P, Erzurum SC, Guignabert C, Michelakis E, et al. Relevant issues in the pathology and pathobiology of pulmonary hypertension. *J Am Coll Cardiol.* (2013) 62:D4–12. doi: 10.1016/j.jacc.2013.10.025
8. Savai R, Pullamsetti SS, Kolbe J, Bieniek E, Voswinckel R, Fink L, et al. Immune and inflammatory cell involvement in the pathology of idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2012) 186:897–908. doi: 10.1164/rccm.201202-0335OC
9. Suzuki J, Isobe M, Morishita R, Nagai R. Characteristics of chronic rejection in heart transplantation: important elements of pathogenesis and future treatments. *Circ J.* (2010) 74:233–9. doi: 10.1253/circj.CJ-09-0809
10. Hunter KS, Lammers SR, Shandas R. Pulmonary vascular stiffness: measurement, modeling, and implications in normal and hypertensive pulmonary circulations. *Compr Physiol.* (2011) 1:1413–35. doi: 10.1002/cphy.c100005
11. Ploegstra MJ, Brokelman JG, Roos-Hesselink JW, Douwes JM, van Osch-Gevers LM, Hoendermis ES, et al. Pulmonary arterial stiffness indices assessed by intravascular ultrasound in children with early pulmonary vascular disease: prediction of advanced disease and mortality during 20-year follow-up. *Eur Heart J Cardiovasc Imaging* (2017) 19:216–24. doi: 10.1093/ehjci/jex015
12. Sanz J, Kariisa M, Dellegrottaglie S, Prat-Gonzalez S, Garcia MJ, Fuster V, et al. Evaluation of pulmonary artery stiffness in pulmonary hypertension with cardiac magnetic resonance. *JACC*

Cardiovasc Imaging (2009) 2:286–95. doi: 10.1016/j.jcmg.2008.08.007
13. Mahapatra S, Nishimura RA, Sorajja P, Cha S, McGoon MD. Relationship of pulmonary arterial capacitance and mortality in idiopathic pulmonary arterial hypertension. *J Am Coll Cardiol.* (2006) 47:799–803. doi: 10.1016/j.jacc.2005.09.054
14. Ghio S, D'Alto M, Badagliacca R, Vitulo P, Argiento P, Mule M, et al. Prognostic relevance of pulmonary arterial compliance after therapy initiation or escalation in patients with pulmonary arterial hypertension. *Int J Cardiol.* (2017) 230:53–8. doi: 10.1016/j.ijcard.2016.12.099
15. Pellegrini P, Rossi A, Pasotti M, Raineri C, Cicoira M, Bonapace S, et al. Prognostic relevance of pulmonary arterial compliance in patients with chronic heart failure. *Chest* (2014) 145:1064–70. doi: 10.1378/chest.13-1510
16. Wagenseil JE, Mecham RP. Vascular extracellular matrix and arterial mechanics. *Physiol Rev.* (2009) 89:957–89. doi: 10.1152/physrev.00041.2008
17. Arciniegas E, Frid MG, Douglas IS, Stenmark KR. Perspectives on endothelial-to-mesenchymal transition: potential contribution to vascular remodeling in chronic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol.* (2007) 293:L1–8. doi: 10.1152/ajplung.00378.2006
18. Chelladurai P, Seeger W, Pullamsetti SS. Matrix metalloproteinases and their inhibitors in pulmonary hypertension. *Eur Respir J.* (2012) 40:766–82. doi: 10.1183/09031936.00209911
19. Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest.* (2012) 122:4306–13. doi: 10.1172/JCI60658
20. Dodson RB, Rozance PJ, Petrash CC, Hunter KS, Ferguson VL. Thoracic and abdominal aortas stiffen through unique extracellular matrix changes in intrauterine growth restricted fetal sheep. *Am J Physiol Heart Circ Physiol.* (2014) 306:H429–37. doi: 10.1152/ajpheart.00472.2013
21. Wetzl V, Tiede SL, Faerber L, Weissmann N, Schermuly RT, Ghofrani HA, et al. Plasma MMP2/TIMP4 ratio at follow-up assessment predicts disease progression of idiopathic pulmonary arterial hypertension. *Lung* (2017) 195:489–96. doi: 10.1007/s00408-017-0014-5
22. Lepetit H, Eddahibi S, Fadel E, Frisdal E, Munaut C, Noel A, et al. Smooth muscle cell matrix metalloproteinases in idiopathic pulmonary arterial hypertension. *Eur Respir J.* (2005) 25:834–42. doi: 10.1183/09031936.05.00072504
23. Wang Z, Lakes RS, Eickhoff JC, Chesler NC. Effects of collagen deposition on passive and active mechanical properties of large pulmonary arteries in hypoxic pulmonary hypertension. *Biomech Model Mechanobiol.* (2013) 12:1115–25. doi: 10.1007/s10237-012-0467-7
24. Schreier D, Hacker T, Song G, Chesler N. The role of collagen synthesis in ventricular and vascular adaptation to hypoxic pulmonary hypertension. *J Biomech Eng.* (2013) 135:021018. doi: 10.1115/1.4023480
25. Zhang L, Li Y, Chen M, Su X, Yi D, Lu P, et al. 15-LO/15-HETE mediated vascular adventitia fibrosis via p38 MAPK-dependent TGF-beta. *J Cell Physiol.* (2014) 229:245–57. doi: 10.1002/jcp.24443
26. Ooi CY, Wang Z, Tabima DM, Eickhoff JC, Chesler NC. The role of collagen in extralobar pulmonary artery stiffening in response to hypoxia-induced pulmonary hypertension. *Am J Physiol Heart Circ Physiol.* (2010) 299:H1823–31. doi: 10.1152/ajpheart.00493.2009
27. Li S, Han D, Zhang Y, Xie X, Ke R, Zhu Y, et al. Activation of AMPK prevents monocrotaline-induced extracellular matrix remodeling of pulmonary artery. *Med Sci Monit Basic Res.* (2016) 22:27–33. doi: 10.12659/MSMBR.897505
28. Wang W, Xiao ZD, Li X, Aziz KE, Gan B, Johnson RL, et al. AMPK modulates Hippo pathway activity to regulate energy homeostasis. *Nat Cell Biol.* (2015) 17:490–9. doi: 10.1038/ncb3113
29. Lai YC, Tabima DM, Dube JJ, Hughan KS, Vanderpool RR, Goncharov DA, et al. SIRT3-AMP-activated protein kinase activation by nitrite and metformin improves hyperglycemia and normalizes pulmonary hypertension associated with heart failure with preserved ejection fraction. *Circulation* (2016) 133:717–31. doi: 10.1161/CIRCULATIONAHA.115.018935
30. Yang J, Li X, Al-Lamki RS, Wu C, Weiss A, Berk J, et al. Sildenafil potentiates bone morphogenetic protein signaling in pulmonary arterial smooth muscle cells and in experimental pulmonary hypertension. *Arterioscler Thromb Vasc Biol.* (2013) 33:34–42. doi: 10.1161/ATVBAHA.112.300121
31. Bertero T, Cotrill KA, Lu Y, Haeger CM, Dieffenbach P, Annis S, et al. Matrix remodeling promotes pulmonary hypertension through feedback mechanoactivation of the YAP/TAZ-miR-130/301 circuit. *Cell Rep.* (2015) 13:1016–32. doi: 10.1016/j.celrep.2015.09.049
32. Bertero T, Cottrill KA, Annis S, Bhat B, Gochuico BR, Osorio JC, et al. A YAP/TAZ-miR-130/301 molecular circuit exerts systems-level control of fibrosis in a network of human diseases and physiologic conditions. *Sci Rep.* (2015) 5:18277. doi: 10.1038/srep18277
33. Bertero T, Oldham WM, Cottrill KA, Pisano S, Vanderpool RR, Yu Q, et al. Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. *J Clin Invest.* (2016) 126:3313–35. doi: 10.1172/JCI86387
34. Fessel JP, Hamid R, Wittmann BM, Robinson LJ, Blackwell T, Tada Y, et al. Metabolomic analysis of bone morphogenetic protein receptor type 2 mutations in human pulmonary endothelium reveals widespread metabolic reprogramming. *Pulm Circ.* (2012) 2:201–13. doi: 10.4103/2045-8932.97606
35. Piao L, Fang YH, Parikh K, Ryan JJ, Toth PT, Archer SL. Cardiac glutaminolysis: a maladaptive cancer metabolism pathway in the right ventricle in pulmonary hypertension. *J Mol Med.* (2013) 91:1185–97. doi: 10.1007/s00109-013-1064-7
36. Cottrill KA, Chan SY. Metabolic dysfunction in pulmonary hypertension: the expanding relevance of the warburg effect. *Eur J Clin Invest.* (2013) 43:855–65. doi: 10.1111/eci.12104
37. Anwar A, Li M, Frid MG, Kumar B, Gerasimovskaya EV, Riddle SR, et al. Osteopontin is an endogenous modulator of the constitutively activated phenotype of pulmonary adventitial fibroblasts in hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol.* (2012) 303:L1–11. doi: 10.1152/ajplung.00050.2012
38. Li M, Riddle SR, Frid MG, El Kasmi KC, McKinsey TA, Sokol RJ, et al. Emergence of fibroblasts with a proinflammatory epigenetically altered phenotype in severe hypoxic pulmonary hypertension. *J Immunol.* (2011) 187:2711–22. doi: 10.4049/jimmunol.1100479
39. El Kasmi KC, Pugliese SC, Riddle SR, Poth JM, Anderson AL, Frid MG, et al. Adventitial fibroblasts induce a distinct proinflammatory/profibrotic macrophage phenotype in pulmonary hypertension. *J Immunol.* (2014) 193:597–609. doi: 10.4049/jimmunol.1303048
40. Plecita-Hlavata L, Tauber J, Li M, Zhang H, Flockton AR, Pullamsetti SS, et al. Constitutive reprogramming of fibroblast mitochondrial metabolism in pulmonary hypertension. *Am J Respir Cell Mol Biol.* (2016) 55:47–57. doi: 10.1165/rcmb.2015-0142OC
41. El Kasmi KC, Stenmark KR. Contribution of metabolic reprogramming to macrophage plasticity and function. *Semin Immunol.* (2015) 27:267–75. doi: 10.1016/j.smim.2015.09.001
42. Stenmark KR, Tuder RM, El Kasmi KC. Metabolic reprogramming and inflammation act in concert to control vascular remodeling in hypoxic pulmonary hypertension. *J Appl Physiol.* (2015) 119:1164–72. doi: 10.1152/japplphysiol.00283.2015
43. Santinon G, Pocaterra A, Dupont S. Control of YAP/TAZ activity by metabolic and nutrient-sensing pathways. *Trends Cell Biol.* (2016) 26:289–99. doi: 10.1016/j.tcb.2015.11.004
44. Valastyan S, Weinberg RA. Roles for microRNAs in the regulation of cell adhesion molecules. *J Cell Sci.* (2011) 124:999–1006. doi: 10.1242/jcs.081513
45. Chun HJ, Bonnet S, Chan SY. Translating MicroRNA biology in pulmonary hypertension: it will take more than "miR" words. *Am J Respir Crit Care Med.* (2016) 195:167–78. doi: 10.1164/rccm.201604-0886PP
46. Boucherat O, Potus F, Bonnet S. microRNA and pulmonary hypertension. *Adv Exp Med Biol.* (2015) 888:237–52. doi: 10.1007/978-3-319-22671-2_12
47. Ruffenach G, Chabot S, Tanguay VF, Courboulin A, Boucherat O, Potus F, et al. Role for runt-related transcription factor 2 in proliferative and calcified vascular lesions in pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2016) 194:1273–85. doi: 10.1164/rccm.201512-2380OC
48. Liu F, Haeger CM, Dieffenbach PB, Sicard D, Chrobak I, Coronata AM, et al. Distal vessel stiffening is an early and pivotal mechanobiological regulator of vascular remodeling and pulmonary hypertension. *JCI Insight* (2016) 1:e86987. doi: 10.1172/jci.insight.86987
49. Zhang H, Wang D, Li M, Plecita-Hlavata L, D'Alessandro A, Tauber J, et al. The metabolic and proliferative state of vascular adventitial fibroblasts in pulmonary hypertension is regulated through a MiR-124/PTBP1/PKM Axis. *Circulation* (2017) 136:2468–85. doi: 10.1161/CIRCULATIONAHA.117.028069

50. Caruso P, Dunmore BJ, Schlosser K, Schoors S, Dos Santos CC, Perez-Iratxeta C, et al. Identification of miR-124 as a major regulator of enhanced endothelial cell glycolysis in pulmonary arterial hypertension via PTBP1 and PKM2. *Circulation* (2017) 136:2451–67. doi: 10.1161/CIRCULATIONAHA.117.028034
51. Li M, Riddle S, Zhang H, D'Alessandro A, Flockton A, Serkova NJ, et al. Metabolic reprogramming regulates the proliferative and inflammatory phenotype of adventitial fibroblasts in pulmonary hypertension through the transcriptional corepressor C-terminal binding protein-1. *Circulation* (2016) 134:1105–21. doi: 10.1161/CIRCULATIONAHA.116.023171
52. Baldinger A, Brehm BR, Richter P, Bossert T, Gruen K, Hekmat K, et al. Comparative analysis of oncofetal fibronectin and tenascin-C expression in right atrial auricular and left ventricular human cardiac tissue from patients with coronary artery disease and aortic valve stenosis. *Histochem Cell Biol.* (2011) 135:427–41. doi: 10.1007/s00418-011-0809-z
53. Nozato T, Sato A, Hikita H, Takahashi A, Imanaka-Yoshida K, Yoshida T, et al. Impact of serum tenascin-C on the aortic healing process during the chronic stage of type B acute aortic dissection. *Int J Cardiol.* (2015) 191:97–9. doi: 10.1016/j.ijcard.2015.05.009
54. Franz M, Doll F, Grun K, Richter P, Kose N, Ziffels B, et al. Targeted delivery of interleukin-10 to chronic cardiac allograft rejection using a human antibody specific to the extra domain A of fibronectin. *Int J Cardiol.* (2015) 195:311–22. doi: 10.1016/j.ijcard.2015.05.144
55. Casi G, Neri D. Antibody-drug conjugates: basic concepts, examples and future perspectives. *J Control Release* (2012) 161:422–8. doi: 10.1016/j.jconrel.2012.01.026
56. Bootz F, Neri D. Immunocytokines: a novel class of products for the treatment of chronic inflammation and autoimmune conditions. *Drug Discov Today* (2016) 21:180–9. doi: 10.1016/j.drudis.2015.10.012
57. Kerr JS, Riley DJ, Frank MM, Trelstad RL, Frankel HM. Reduction of chronic hypoxic pulmonary hypertension in the rat by beta-aminopropionitrile. *J Appl Physiol Respir Environ Exerc Physiol.* (1984) 57:1760–6. doi: 10.1152/jappl.1984.57.6.1760
58. Kerr JS, Ruppert CL, Tozzi CA, Neubauer JA, Frankel HM, Yu SY, et al. Reduction of chronic hypoxic pulmonary hypertension in the rat by an inhibitor of collagen production. *Am Rev Respir Dis.* (1987) 135:300–6.
59. Nave AH, Mizikova I, Niess G, Steenbock H, Reichenberger F, Talavera ML, et al. Lysyl oxidases play a causal role in vascular remodeling in clinical and experimental pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol.* (2014) 34:1446–58. doi: 10.1161/ATVBAHA.114.303534
60. Kudryashova TV, Goncharov DA, Pena A, Kelly N, Vanderpool R, Baust J, et al. HIPPO-integrin-linked kinase cross-talk controls self-sustaining proliferation and survival in pulmonary hypertension. *Am J Respir Crit Care Med.* (2016) 194:866–77. doi: 10.1164/rccm.201510-2003OC
61. Bertero T, Lu Y, Annis S, Hale A, Bhat B, Saggar R, et al. Systems-level regulation of microRNA networks by miR-130/301 promotes pulmonary hypertension. *J Clin Invest.* (2014) 124:3514–28. doi: 10.1172/JCI74773
62. Im SS, Osborne TF. Liver x receptors in atherosclerosis and inflammation. *Circ Res.* (2011) 108:996–1001. doi: 10.1161/CIRCRESAHA.110.226878
63. Kent DL. Age-related macular degeneration: beyond anti-angiogenesis. *Mol Vis.* (2014) 20:46–55.
64. Zhou Z, Hu T, Xu Z, Lin Z, Zhang Z, Feng T, et al. Targeting Hippo pathway by specific interruption of YAP-TEAD interaction using cyclic YAP-like peptides. *FASEB J.* (2015) 29:724–32. doi: 10.1096/fj.14-262980
65. Katt WP, Lukey MJ, Cerione RA. A tale of two glutaminases: homologous enzymes with distinct roles in tumorigenesis. *Future Med Chem.* (2017) 9:223–43. doi: 10.4155/fmc-2016-0190
66. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest.* (2001) 108:1167–74. doi: 10.1172/JCI13505

Conflict of Interest Statement: SYC has served as a consultant for Actelion (Significant), Gilead, Pfizer, and Vivus (Modest).

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 27 July 2018
doi: 10.3389/fmed.2018.00212

Divergent Roles for TRAIL in Lung Diseases

Adam T. Braithwaite, Helen M. Marriott and Allan Lawrie*

Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Medical School, Sheffield, United Kingdom

The tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is a widely expressed cytokine that can bind five different receptors. TRAIL has been of particular interest for its proposed ability to selectively induce apoptosis in tumour cells. However, it has also been found to regulate a wide variety of non-canonical cellular effects including survival, migration and proliferation via kinase signalling pathways. Lung diseases represent a wide range of conditions affecting multiple tissues. TRAIL has been implicated in several biological processes underlying lung diseases, including angiogenesis, inflammation, and immune regulation. For example, TRAIL is detrimental in pulmonary arterial hypertension—it is upregulated in patient serum and lungs, and drives the underlying proliferative pulmonary vascular remodelling in rodent models. However, TRAIL protects against pulmonary fibrosis in mice models—by inducing apoptosis of neutrophils—and reduced serum TRAIL is found in patients. Conversely, in the airways TRAIL positively regulates inflammation and immune response. In COPD patients and asthmatic patients challenged with antigen, TRAIL and its death receptors are upregulated in serum and airways. Furthermore, TRAIL-deleted mouse models have reduced airway inflammation and remodelling. In the context of respiratory infections, TRAIL assists in immune response, e.g., via T-cell toxicity in influenza infection, and neutrophil killing in S. pneumoniae infection. In this mini-review, we examine the functions of TRAIL and highlight the diverse roles TRAIL has in diseases affecting the lung. Disentangling the facets of TRAIL signalling in lung diseases could help in understanding their pathogenic processes and targeting novel treatments.

Keywords: TRAIL, TNF-related apoptosis-inducing ligand, pulmonary arterial hypertension, immune regulation, pulmonary vascular disease, pulmonary fibrosis, respiratory tract infections, chronic obstructive pulmonary diseases

OPEN ACCESS

Edited by:
Kian Fan Chung,
Imperial College London,
United Kingdom

Reviewed by:
Eleni Papakonstantinou,
Aristotle University of Thessaloniki,
Greece
Megan Noelle Ballinger,
The Ohio State University,
United States

****Correspondence:***
Adam T. Braithwaite
a.braithwaite@sheffield.ac.uk

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *28 February 2018*
Accepted: *10 July 2018*
Published: *27 July 2018*

Citation:
Braithwaite AT, Marriott HM and Lawrie A (2018) Divergent Roles for TRAIL in Lung Diseases. Front. Med. 5:212. doi: 10.3389/fmed.2018.00212

INTRODUCTION

The tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), also known as Apo2 ligand is an apoptosis-inducing cytokine that is expressed in most cell types. As its name suggests, TRAIL was primarily of particular interest for its ability to selectively induce apoptosis in tumour cells *in vitro* and *in vivo*, while apparently exhibiting minimal off-target effects (1–3). TRAIL-deficient mice are also more susceptible to tumour formation and metastasis (4), suggesting TRAIL has a protective role in cancer suppression. Consequently TRAIL signalling has been targeted for use in several anticancer therapies (5), however several types of cancer cells are resistant to TRAIL-induced apoptosis. In these cells, TRAIL can activate pro-inflammatory signalling pathways (6, 7),

proliferation (8–10) and metastasis (11). The purpose of this mini-review is to discuss how the known function of TRAIL has evolved beyond apoptosis to these alternative effects and highlight the different roles TRAIL has in diseases affecting the lung (**Figure 1**), where TRAIL is widely expressed (12, 13). The better understanding of the diverse roles for TRAIL in lung disease could lead to the development of more effective, and novel treatments.

TRAIL MOLECULAR SIGNALLING

TRAIL, a type II transmembrane protein, is a member of the death receptor ligand family; a subclass of the tumour necrosis factor family (14) and is widely expressed in a variety of human tissues, most predominantly in lung, spleen and prostate (14). TRAIL is proteolytically-cleaved and its extracellular domain can bind five TRAIL receptors: membrane-bound death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2), membrane-bound decoy receptors DcR1 (TRAIL-R3), and DcR2 (TRAIL-R4) and the soluble decoy osteoprotegerin (OPG) (15–21) [TRAIL is conserved in mice—they have two decoy receptors and a single TRAIL death receptor, mDR5, which is more similar to DR5 than DR4 (22)].

TRAIL is composed of 281 amino acids and forms a homotrimeric structure upon binding three receptor molecules (23). The death receptors DR4 and DR5 are type I transmembrane proteins containing a cytoplasmic death domain. In the canonical TRAIL apoptosis signalling pathway (**Figure 2A**), binding of death receptors by TRAIL leads to recruitment of Fas-associated protein with death domain (FADD), formation of a complex known as death-inducing signalling complex (DISC), activation of caspase-8 and subsequently downstream caspase-3 dependent apoptosis of the cell [**Figure 1**; (24–26)]. Unlike the TRAIL death receptors, the decoy receptor DcR1 has no death domain (15, 18) and DcR2 has a truncated, non-functional death domain (15, 17, 27). These decoy receptors, and additionally binding with lower affinity, the soluble OPG, are suggested to suppress apoptotic signalling by competitively binding TRAIL (28, 29).

Conversely, TRAIL can also stimulate pathways promoting cell survival, proliferation and migration via activation of kinase signalling pathways (**Figure 2B**) (30). This non-canonical signalling may depend on the formation of a secondary signalling complex after initial DISC assembly (31), recruiting other factors including FADD, Caspase 8, RIPK1, TNF receptor-associated factor 2 (TRAF2) and inhibitor of NF-κB kinase subunit gamma (IKK-γ). Activation of non-canonical TRAIL signalling pathways may also be regulated by expression of DcR1, as antibody neutralisation of this decoy receptor can inhibit TRAIL-induced cell proliferation (30). Downstream non-canonical signalling by TRAIL has been shown to be effected by activation of kinase signalling e.g., NF-κB, p38, c-Jun N-terminal kinase (JNK), phosphatidylinositide 3-kinases (PI3K), Akt, and extracellular signal-regulated kinases (ERK); leading to activation of gene transcription (32). By activating NF-κB, TRAIL can also modulate levels of FADD-like interleukin-1β-converting enzyme)-inhibitory protein [c-FLIP; (33)], a negative regulator of caspase-mediated apoptosis—a further mechanism by which a cell may deviate from pro-apoptotic to pro-survival signalling in response to TRAIL.

Abbreviations: COPD, Chronic obstructive pulmonary disease; DcR1/2, Decoy receptor 1/2; DISC, Death-inducing signalling complex; DR4/5, Death receptor 4/5; EC, Endothelial cell; PAH, Pulmonary arterial hypertension; PF, Pulmonary fibrosis; SMC, Smooth muscle cell; SSc, Systemic sclerosis.

PULMONARY ARTERIAL HYPERTENSION

TRAIL has been implicated in the pathobiology of pulmonary arterial hypertension (30, 34). This is indicated by elevated levels of soluble TRAIL found in the serum of PAH patients, and increased abundance of serum TRAIL, which is associated with worsened clinical severity (35). The pulmonary vasculature is complex, and many aberrant processes can lead to disease. PAH is a multifactorial disorder characterised by remodelling of the pulmonary arteries and a progressive increase in pulmonary vascular resistance, leading to raised afterload on the right ventricle and ultimately right heart failure (36). The most frequent alterations are sustained pulmonary vasoconstriction and remodelling of the pulmonary arteries and arterioles. The arterial remodelling is characterised by medial hypertrophy, intimal fibrosis and often the development of thrombotic or plexiform lesions (37). Together, these processes cause the occlusion of small pulmonary arteries. Combined with the muscularisation and progressive obliteration of distal vessels, the subsequent loss of cross-sectional area generates increased right ventricular afterload. At the cellular level, the neoplastic pathologies of PAH are thought to be driven by excessive proliferation of apoptosis-resistant endothelial cells (ECs), together with proliferation and migration of medial smooth muscle cells (SMCs) and fibroblasts.

TRAIL immunoreactivity has been shown in pulmonary vascular lesions from idiopathic PAH patients (13) and increased TRAIL mRNA expression is detected in the lungs of rodent models of PAH (35, 38). Furthermore, TRAIL has been demonstrated—by knockout and by inactivation—as necessary for the development of PAH in multiple pre-clinical models of PAH (30). Reversal of established PAH in rodent models was also demonstrated by administration of an anti-TRAIL antibody (30). TRAIL knockout also had a similar protective effect in a Sugen5416 and hypoxia mouse model of PAH (34). Increased TRAIL, DR4 and DcR1 mRNA levels have been detected in explanted pulmonary artery SMCs from idiopathic PAH patients, compared to healthy control cells (30). Additionally, TRAIL depletion or blockade in rodent models of PAH is associated with reduced pulmonary arterial remodelling with fewer proliferating pulmonary artery SMCs (30, 34). This evidence indicates that TRAIL is a key promoter of the pulmonary arterial SMC proliferation associated with the pathogenic vascular remodelling in PAH. Recombinant TRAIL was also shown to induce proliferation and migration of idiopathic PAH patient pulmonary artery SMCs *in vitro*, via phosphorylation of ERK1/2 (30). The

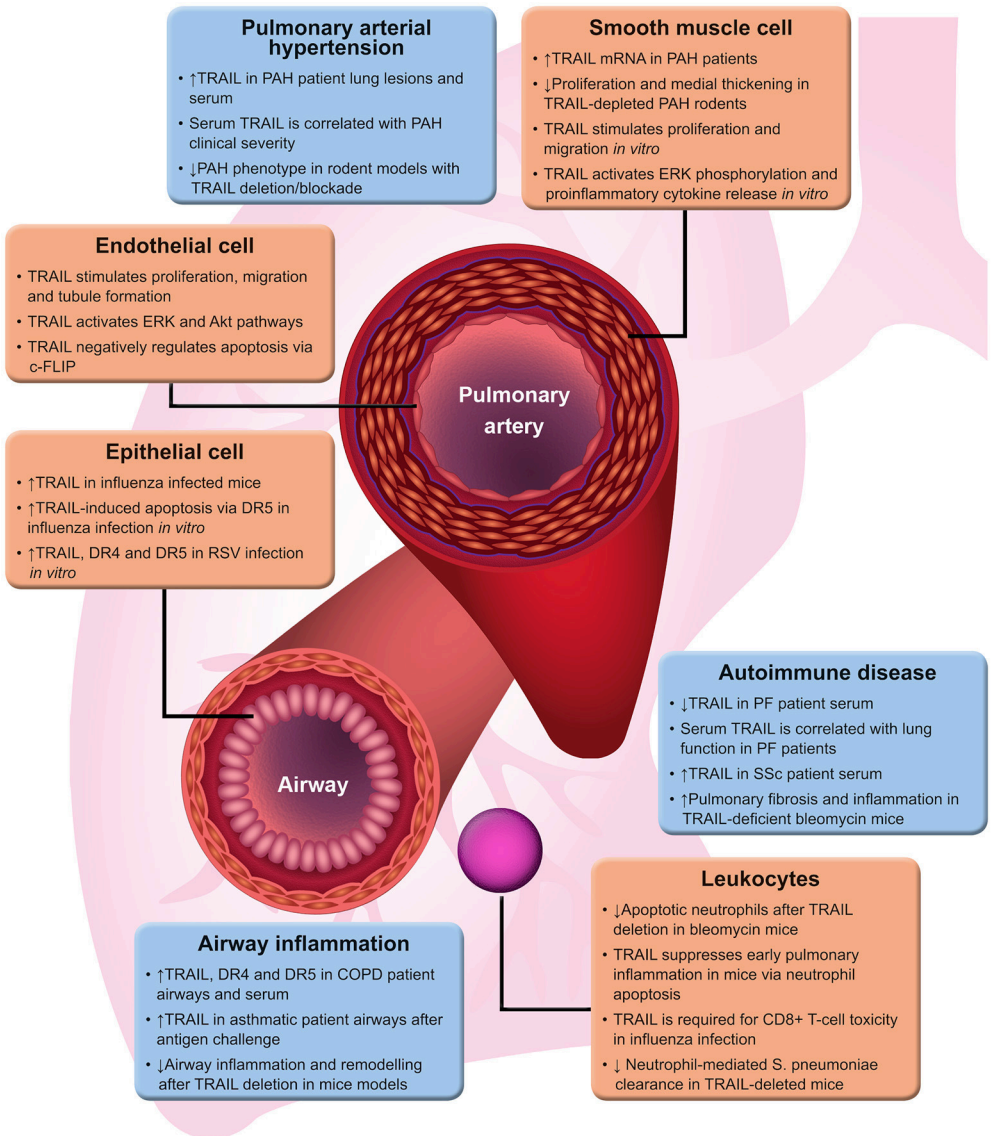


FIGURE 1 | TRAIL functions in lung disease. A brief summary of evidence for the varied roles of TRAIL in different lung diseases. ↑ and ↓ represent up- and down-regulation, respectively. Akt, protein kinase B; COPD, chronic obstructive pulmonary disease; DR4/5, TRAIL death receptor 4/5; ERK, extracellular signal-regulated kinase; PAH, pulmonary arterial hypertension; PF, pulmonary fibrosis; RSV, respiratory syncytial virus; SSc, systemic sclerosis; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.

pro-proliferative effect of TRAIL was reversed by the addition of DcR1 neutralising antibody, suggesting this decoy receptor is essential to non-canonical TRAIL signalling in pulmonary artery SMCs. Other studies have similarly demonstrated that TRAIL can stimulate proliferation and migration of vascular SMCs via non-canonical kinase signalling cascades (39, 40). Additionally, following activation of NF-κB, TRAIL has been shown to stimulate production and release of pro-inflammatory cytokines in vascular SMCs (41).

EC dysfunction is another key aspect of the angioproliferative state of pulmonary arteries in PAH. Several studies have demonstrated that TRAIL can stimulate angiogenic processes in vascular ECs *in vitro*, including proliferation (33, 42, 43), migration (33, 43, 44) and tubule formation (43). Similarly to non-canonical TRAIL signalling in SMCs, its angioproliferative effect in ECs has been linked to activation of Akt and ERK pathways (42), as well as upregulation of DcR2 (45). Conversely, TRAIL has also been demonstrated to have apoptotic (12, 46) and anti-angiogenic (47) effects on vascular ECs. The reason for this disparity is unclear, although each of these studies used a relatively high concentration of recombinant TRAIL (100 ng/ml), suggesting the pro-angiogenic signalling in endothelium may preferentially occur at lower TRAIL concentrations. In Cantarella et al. (33), high levels of TRAIL were shown to induce caspase

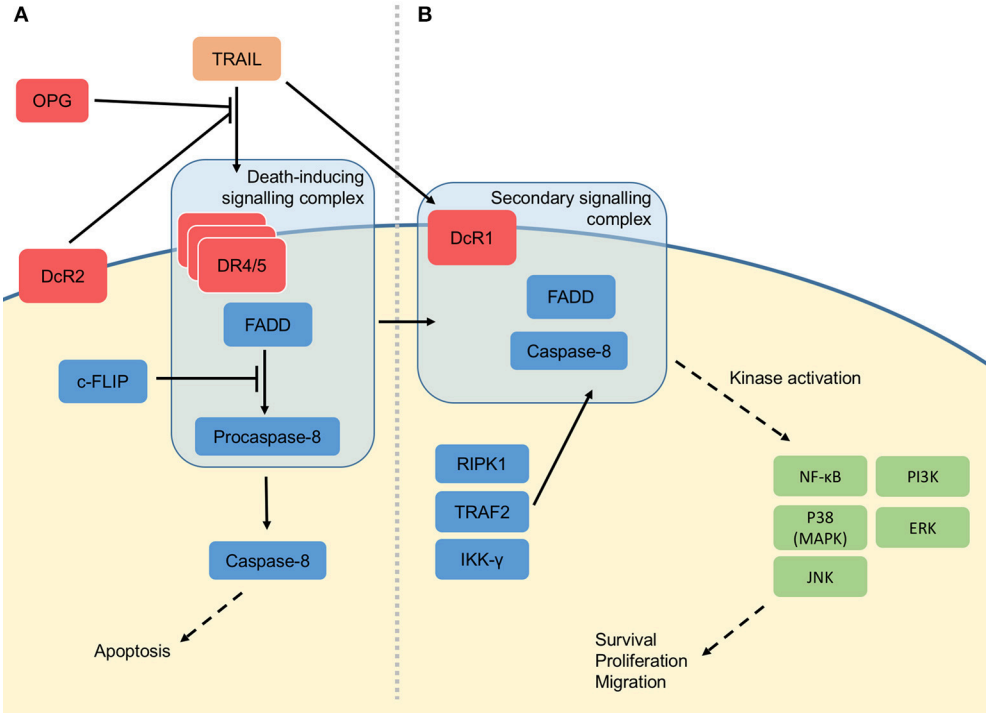


FIGURE 2 | Molecular signalling of TRAIL. **(A)** Three proteolytically-cleaved tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) proteins form a homotrimeric structure when binding death receptor 4 (DR4) or death receptor 5 (DR5) at the cell membrane. These are joined by Fas-associated death domain (FADD) and procaspase-8 to form the so-called death-inducing signalling complex (DISC). The DISC causes activation of the caspase cascade, leading to apoptosis of the cell. TRAIL may also bind the membrane decoy receptors (DcR1/2) or soluble osteoprotegerin (OPG), which do not contain a death domain, thus preventing TRAIL-induced apoptosis. Apoptosis can also be suppressed by (FADD-like interleukin-1β-converting enzyme)-inhibitory protein (c-FLIP), which inhibits the function of the DISC. **(B)** In the non-canonical signalling pathway, the receptor and ligand are thought to be lost, leaving FADD and caspase-8 to be joined by receptor-interacting serine/threonine-protein kinase 1 (RIPK1), TNF receptor-associated factor 2 (TRAF2) and inhibitor of NF-κB kinase subunit gamma (IKK-γ). This secondary signalling complex initiates protein kinase signalling pathways, leading to activation of kinases including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), p38 (mitogen-activated protein kinase; MAPK), c-Jun N-terminal kinase (JNK), phosphatidylinositide 3-kinases (PI3K) and extracellular signal-regulated kinase (ERK). The effects of these kinases include survival, proliferation and migration.

8-mediated apoptosis of ECs, whereas low levels of TRAIL were pro-angiogenic. Interestingly, these dose-dependent opposing effects of TRAIL in ECs were linked to modulation of levels of c-FLIP, a procaspase-8 homolog and negative regulator of apoptosis (33).

AUTOIMMUNE DISEASE

TRAIL is now known to have crucial functions in regulation of inflammation and immune response. These systems are significant in the pathogenesis of many forms of lung disease, including autoimmune disorders and respiratory infection in addition to pulmonary vascular disease (**Figure 2**). A role for TRAIL in regulating inflammation via apoptosis was highlighted in a knockout of the mouse TRAIL death receptor, as in addition to tumour formation, the mice were prone to chronic inflammation (48). Additionally, TRAIL has been demonstrated to suppress the early inflammatory response via apoptosis of neutrophils (49).

PAH is an associated complication in autoimmune disease, e.g., 7–12% of patients with systemic sclerosis (SSc) develop PAH (50, 51). SSc is a heterogeneous autoimmune disorder, characterised by tissue fibrosis and vascular injury. Pulmonary fibrosis (PF) is a condition often found in interstitial lung disease and autoimmune disorders of the connective tissue, including SSc and rheumatoid arthritis. Elevated serum TRAIL levels have been found in SSc patients compared to healthy controls, in addition to being elevated in SSc patients with either PAH or PF compared to those without pulmonary involvement (52), suggesting that TRAIL may also may play a key role. In contrast, soluble TRAIL has been found at lower levels in the serum of patients with the idiopathic form of PF than health controls (53). Within the idiopathic PF patient group, lung function—shown by transfer factor of the lung for carbon monoxide—was correlated with serum levels of TRAIL, suggesting it may have a protective role in idiopathic PF (53). Furthermore, a direct link to PF pathobiology is illustrated in TRAIL-deficient mice, where fibrosis in the bleomycin model of PF was enhanced in comparison to wild-type mice (53). In this model, TRAIL deletion also increased pulmonary inflammation (neutrophil counts in bronchoalveolar lavage fluid). The inflammatory phenotype in TRAIL knockout

mice was accompanied by a reduced number of apoptotic cells in lung tissue, with a corresponding reduction of apoptotic neutrophils in bronchoalveolar lavage fluid. This suggests that TRAIL-mediated apoptosis of neutrophils is a protective process in this form of PF.

AIRWAY INFLAMMATION

Contrary to its protective effect in idiopathic PF, TRAIL appears to have a detrimental role in the context of both acute and chronic airway inflammation, by upregulating inflammation and autoimmune responses (**Figure 2**). TRAIL is elevated in bronchoalveolar lavage fluid from asthmatic patients following antigen challenge, and isolated eosinophils express more TRAIL and DcR2, but less DR4 and DR5 (54). Deletion of the TRAIL gene in mice diminishes airway hyper-reactivity, inflammation and remodelling in an ovalbumin-induced model of allergic asthma (55, 56) and a rhinovirus-induced asthma model (57). Additionally, chronic asthmatic inflammation, remodelling and lung function are worsened by TRAIL deletion in mice infected as neonates with chlamydia (58).

Prolonged exposure to irritants and inflammation can lead to chronic obstructive pulmonary disease (COPD). A role for TRAIL in COPD has been highlighted by its elevated levels in the lungs of COPD patients. One study found increased TRAIL, DR4, DR5, and DcR1 protein in lung parenchyma from COPD patients (59). Higher levels of TRAIL, DR4, and DR5 mRNA were also found in airway epithelial brushing of COPD patients compared to healthy controls (60). Another study found increased levels of serum TRAIL and DR5 in COPD patients compared to healthy controls (61). Additionally, with the COPD patient group serum levels of TRAIL and DR5 were found to be inversely correlated with forced expiratory volume (61). Inflammation and alveolar cell apoptosis are key processes in many forms of COPD. A pro-apoptotic function of TRAIL in COPD was originally suggested, as emphysematous lung tissue is more sensitive to TRAIL-induced apoptosis than health lung (62). However, a pro-inflammatory element may also be important. In a chronic cigarette smoke-exposure mouse model of COPD, TRAIL mRNA, and protein expression was increased in the airway epithelium and parenchyma, and in mice with TRAIL deletion, airway inflammation—as well as remodelling—was reduced (60). The activation by TRAIL of both apoptotic and inflammatory pathways within COPD highlights its varied roles and how specific cell types are targeted—whether or not this is this mediated by differential receptor expression or some other mechanism remains unclear.

RESPIRATORY INFECTION

In lower respiratory tract infections, TRAIL has differing roles in immune response and damage to host tissues (**Figure 2**). Apoptosis of virus-infected cells is a key mechanism for clearance of viral infection and *in vitro*. In the context of influenza infection, TRAIL-induced apoptosis of human lung alveolar epithelial cells is enhanced; an effect which is inhibited by blocking DR5 (63). Similarly, TRAIL, DR4, and DR5 are strongly upregulated in response to respiratory syncytial virus infection in pulmonary epithelial cells, leading to increased sensitivity to apoptosis (64). In animal models, TRAIL expressed by CD8+ T-cells has been demonstrated as essential for viral immunity, with TRAIL knockout mice exhibiting increased influenza-associated morbidity and reduced CD8+ T-cell cytotoxicity (65–67). DR5 expression was also shown to be upregulated in influenza-infected pulmonary epithelial cells *in vivo* (63, 65).

In opposition to its protective role in viral clearance, other studies have shown that TRAIL expressed by macrophages is instrumental in damage to airways caused by apoptosis of alveolar epithelial cells in influenza infection (68, 69). Deletion of TRAIL in mice led to a reduction in mortality and the alveolar epithelial apoptosis and alveolar leakage associated with influenza virus pneumonia (68). This highlights an interesting situation whereby TRAIL death signalling may be used for host for viral clearance, while also assisting in viral infection via tissue damage. TRAIL has also been demonstrated as important in immune response to bacterial respiratory infection. In the context of Streptococcus pneumoniae infection, deletion of TRAIL in mice reduces bacterial clearance in the lungs and worsens survival—an effect that is reversed by treatment with TRAIL or DR5 agonist antibody (70). In the same study, neutrophils were found to be the key source of TRAIL (70).

CONCLUSIONS

As highlighted in this mini-review, TRAIL is multifaceted in a variety of lung diseases. TRAIL also has the ability to function as either pro-apoptotic or pro-survival depending on the cells type, and receptor expression on local tissue to mediate either protective or pathogenic mechanisms. The exact mechanism by which TRAIL modulates these functions is not fully understood, although regulation of TRAIL, and its cleavage, as well as the expression of receptors by specific cell types is clearly important in determining its effects. Further work is required to fully elucidate the divergent roles of TRAIL to gain a better understanding of the role it plays in underlying processes of lung disease, and its potential as a therapeutic agent—or target—depending on disease context.

AUTHOR CONTRIBUTIONS

AB was involved in conception and design of the work, drafting the article and final approval of the version to be published. HM and AL were involved in critical revision of the article and final approval of the version to be published.

FUNDING

AL is supported by British Heart Foundation Senior Basic Science Research Fellowship (FS/13/48/30453). AB is supported by Donald Heath Doctoral Training Fellowship.

REFERENCES

1. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. *J Clin Invest*. (1999) 2:155–62. doi: 10.1172/JCI6926
2. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. *Nat Med*. (1999) 2:157–63. doi: 10.1038/5517
3. Chinnaiyan AM, Prasad U, Shankar S, Hamstra DA, Shanaiah M, Chenevert TL, et al. Combined effect of tumor necrosis factor-related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy. *Proc Natl Acad Sci USA*. (2000) 4:1754–9. doi: 10.1073/pnas.030545097
4. Cretney E, Takeda K, Yagita H, Glaccum M, Peschon JJ, Smyth MJ. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. *J Immunol*. (2002) 3:1356–61. doi: 10.4049/jimmunol.168.3.1356
5. Johnstone RW, Frew AJ, Smyth MJ. The TRAIL apoptotic pathway in cancer onset, progression and therapy. *Nat Rev Cancer* (2008) 10:782–98. doi: 10.1038/nrc2465
6. Berg D, Stuhmer T, Siegmund D, Muller N, Giner T, Dittrich-Breiholz O, et al. Oligomerized tumor necrosis factor-related apoptosis inducing ligand strongly induces cell death in myeloma cells, but also activates proinflammatory signaling pathways. *FEBS J*. (2009) 23:6912–27. doi: 10.1111/j.1742-4658.2009.07388.x
7. Nguyen V, Cudrici C, Zernetkina V, Niculescu F, Rus H, Drachenberg C, et al. TRAIL, DR4 and DR5 are upregulated in kidneys from patients with lupus nephritis and exert proliferative and proinflammatory effects. *Clin Immunol*. (2009) 1:32–42. doi: 10.1016/j.clim.2009.02.011
8. Ehrhardt H, Fulda S, Schmid I, Hiscott J, Debatin KM, Jeremias I. TRAIL induced survival and proliferation in cancer cells resistant towards TRAIL-induced apoptosis mediated by NF-kappa B. *Oncogene* (2003) 25:3842–52. doi: 10.1038/sj.onc.1206520
9. Baader E, Toloczko A, Fuchs U, Schmid I, Beltinger C, Ehrhardt H, et al. Tumor necrosis factor-related apoptosis-inducing ligand-mediated proliferation of tumor cells with receptor-proximal apoptosis defects. *Cancer Res*. (2005) 17:7888–95. doi: 10.1158/0008-5472.CAN-04-4278
10. Azijli K, Yuvaraj S, Peppelenbosch MP, Wurdinger T, Dekker H, Joore J, et al. Kinome profiling of non-canonical TRAIL signaling reveals RIP1-Src-STAT3-dependent invasion in resistant non-small cell lung cancer cells. *J Cell Sci*. (2012) (Pt 19):4651–61. doi: 10.1242/jcs.109587
11. Trauzold A, Siegmund D, Schniewind B, Sipos B, Egberts J, Zorenkov D, et al. TRAIL promotes metastasis of human pancreatic ductal adenocarcinoma. *Oncogene* (2006) 56:7434–39. doi: 10.1038/sj.onc.1209719
12. Gochuico BR, Zhang J, Ma BY, Marshak-Rothstein A, Fine A. TRAIL expression in vascular smooth muscle. *Am J Physiol Lung Cell Mol Physiol*. (2000) 5:L1045–50. doi: 10.1152/ajplung.2000.278.5.L1045
13. Lawrie A, Waterman E, Southwood M, Evans D, Suntharalingam J, Francis S, et al. Evidence of a role for osteoprotegerin in the pathogenesis of pulmonary arterial hypertension. *Am J Pathol*. (2008) 1:256–64. doi: 10.2353/ajpath.2008.070395
14. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* (1995) 6:673–82. doi: 10.1016/1074-7613(95)90057-8
15. Degli-Esposti MA, Smolak PJ, Walczak H, Waugh J, Huang CP, DuBose RF, et al. Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J Exp Med*. (1997) 7:1165–70. doi: 10.1084/jem.186.7.1165
16. MacFarlane M, Ahmad M, Srinivasula SM, Fernandes-Alnemri T, Cohen GM, Alnemri ES. Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL. *J Biol Chem*. (1997) 41:25417–20. doi: 10.1074/jbc.272.41.25417
17. Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr Biol*. (1997) 12:1003–6. doi: 10.1016/S0960-9822(06)00422-2
18. Pan G, Ni J, Wei YF, Yu G, Gentz R, Dixit VM. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* (1997) 5327:815–8.
19. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, et al. The receptor for the cytotoxic ligand TRAIL. *Science* (1997) 5309:111–3.
20. Screaton GR, Mongkolsapaya J, Xu XN, Cowper AE, McMichael AJ, Bell JI. TRICK2, a new alternatively spliced receptor that transduces the cytotoxic signal from TRAIL. *Curr Biol*. (1997) 9:693–6.
21. Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, et al. Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J Biol Chem*. (1998) 23:14363–7. doi: 10.1074/jbc.273.23.14363
22. Schneider P, Olson D, Tardivel A, Browning B, Lugovskoy A, Gong D, et al. Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J Biol Chem*. (2003) 7:5444–54. doi: 10.1074/jbc.M210783200
23. Hymowitz SG, Christinger HW, Fuh G, Ultsch M, O'Connell M, Kelley RF, et al. Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. *Mol Cell*. (1999) 4:563–71. doi: 10.1016/S1097-2765(00)80207-5
24. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A, Ashkenazi A. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J Biol Chem*. (1996) 22:12687–90. doi: 10.1074/jbc.271.22.12687
25. Suliman A, Lam A, Datta R, Srivastava RK. Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. *Oncogene* (2001) 17:2122–33. doi: 10.1038/sj.onc.1204282
26. Dickens LS, Boyd RS, Jukes-Jones R, Hughes MA, Robinson GL, Fairall L, et al. A death effector domain chain DISC model reveals a crucial role for caspase-8 chain assembly in mediating apoptotic cell death. *Mol Cell*. (2012) 2:291–305. doi: 10.1016/j.molcel.2012.05.004
27. Pan G, Ni J, Yu G, Wei YF, Dixit VM. TRUNDD, a new member of the TRAIL receptor family that antagonizes TRAIL signalling. *FEBS Lett*. (1998) 1–2:41–45. doi: 10.1016/S0014-5793(98)00135-5
28. Miyashita T, Kawakami A, Nakashima T, Yamasaki S, Tamai M, Tanaka F, et al. Osteoprotegerin (OPG) acts as an endogenous decoy receptor in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis of fibroblast-like synovial cells. *Clin Exp Immunol*. (2004) 2:430–6. doi: 10.1111/j.1365-2249.2004.02534.x
29. Daniels RA, Turley H, Kimberley FC, Liu XS, Mongkolsapaya JP, Ch'En, et al. Expression of TRAIL and TRAIL receptors in normal and malignant tissues. *Cell Res*. (2005) 6:430–8. doi: 10.1038/sj.cr.7290311
30. Hameed AG, Arnold ND, Chamberlain J, Pickworth JA, Paiva C, Dawson S, et al. Inhibition of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) reverses experimental pulmonary hypertension. *J Exp Med*. (2012) 11:1919–35. doi: 10.1084/jem.20112716
31. Varfolomeev E, Maecker H, Sharp D, Lawrence D, Renz M, Vucic D, et al. Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand. *J Biol Chem*. (2005) 49:40599–608. doi: 10.1074/jbc.M509560200
32. Russo M, Mupo A, Spagnuolo C, Russo GL. Exploring death receptor pathways as selective targets in cancer therapy. *Biochem Pharmacol*. (2010) 5:674–82. doi: 10.1016/j.bcp.2010.03.011
33. Cantarella G, Di Benedetto G, Ribatti D, Saccani-Jotti G, Bernardini R. Involvement of caspase 8 and c-FLIPL in the proangiogenic effects of the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). *FEBS J*. (2014) 5:1505–13. doi: 10.1111/febs.12720
34. Dawson S, Arnold N, Pickworth J, Francis S, and Lawrie A. TRAIL deficient mice are protected from sugen/hypoxia induced pulmonary arterial hypertension. *Diseases* (2014) 3:260. doi: 10.3390/diseases3030260
35. Liu H, Yang E, Lu X, Zuo C, He Y, Jia D, et al. Serum levels of tumor necrosis factor-related apoptosis-inducing ligand correlate with the severity of pulmonary hypertension. *Pulm Pharmacol Ther*. (2015) 33:39–46. doi: 10.1016/j.pupt.2015.06.002

36. Hoeper MM, Bogaard HJ, Condliffe R, Frantz R, Khanna D, Kurzyna M, et al. Definitions and diagnosis of pulmonary hypertension. *J Am Coll Cardiol*. (2013) 25 (Suppl.):D42–50. doi: 10.1016/j.jacc.2013.10.032
37. Pietra GG, Edwards WD, Kay JM, Rich S, Kernis J, Schloo B, et al. Histopathology of primary pulmonary hypertension. A qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. *Circulation* (1989) 5:1198–206. doi: 10.1161/01.CIR.80.5.1198
38. Lawrie A, Hameed AG, Chamberlain J, Arnold N, Kennerley A, Hopkinson K, et al. Paigen diet-fed apolipoprotein E knockout mice develop severe pulmonary hypertension in an interleukin-1-dependent manner. *Am J Pathol*. (2011) 4:1693–705. doi: 10.1016/j.ajpath.2011.06.037
39. Secchiero P, Zerbinati C, Rimondi E, Corallini F, Milani D, Grill V, et al. TRAIL promotes the survival, migration and proliferation of vascular smooth muscle cells. *Cell Mol Life Sci*. (2004) 15:1965–74. doi: 10.1007/s00018-004-4197-6
40. Kavurma MM, Schoppet M, Bobryshev YV, Khachigian LM, Bennett MR. TRAIL stimulates proliferation of vascular smooth muscle cells via activation of NF-kappaB and induction of insulin-like growth factor-1 receptor. *J Biol Chem*. (2008) 12:7754–62. doi: 10.1074/jbc.M706927200
41. Song S, Choi K, Ryu SW, Kang SW, Choi C. TRAIL promotes caspase-dependent pro-inflammatory responses via PKCdelta activation by vascular smooth muscle cells. *Cell Death Dis*. (2011) 2:*e*223. doi: 10.1038/cddis.2011.103
42. Secchiero P, Gonelli A, Carnevale E, Milani D, Pandolfi A, Zella D, et al. TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. *Circulation* (2003) 17:2250–56. doi: 10.1161/01.CIR.0000062702.60708.C4
43. Cartland SP, Genner SW, Zahoor A, Kavurma MM. Comparative evaluation of TRAIL, FGF-2 and VEGF-a-induced angiogenesis *in vitro* and *in vivo*. *Int J Mol Sci*. (2016) 12:E2025. doi: 10.3390/ijms17122025
44. Zauli G, Pandolfi A, Gonelli A, Di Pietro R, Guarnieri S, Ciabattoni G, et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) sequentially upregulates nitric oxide and prostanoid production in primary human endothelial cells. *Circ Res*. (2003) 7:732–40. doi: 10.1161/01.RES.0000067928.83455.9C
45. Harith HH, Di Bartolo BA, Cartland SP, Genner S, Kavurma MM. Insulin promotes vascular smooth muscle cell proliferation and apoptosis via differential regulation of tumor necrosis factor-related apoptosis-inducing ligand. *J Diabetes*. (2016) 4:568–78. doi: 10.1111/1753-0407.12339
46. Alladina SJ, Song JH, Davidge ST, Hao C, Easton AS. TRAIL-induced apoptosis in human vascular endothelium is regulated by phosphatidylinositol 3-kinase/Akt through the short form of cellular FLIP and Bcl-2. *J Vasc Res*. (2005) 4:337–47. doi: 10.1159/000086599
47. Cantarella G, Risuglia NR, Dell'eva, Lempereur L, Albini A, Pennisi G, et al. TRAIL inhibits angiogenesis stimulated by VEGF expression in human glioblastoma cells. *Br J Cancer* (2006) 10:1428–35. doi: 10.1038/sj.bjc.6603092
48. Finnberg N, Klein-Szanto AJ, El-Deiry W S. TRAIL-R deficiency in mice promotes susceptibility to chronic inflammation and tumorigenesis. *J Clin Invest*. (2008) 1:111–23. doi: 10.1172/JCI29900
49. McGrath EE, Marriott HM, Lawrie A, Francis SE, Sabroe I, Renshaw SA, et al. TNF-related apoptosis-inducing ligand (TRAIL) regulates inflammatory neutrophil apoptosis and enhances resolution of inflammation. *J Leukoc Biol*. (2011) 5:855–65. doi: 10.1189/jlb.0211062
50. Mukerjee D, St George D, Coleiro B, Knight C, Denton CP, Davar J, et al. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hypertension: application of a registry approach. *Ann Rheum Dis*. (2003) 11:1088–93. doi: 10.1136/ard.62.11.1088
51. Hachulla E, Gressin V, Guillevin L, Carpentier P, Diot E, Sibilia J, et al. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthritis Rheum*. (2005) 12:3792–800. doi: 10.1002/art.21433
52. Azab NA, Rady HM, Marzouk S A. Elevated serum TRAIL levels in scleroderma patients and its possible association with pulmonary involvement. *Clin Rheumatol*. (2012) 9:1359–64. doi: 10.1007/s10067-012-2023-3
53. McGrath EE, Lawrie A, Marriott HM, Mercer P, Cross SS, Arnold N, et al. Deficiency of tumour necrosis factor-related apoptosis-inducing ligand exacerbates lung injury and fibrosis. *Thorax*. (2012) 9:796–803. doi: 10.1136/thoraxjnl-2011-200863
54. Robertson NM, Zangrilli JG, Steplewski A, Hastie A, Lindemeyer RG, Planeta MA, et al. Differential expression of TRAIL and TRAIL receptors in allergic asthmatics following segmental antigen challenge: evidence for a role of TRAIL in eosinophil survival. *J Immunol*. (2002) 10:5986–96. doi: 10.4049/jimmunol.169.10.5986
55. Weckmann M, Collison A, Simpson JL, Kopp MV, Wark PA, Smyth MJ, et al. Critical link between TRAIL and CCL20 for the activation of TH2 cells and the expression of allergic airway disease. *Nat Med*. (2007) 11:1308–15. doi: 10.1038/nm1660
56. Collison A, Li JA, Pereira de Siqueira, Zhang J, Toop HD, Morris JC, et al. Tumor necrosis factor-related apoptosis-inducing ligand regulates hallmark features of airways remodeling in allergic airways disease. *Am J Respir Cell Mol Biol*. (2014) 1:86–93. doi: 10.1165/rcmb.2013-0490OC
57. Girkin JL, Hatchwell LM, Collison AM, Starkey MR, Hansbro PM, Yagita H, et al. TRAIL signaling is proinflammatory and proviral in a murine model of rhinovirus 1B infection. *Am J Physiol Lung Cell Mol Physiol*. (2017) 1:L89–99. doi: 10.1152/ajplung.00200.2016
58. Starkey MR, Nguyen DH, Essilfie AT, Kim RY, Hatchwell LM, Collison AM, et al. Tumor necrosis factor-related apoptosis-inducing ligand translates neonatal respiratory infection into chronic lung disease. *Mucosal Immunol*. (2014) 3:478–88. doi: 10.1038/mi.2013.65
59. Morissette MC, Vachon-Beaudoin G, Parent J, Chakir J, Milot J. Increased p53 level, Bax/Bcl-x(L) ratio, and TRAIL receptor expression in human emphysema. *Am J Respir Crit Care Med*. (2008) 3:240–7. doi: 10.1164/rccm.200710-1486OC
60. Haw TJ, Starkey MR, Nair PM, Pavlidis S, Liu G, Nguyen DH, et al. A pathogenic role for tumor necrosis factor-related apoptosis-inducing ligand in chronic obstructive pulmonary disease. *Mucosal Immunol*. (2016) 4:859–72. doi: 10.1038/mi.2015.111
61. Wu Y, Shen Y, Zhang J, Wan C, Wang T, Xu D, et al. Increased serum TRAIL and DR5 levels correlated with lung function and inflammation in stable COPD patients. *Int J Chron Obstruct Pulmon Dis*. (2015) 2015:2405–2412. doi: 10.2147/COPD.S92260
62. Morissette MC, Parent J, Milot J. The emphysematous lung is abnormally sensitive to TRAIL-mediated apoptosis. *Respir Res*. (2011) 12:105. doi: 10.1186/1465-9921-12-105
63. Brincks EL, Kucaba TA, Legge KL, Griffith TS. Influenza-induced expression of functional tumor necrosis factor-related apoptosis-inducing ligand on human peripheral blood mononuclear cells. *Hum Immunol*. (2008b) 10:634–46. doi: 10.1016/j.humimm.2008.07.012
64. Kotelkin A, Prikhod'ko EA, Cohen JI, Collins PL, Bukreyev A. Respiratory syncytial virus infection sensitizes cells to apoptosis mediated by tumor necrosis factor-related apoptosis-inducing ligand. *J Virol*. (2003) 17:9156–72. doi: 10.1128/JVI.77.17.9156-9172.2003
65. Ishikawa E, Nakazawa M, Yoshinari M, Minami M. Role of tumor necrosis factor-related apoptosis-inducing ligand in immune response to influenza virus infection in mice. *J Virol*. (2005) 12:7658–63. doi: 10.1128/JVI.79.12.7658-7663.2005
66. Brincks EL, Katewa A, Kucaba TA, Griffith TS, Legge KL. CD8 T cells utilize TRAIL to control influenza virus infection. *J Immunol*. (2008) 7:4918–25. doi: 10.4049/jimmunol.181.7.4918
67. Brincks EL, Gurung P, Langlois RA, Hemann EA, Legge KL, Griffith TS. The magnitude of the T cell response to a clinically significant dose of influenza virus is regulated by TRAIJL. *J Immunol*. (2011) 9:4581–8. doi: 10.4049/jimmunol.1002241
68. Herold S, Steinmueller M, von Wulffen W, Cakarova L, Pinto R, Pleschka S, et al. Lung epithelial apoptosis in influenza virus pneumonia: the role of

macrophage-expressed TNF-related apoptosis-inducing ligand. *J Exp Med*. (2008) 13:3065–77. doi: 10.1084/jem.20080201

69. Peteranderl C, Morales-Nebreda L, Selvakumar B, Lecuona E, Vadasz I, Morty RE, et al. Macrophage-epithelial paracrine crosstalk inhibits lung edema clearance during influenza infection. *J Clin Invest*. (2016) 4:1566–80. doi: 10.1172/JCI83931
70. Steinwede K, Henken S, Bohling J, Maus R, Ueberberg B, Brumshagen C, et al. TNF-related apoptosis-inducing ligand (TRAIL) exerts therapeutic efficacy for the treatment of pneumococcal pneumonia in mice. *J Exp Med*. (2012) 11:1937–52. doi: 10.1084/jem.201 20983

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 03 August 2018
doi: 10.3389/fmed.2018.00217

The Role of Neutrophils and Neutrophil Elastase in Pulmonary Arterial Hypertension

Shalina Taylor[1,2†], Omar Dirir[3†], Roham T. Zamanian[2,4], Marlene Rabinovitch[1,2] and A. A. Roger Thompson[3]*

[1] Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, United States, [2] Vera Moulton Wall Center for Pulmonary Vascular Disease, Stanford University, Stanford, CA, United States, [3] Infection, Immunity, and Cardiovascular Disease, University of Sheffield, Sheffield, United Kingdom, [4] Division of Pulmonary and Critical Care Medicine, Stanford University School of Medicine, Stanford, CA, United States

Pulmonary arterial hypertension (PAH) is a severe vasculopathy characterized by the presence of fibrotic lesions in the arterial wall and the loss of small distal pulmonary arteries. The vasculopathy is accompanied by perivascular inflammation and increased protease levels, with neutrophil elastase notably implicated in aberrant vascular remodeling. However, the source of elevated elastase levels in PAH remains unclear. A major source of neutrophil elastase is the neutrophil, an understudied cell population in PAH. The principal function of neutrophils is to destroy invading pathogens by means of phagocytosis and NET formation, but proteases, chemokines, and cytokines implicated in PAH can be released by and/or prime and activate neutrophils. This review focuses on the contribution of inflammation to the development and progression of the disease, highlighting studies implicating neutrophils, neutrophil elastase, and other neutrophil proteases in PAH. The roles of cytokines, chemokines, and neutrophil elastase in the disease are discussed and we describe new insight into the role neutrophils potentially play in the pathogenesis of PAH.

Keywords: neutrophils, neutrophil elastase, pulmonary hypertension, pulmonary arterial hypertension, vascular remodeling

OPEN ACCESS

Edited by:
Paul Anthony Corris, Newcastle University, United Kingdom

Reviewed by:
Eleni Papakonstantinou, Aristotle University of Thessaloniki, Greece
John Simpson, Newcastle University, United Kingdom

***Correspondence:**
A. A. Roger Thompson
r.thompson@sheffield.ac.uk

† These authors have contributed equally to this work.

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *26 April 2018*
Accepted: *16 July 2018*
Published: *03 August 2018*

Citation:
Taylor S, Dirir O, Zamanian RT, Rabinovitch M and Thompson AAR (2018) The Role of Neutrophils and Neutrophil Elastase in Pulmonary Arterial Hypertension. Front. Med. 5:217. doi: 10.3389/fmed.2018.00217

PULMONARY ARTERIAL HYPERTENSION AND INFLAMMATION

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by thickening, and progressive occlusion of distal arteries in the lung related to vascular cell dysfunction, and perivascular inflammation. As a consequence of elevation in pulmonary arterial pressure, the right ventricle hypertrophies and later becomes dysfunctional leading to right heart failure (1, 2). Perivascular inflammation has been observed in all subsets of PAH and correlates with clinical markers of disease progression such as an increase in pulmonary vascular resistance and a decrease in the 6-min walk test (3, 4). The consequences of perivascular inflammation include cytokine production by vascular and inflammatory cells, and degradation of the extracellular matrix (ECM) by proteases (5, 6). Both the increased cytokine production and the peptides that are released as a result of ECM degradation cause activation and recruitment of circulating immune cells (6, 7). Neutrophils are among the cells that are recruited, and these cells release proteolytic enzymes, including neutrophil elastase (NE), that cause vascular injury (8). Although many aspects of perivascular inflammation can cause progressive PAH, this review will focus on neutrophils and NE. We will review evidence of neutrophil accumulation in PAH, discuss the role of NE and other proteases in driving vascular remodeling and highlight potential

interactions between neutrophils, NE and the key genetic driver of PAH, bone morphogenetic protein receptor type 2 (BMPR2).

NEUTROPHILS AND PAH

Neutrophils are the predominant circulating leukocyte population and are important in modulating innate and adaptive immunity. They are early responders and are recruited to sites of sterile inflammation and infection by environmental cues.

Relatively little attention has been given to the role of neutrophils in the pathogenesis of PAH. Neutrophil and macrophage perivascular accumulation has been observed in murine lungs in association with hypoxic pulmonary hypertension (PH) and in monocrotaline-induced PH in rats (9, 10). It has also been established that the neutrophil to lymphocyte ratio is increased in PAH patients when compared to healthy controls (11) and that increased neutrophil to lymphocyte ratio positively correlates with New York Heart Association (NYHA) functional class (FC) and predicts event free survival (12, 13). It is not clear why this relative increase in circulating neutrophils is associated with PAH progression. However, neutrophils produce a wide range of substances that could contribute to vascular remodeling and promote inflammation in PAH. For example, myeloperoxidase (MPO), a catalyst for reactive oxygen species (ROS) formation, has recently been implicated in the pathophysiology of PAH (14). Two independent cohorts of PAH patients had increased plasma MPO relative to healthy controls and $Mpo^{-/-}$ mice displayed reduced right ventricular pressures following hypoxic exposure (14). Interestingly, although MPO can reduce nitric oxide (NO) bioavailability in other vascular beds (15), there was no evidence supporting a difference in NO availability between wild-type and MPO-deficient mice (14). Instead, activation of a Rho kinase pathway by MPO was found to drive pulmonary vasoconstriction and SMC proliferation (14).

In addition to MPO and ROS, neutrophils produce proteolytic enzymes. Activity of these enzymes is tightly controlled by endogenous inhibitors, but excessive protease activity has the potential to destroy tissue and cause extensive fibrotic remodeling, leading to organ failure (16). Indeed neutrophil proteases are implicated in airway and parenchymal lung diseases [reviewed by Taggart et al. (17)] and are known to have roles in systemic vascular pathology (18, 19).

Neutrophil granule proteases include members of the serine protease family (NE, cathepsin G, proteinase 3) and matrix metalloproteinases [neutrophil collagenase (MMP-8) and gelatinase (MMP-9)] (20). Of these proteases, NE has been most heavily implicated in the pathogenesis of PAH, as described below.

SOURCES OF NEUTROPHIL ELASTASE IN PAH

Neutrophils are the dominant cellular source of NE but it is also produced by macrophages and smooth muscle cells (SMC) (21–24). There is evidence of both augmented NE release from neutrophils isolated from PAH patients (25) and upregulation of endogenous NE in PAH patient SMCs and in experimental animal models of PH (23, 26, 27). Macrophages are observed in the plexiform lesions of PAH patient lungs and activated macrophages release leukotriene B4, which induces endothelial cell (EC) injury and results in EC apoptosis, but could also promote neutrophil recruitment (28). Moreover, *in vitro* work suggests that human alveolar macrophages internalize NE through ingestion of apoptotic neutrophils, and act as a vehicle for the enzyme, transporting it to the tissues and subsequently releasing the active form (29). However, further work is required to show conservation of this mechanism in perivascular or interstitial lung macrophages.

NE is dispensed by neutrophils during degranulation and upon release of neutrophil extracellular traps (NETs). NETs consist of decondensed chromatin decorated with NE and other antimicrobial proteases. NE plays an important role in NET release, evidenced by reduced NET formation in the presence of NE inhibitors and in NE knockout mice (30). Upon stimulation, NE is discharged from azurophilic granules and translocates to the nucleus where it contributes to histone degradation, facilitating chromatin decondensation (30). Depending on the stimulus, chromatin is then expelled via vesicles (31) or suicidal NETosis (32), reviewed by Jorch et al. (33).

NETs assist in microbial trapping and killing (32), but non-infectious roles for NETs are emerging and NETs have been identified in vascular pathologies such as atherosclerosis and PAH (34, 35). Detrimental consequences attributed to NETs include nuclear factor kappa-light-chain-enhancer of activated B cells-dependent PA EC pathological angiogenesis, release of the vasoactive agent endothelin-1, and promotion of SMC proliferation (34). NETs can also directly induce EC cell death (36) and promote thrombus formation (37). In PAH lung tissue, markers of NET formation (DNA, myeloperoxidase, and citrullinated histone H3) have been identified in proximity to plexiform lesions and circulating DNA levels were elevated in IPAH patient plasma (34), although this study did not investigate whether IPAH patient neutrophils are more prone to releasing NETs.

Studies prior to the identification of NETs had suggested that binding of NE to DNA inhibited the activity of the protease (38). However, Kolaczkowska et al. used an *in vivo* zymography assay to demonstrate that NE associated with NETs remains proteolytically active (39). This implies that NE attached to NETs is shielded from endogenous anti-protease activity and thus high NET levels in PAH may represent an important source of active NE.

NEUTROPHIL ELASTASE IN THE PATHOLOGY OF PAH

Elastolytic activity has been implicated in the pathogenesis of PAH for over three decades, with evidence of fragmented internal elastic laminae in the pulmonary arteries of children with congenital heart defects (40). There is a clear temporal

relationship between increased NE activity and vascular changes in PAH experimental rat models (41). Furthermore, inhibition of NE enzymatic activity not only attenuates the progression of PAH but can also reverse the disease process in experimental models. This was first shown in the monocrotaline model where a progressive and fatal form of PH was reversed by elastase inhibitors in association with normalization of hemodynamic changes of PH and structural abnormalities that included extensive occlusive muscularization (26). Nickel et al. also demonstrated reversal of hemodynamic and histological measures of PH in the Sugen/Hypoxia rat model following treatment with recombinant human elafin (42). Increased NE activity was demonstrated in the lungs of rats with Sugen/Hypoxia-induced PH and this was attenuated by elafin (42). Importantly, relevance to human disease was confirmed through use of a human pulmonary artery explant model. Tissue sections containing pulmonary arteries were isolated from the explanted lungs of PAH patients and treatment with elafin induced regression of neointimal changes and improved measures of vessel lumen size (42).

Release of NE can stimulate adverse remodeling by degrading virtually all the components of the ECM in addition to elastin, and including collagen, fibronectin, and laminin (43). As a consequence, degraded ECM releases bioactive peptides and growth factors such as epidermal growth factor (EGF) and fibroblast growth factor (FGF), which have both mitogenic and motogenic effects on SMCs and fibroblasts (44–47). Additionally, heightened NE activity leads to the activation of matrix metalloproteinases (MMPs), which could potentiate ECM degradation, and induction of tenascin C, a glycoprotein associated with the upregulation of growth factor receptors and proliferation of SMCs (48). The sub-endothelial deposition of tenascin C and fibronectin appears to be a chemotactic factor for PA SMCs, facilitating their migration and the formation of neointimal lesions (48, 49). Conversely, inhibition of NE leads to reduced tenascin C, induction of SMC apoptosis, and regression of pulmonary artery (PA) medial hypertrophy (26).

The role of elastase in vascular remodeling was further explored using transgenic mice that over-express the S100A4 protein (23, 27). Following infection with the murine herpes virus, MHV-68, S100A4 mice develop histological features of human PAH including neointima formation (27). These vascular lesions display fragmented elastic laminae associated with heightened lung elastase activity (27). Infusion of recombinant human elafin, an endogenous elastase inhibitor, reduced the number and severity of neointimal lesions in this model (23). Furthermore, using FLAG-tagged elafin, NE was identified as the serine elastase responsible for the elevation in elastase activity in the S100A4 lungs (23). While this finding implies that NE is the dominant target in this model, the relative importance of NE above other proteases in human disease has not been confirmed and the potential roles of other proteases will be discussed below.

The source of NE in the S100A4 lungs was localized not only to neutrophils but also to PA SMCs and, as indicated above, this finding was conserved in human disease, evidenced by cultured PA SMCs from IPAH patients expressing higher levels of NE than cells from control donor lungs (23). Interestingly, *ex vivo* perfusion of S100A4 lungs with porcine pancreatic elastase suggested that the elastin fibers in these mice were more prone to degradation (23). This implies that certain viral infections, and potentially other inflammatory stimuli, may predispose the vasculature to pathological remodeling upon exposure to elastase.

NEUTROPHIL ELASTASE AND PERPETUATION OF INFLAMMATION IN PAH

In addition to the impact of NE on SMC migration and proliferation, this enzyme contributes to PAH pathogenesis by proteolytic modification of cytokines. For example, NE promotes IL-1β activity by cleaving the pro-isoform of IL-1β in human coronary ECs, thereby increasing the secretion of the bioactive form in extracellular vesicles (50). Notably, secretion of active IL-1β could promote neutrophil survival, an example of positive regulation of neutrophil activity by cytokines (51).

NE also cleaves CXCL12 (SDF-1 alpha), a chemokine involved in the regulated release of neutrophils from the bone marrow (52–54). CXCL12 inactivation would promote mobilization of neutrophils into the circulation, particularly in the context of elevated plasma IL-8 levels, as observed in PAH patients (4, 54). On the other hand, NE can inactivate tumor-necrosis factor (55) and IL-6 (56), suggesting that there is a balance that must be maintained between elastase and anti-elastase activity. The chronic perivascular inflammatory phenotype of PAH patients characterized by elevated levels of circulating and tissue cytokines, is consistent with persistent neutrophil activation. Indeed, many of the cytokines elevated in PAH are known to enhance neutrophil function and survival and may thus perpetuate neutrophil-mediated inflammation (**Table 1**).

OTHER PROTEASES IMPLICATED IN TISSUE REMODELING IN PAH

As mentioned above, other neutrophil proteases may contribute to the vascular remodeling observed in PAH. For example, MMP-9 expression is upregulated in human plexiform pulmonary arterial lesions (61) and in lungs isolated from rats with monocrotaline-induced pulmonary hypertension (62). Furthermore, transgenic overexpression of human MMP-9 exacerbated monocrotaline-induced pulmonary hypertension in mice (63). The role of other MMPs and MMP inhibitors in PAH has been reviewed by Chelladuri et al. (64).

This review has focused mainly on neutrophil proteases, but it should be noted that many other cells release proteases. For example, upregulation of MMP-9 has been detected in natural killer cells from patients with PAH (65) and it is also released by monocytes and macrophages (66). Further examples of important non-neutrophil proteases are chymase and tryptase, the main proteases released by mast cells. Heath and Yacoub (67) noted increased perivascular mast cell numbers in both primary and secondary forms of pulmonary hypertension compared to controls, an observation confirmed more recently by others (68,

TABLE 1 | Cytokines elevated in PAH and known to alter neutrophil function.

Cytokine	Release	Priming	Adhesion	Chemotaxis	ROS	Survival	References
CCL2	x		x	x		x	(57)
IL-1α	x						(58)
IL-1β	x	x	x	x	x	x	(4, 58, 59)
IL-4	x						(4)
IL-6	x		x	x			(4, 58–60)
IL-8	x	x	x	x			(4)
IL-10	x						(4)
IL-12	x						(4)
Interferon- γ	x	x				x	(4)
TNF-α	x	x	x	x	x	x	(4, 58)

The x symbols indicate which functions are altered by each cytokine. ROS, reactive oxygen species generation; CCL2, C-C Motif Chemokine Ligand 2, also known as monocyte chemoattractant protein 1; IL, interleukin; TNF, tumor necrosis factor.

69). Circulating tryptase levels were elevated in PAH patients compared to controls and correlated with disease severity as assessed by brain natriuretic peptide level (68). In this study a small number of patients were treated with mast cell stabilizers, but there were no changes in clinical endpoints such as 6-min walk distance or BNP level.

A key question about protease activity in any disease process is how the protease evades suppression by endogenous inhibitors. NE is inhibited by several anti-proteases including α1-antitrypsin, secretory leucocyte peptidase inhibitor (SLPI) and elafin. On the one hand it is possible that localized release of the protease simply overcomes anti-protease activity in the immediate microenvironment. Indeed, markers of pre-inhibited elastase activity have been reported including the cleaved fibrinogen product, Aα-Val^{360} (70). On the other hand, proteases may be shielded from their inhibitors by attachment to NETS (discussed above), or via transport in exosomes. Neutrophil-derived exosomes degrade NE substrates and induce emphysematous changes in murine lungs following intra-tracheal administration (71). It is also possible that proteases have enzyme-independent functions but the beneficial consequences of elafin treatment in animal models of PH and in lung explant models (23, 42) would favor the hypothesis that there is an excess of protease activity. Interestingly, however, elafin may exert protective effects independently of protease inhibition as described below.

NEUTROPHILS AND PAH RELATED TO BMPR2 DEFICIENCY

The bone morphogenetic protein receptor 2 (BMPR2) signaling pathway has become a key focus of investigation since *BMPR2* gene mutations were identified as the main predisposing risk factor in the heritable forms of PAH (HPAH) (72), with dysfunction in the signaling pathway present in all subtypes of PAH (73). However, although present in a high proportion of HPAH cases—identified in 70% of familial PAH cases (74)—the autosomal dominant *BMPR2* mutation exhibits low penetrance with 70–80% of those carrying the mutation never developing PAH (75). This, and the lack of spontaneous PAH in most heterozygous *BMPR2* animal models, implies that a second insult or background genetic variants are needed for predisposed individuals to develop PAH. Inflammation has been proposed as a second hit which promotes adverse remodeling when there is a loss of BMPR2.

BMPR2 is expressed by all cells in the arterial wall but by far the highest level of expression is present in endothelial cells (73). Reduced BMPR2 signaling is related both to excessive vascular smooth muscle proliferation (76) and exaggerated PA EC apoptosis (77). A study by Burton et al. (78) investigated the role of BMPR2 in maintaining the barrier function of PA ECs and in suppressing inflammation within the pulmonary vasculature. Using static and flow-based *in vitro* systems, they were able to demonstrate that a reduction in BMPR2 expression facilitated neutrophil transmigration across the PA EC monolayer and reported that a lack of BMPR2 led to overexpression of IL-8, which in turn led to the recruitment of neutrophils. Similarly, loss of BMPR2 can lead to heightened expression of IL-6, an inducer of SMC proliferation (79). Taken together, BMPR2 plays a role in dampening inflammatory signals in the pulmonary vasculature that could influence neutrophil recruitment and elastase activity.

Conversely, neutrophils may also impact BMPR2 function by releasing NE and degrading BMP9, an anti-angiogenic ligand for the BMPR2/ALK1 heterodimer present on PA ECs (80). BMP9 has been shown to circulate in humans at biologically active levels (81), maintaining vascular quiescence. Li et al. showed that BMP9 is readily cleaved by NE, released by activated neutrophils (80). Further work by this group demonstrated that the administration of recombinant BMP9 reversed established PH in *BMPR2*-deficient mice, overcoming reduced BMPR2 levels, and preventing lung vascular leakage (82). Paradoxically, Appleby et al. demonstrated that BMP9 could have a pro-inflammatory role and, in fact, facilitate neutrophil recruitment to the pulmonary vasculature by activated endothelial cells (83). However, the mouse model used in that study was acute endotoxemia, so this may not relate directly to PAH pathogenesis.

Overall, it appears that inflammation-driven release of NE can suppress BMPR2 signaling. Nickel and colleagues investigated the impact of the NE inhibitor elafin on BMPR2 signaling in PAH (42). In the Sugen/Hypoxia rat model of PH, elafin improved

pulmonary endothelial expression of apelin, a BMPR2 target gene. Loss of apelin expression in the pulmonary endothelium during PAH is associated with the failure to repress the release of fibroblast growth factor-2 (FGF2) (84). Nickel et al. also found that in human cells, elafin augmented BMPR2 interactions with caveolin-1, another downstream target of BMPR2 signaling (42). Unfortunately, changes in BMP9 levels following elafin administration were not measured. Nonetheless, the findings suggest that improving BMPR2 signaling in addition to the beneficial sequelae of inhibiting NE, described earlier in this review, could reverse the vascular remodeling observed in PAH. Interestingly, targeting NE activity may be of particular importance in patients with BMPR2 mutations, as histological assessment of pulmonary vessels in such patients demonstrates a reduction in elastin and fibrillin-1, the two major constituents of elastic fibers (85). Furthermore, pulmonary artery elastic fibers from mice with compound *Bmpr2/1a* mutations were more susceptible to elastase-mediated degradation of elastic fibers compared to wild-type mice (85).

SUMMARY

Collectively the studies discussed above implicate the neutrophil and neutrophil products such as MPO, proteases and NETs in the pathogenesis of PAH. Neutrophils are attractive candidates in contributing to vascular changes seen in PAH because they are among the first cells to arrive at sites of inflammation. The evidence of increased circulating elastase in PAH patients and fragmented elastic lamina in PAH vessels, highlights a key pathogenic role for proteolytic enzymes and in particular NE. NE has a wide range of targets, but we describe evidence for how it could contribute to PAH pathogenesis by modulating the activity of cytokines and degrading ECM releasing growth factors that promote remodeling. Moreover, the release of NE and NETs is likely to alter the local inflammatory environment, augmenting leukocyte responses and further driving inflammation in PAH. It is also evident that reduced BMPR2 receptor signaling cooperatively interacts with the sequelae of inflammation and neutrophil activation in contributing to adverse vascular remodeling. **Figure 1** provides a summary of the role of neutrophils, NE and NETs in PAH.

While increases in NE activity coincide with changes in vascular remodeling in animal models, it remains unclear whether intrinsic neutrophil abnormalities are necessary to initiate aberrant remodeling in human lungs or whether alterations in NET and NE release by neutrophils are a consequence of other features of the disease. To better understand the role of neutrophils at different stages of PAH pathogenesis, a longitudinal study to evaluate how they become increased and activated would be important. However,

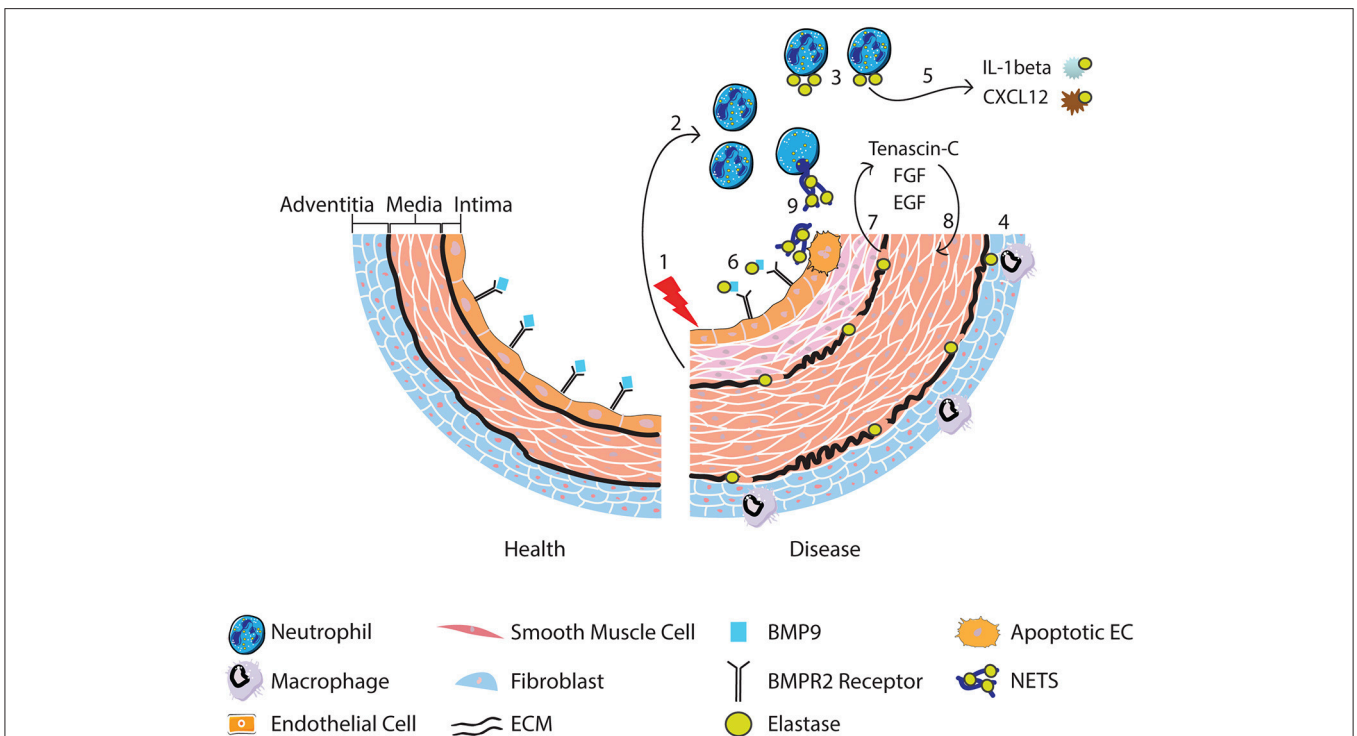


FIGURE 1 | Following vascular injury (1), the release of chemokines and chemotactic peptides from surrounding tissue recruits and activates neutrophils (2). Neutrophils release elastase (3) although it can also be released from activated macrophages that engulf neutrophil elastase (4) and from smooth muscle cells. Release of neutrophil elastase leads to cleavage of cytokines resulting in the conversion of the pro-to active form of IL-1b, promoting neutrophil survival, and degradation of CXCL12, favoring release of neutrophils from the bone marrow (5). BMP9 is also cleaved, impacting upon BMPR2 receptor signaling (6). Degradation of the ECM by elastase (7) releases SMC mitogenic growth factors promoting SMC proliferation (8). Neutrophil elastase is also involved in the release of NETs, which may induce EC apoptosis (9) and therefore contribute to endothelial dysfunction.

directly targeting neutrophils as a therapeutic strategy would be challenging given their vital role in host defense and as mentioned above, cells other than neutrophils can produce damaging proteases. Of these proteases, NE provides an attractive target as evidence from animal models suggests that NE inhibition has the potential to inhibit aberrant remodeling of the pulmonary vessels and indirectly dampen persistent inflammation in PAH. These findings have encouraged initiation of clinical trials of elastase inhibitors such as elafin for PAH (NCT03522935). Further, the potential for NE inhibitors such as elafin to enhance BMPR2 signaling and target pathology driven by BMPR2 deficiency, identifies these drugs as promising novel therapies for PAH.

AUTHOR CONTRIBUTIONS

ST and OD wrote sections of the manuscript; AT initiated the work and drafted sections of the manuscript; AT, MR and RZ revised the manuscript critically for important intellectual content.

FUNDING

AT was supported by a JG Graves Fellowship (University of Sheffield) and a British Heart Foundation-Fulbright Award. ST was supported by the T32 NIH/NHLBI Stanford Training Program in Lung Biology (HL129970-02).

REFERENCES

1. Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest.* (2008) 118:2372–9. doi: 10.1172/JCI33452
2. Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest.* (2012) 122:4306–13. doi: 10.1172/JCI60658
3. 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) 186:261–72. doi: 10.1164/rccm.201201-0164OC
4. Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, et al. Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. *Circulation* (2010) 122:920–7. doi: 10.1161/CIRCULATIONAHA.109.933762
5. Humbert M, Morrell NW, Archer SL, Stenmark KR, MacLean MR, Lang IM, et al. Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol.* (2004) 43(12 Suppl. S):13S−24. doi: 10.1016/j.jacc.2004.02.029
6. Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res.* (2014) 115:165–75. doi: 10.1161/CIRCRESAHA.113.301141
7. Senior RM, Griffin GL, Mecham RP. Chemotactic activity of elastin-derived peptides. *J Clin Invest.* (1980) 66:859–62. doi: 10.1172/JCI109926
8. Korkmaz B, Horwitz MS, Jenne DE, Gauthier F. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. *Pharmacol Rev.* (2010) 62:726–59. doi: 10.1124/pr.110.002733
9. Frid MG, Brunetti JA, Burke DL, Carpenter TC, Davie NJ, Reeves JT, et al. Hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal precursors of a monocyte/macrophage lineage. *Am J Pathol.* (2006) 168:659–69. doi: 10.2353/ajpath.2006.050599
10. Schultze AE, Wagner JG, White SM, Roth RA. Early indications of monocrotaline pyrrole-induced lung injury in rats. *Toxicol Appl Pharmacol.* (1991) 109:41–50.
11. Yildiz A, Kaya H, Ertas F, Oylumlu M, Bilik MZ, Yuksel M, et al. Association between neutrophil to lymphocyte ratio and pulmonary arterial hypertension. *Turk Kardiyol Dern Ars.* (2013) 41:604–9. doi: 10.5543/tkda.2013.93385
12. Ozpelit E, Akdeniz B, Ozpelit ME, Tas S, Bozkurt S, Tertemiz KC, et al. Prognostic value of neutrophil-to-lymphocyte ratio in pulmonary arterial hypertension. *J Int Med Res.* (2015) 43:661–71. doi: 10.1177/0300060515589394
13. Harbaum L, Baaske KM, Simon M, Oqueka T, Sinning C, Glatzel A, et al. Exploratory analysis of the neutrophil to lymphocyte ratio in patients with pulmonary arterial hypertension. *BMC Pulm Med.* (2017) 17:72. doi: 10.1186/s12890-017-040
14. Klinke A, Berghausen E, Fr7-5iedrichs K, Molz S, Lau D, Remane L, et al. Myeloperoxidase aggravates pulmonary arterial hypertension by activation of vascular Rho-kinase. *JCI Insight* (2018) 3:530. doi: 10.1172/jci.insight.97530
15. Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, et al. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* (2002) 296:2391–4. doi: 10.1126/science.1106830
16. Segal AW. How neutrophils kill microbes. *Annu Rev Immunol.* (2005) 23:197–223. doi: 10.1146/annurev.immunol.23.021704.115653
17. Taggart C, Mall MA, Lalmanach G, Cataldo D, Ludwig A, Janciauskiene S, et al. Protean proteases: at the cutting edge of lung diseases. *Eur Respir J.* (2017) 49:2015. doi: 10.1183/13993003.01200-2015
18. Yan H, Zhou HF, Akk A, Hu Y, Springer LE, Ennis TL, et al. Neutrophil proteases promote experimental abdominal aortic aneurysm via extracellular trap release and plasmacytoid dendritic cell activation. *Arterioscler Thromb Vasc Biol.* (2016) 36:1660–9. doi: 10.1161/ATVBAHA.116.307786
19. Soehnlein O. Multiple roles for neutrophils in atherosclerosis. *Circ Res.* (2012) 110:875–88. doi: 10.1161/CIRCRESAHA.111.257535
20. Cowland JB, Borregaard N. Granulopoiesis and granules of human neutrophils. *Immunol Rev.* (2016) 273:11–28. doi: 10.1111/imr.12440
21. Belaaouaj A, Kim KS, Shapiro SD. Degradation of outer membrane protein A in *Escherichia coli* killing by neutrophil elastase. *Science* (2000) 289:1185–8. doi: 10.1126/science.289.5482.1185
22. Dollery CM, Owen CA, Sukhova GK, Krettek A, Shapiro SD, Libby P. Neutrophil elastase in human atherosclerotic plaques: production by macrophages. *Circulation* (2003) 107:2829–36. doi: 10.1161/01.CIR.0000072792.65250.4A
23. Kim YM, Haghighat L, Spiekerkoetter E, Sawada H, Alvira CM, Wang L, et al. Neutrophil elastase is produced by pulmonary artery smooth muscle cells and is linked to neointimal lesions. *Am J Pathol.* (2011) 179:1560–72. doi: 10.1016/j.ajpath.2011.05.051
24. Kobayashi J, Wigle D, Childs T, Zhu L, Keeley FW, Rabinovitch M. Serum-induced vascular smooth muscle cell elastolytic activity through tyrosine kinase intracellular signalling. *J Cell Physiol.* (1994) 160:121–31. doi: 10.1002/jcp.1041600115
25. Rose F, Hattar K, Gakisch S, Grimminger F, Olschewski H, Seeger W, et al. Increased neutrophil mediator release in patients with pulmonary hypertension–suppression by inhaled iloprost. *Thromb Haemost.* (2003) 90:1141–9. doi: 10.1160/TH03-03-0173
26. Cowan KN, Heilbut A, Humpl T, Lam C, Ito S, Rabinovitch M. Complete reversal of fatal pulmonary hypertension in rats by a serine elastase inhibitor. *Nat Med* (2000) 6:698–702. doi: 10.1038/76282
27. Spiekerkoetter E, Alvira CM, Kim YM, Bruneau A, Pricola KL, Wang L, et al. Reactivation of gammaHV68 induces neointimal lesions in pulmonary arteries of S100A4/Mts1-overexpressing mice in association with degradation of elastin. *Am J Physiol Lung Cell Mol Physiol.* (2008) 294:L276–89. doi: 10.1152/ajplung.00414.2007
28. Tian W, Jiang X, Tamosiuniene R, Sung YK, Qian J, Dhillon G, et al. Blocking macrophage leukotriene b4 prevents endothelial injury and reverses pulmonary hypertension. *Sci Transl Med.* (2013) 5:200ra117. doi: 10.1126/scitranslmed.3006674
29. Campbell EJ, Wald MS. Hypoxic injury to human alveolar macrophages accelerates release of previously bound neutrophil elastase. Implications for lung connective tissue injury including pulmonary emphysema. *Am Rev Respir Dis.* (1983) 127:631–5. doi: 10.1164/arrd.1983.127.5.631

30. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol.* (2010) 191:677–91. doi: 10.1083/jcb.201006052
31. Pilsczek FH, Salina D, Poon KK, Fahey C, Yipp BG, Sibley CD, et al. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. *J Immunol.* (2010) 185:7413–25. doi: 10.4049/jimmunol.1000675
32. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science* (2004) 303:1532–5. doi: 10.1126/science.1092385
33. Jorch SK, Kubes P. An emerging role for neutrophil extracellular traps in noninfectious disease. *Nat Med.* (2017) 23:279–87. doi: 10.1038/nm.4294
34. Aldabbous L, Abdul-Salam V, McKinnon T, Duluc L, Pepke-Zaba J, Southwood M, et al. Neutrophil extracellular traps promote angiogenesis: evidence from vascular pathology in pulmonary hypertension. *Arterioscler Thromb Vasc Biol.* (2016) 36:2078–87. doi: 10.1161/ATVBAHA.116.307634
35. Borissoff JI, Joosen IA, Versteylen MO, Brill A, Fuchs TA, Savchenko AS, et al. Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. *Arterioscler Thromb Vasc Biol.* (2013) 33:2032–40. doi: 10.1161/ATVBAHA.113.301627
36. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS ONE* (2012) 7:e32366. doi: 10.1371/journal.pone.0032366
37. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr., et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci USA.* (2010) 107:15880–5. doi: 10.1073/pnas.1005743107
38. Belorgey D, Bieth JG. DNA binds neutrophil elastase and mucus proteinase inhibitor and impairs their functional activity. *FEBS Lett.* (1995) 361:265–8.
39. Kolaczkowska E, Jenne CN, Surewaard BG, Thanabalasuriar A, Lee WY, Sanz MJ, et al. Molecular mechanisms of NET formation and degradation revealed by intravital imaging in the liver vasculature. *Nat Commun.* (2015) 6:6673. doi: 10.1038/ncomms7673
40. Rabinovitch M, Bothwell T, Hayakawa BN, Williams WG, Trusler GA, Rowe RD, et al. Pulmonary artery endothelial abnormalities in patients with congenital heart defects and pulmonary hypertension. A correlation of light with scanning electron microscopy and transmission electron microscopy. *Lab Invest.* (1986) 55:632–53.
41. Zhu L, Wigle D, Hinek A, Kobayashi J, Ye C, Zuker M, et al. The endogenous vascular elastase that governs development and progression of monocrotaline-induced pulmonary hypertension in rats is a novel enzyme related to the serine proteinase adipsin. *J Clin Invest.* (1994) 94:1163–71. doi: 10.1172/JCI117432
42. Nickel NP, Spiekerkoetter E, Gu M, Li CG, Li H, Kaschwich M, et al. Elafin reverses pulmonary hypertension via caveolin-1-dependent bone morphogenetic protein signaling. *Am J Respir Crit Care Med.* (2015) 191:1273–86. doi: 10.1164/rccm.201412-2291OC
43. Lee KM, Tsai KY, Wang N, Ingber DE. Extracellular matrix and pulmonary hypertension: control of vascular smooth muscle cell contractility. *Am J Physiol.* (1998) 274(1 Pt 2):H76–82.
44. Tsukamoto Y, Helsel WE, Wahl SM. Macrophage production of fibronectin, a chemoattractant for fibroblasts. *J Immunol.* (1981) 127:673–8.
45. Thompson K, Rabinovitch M. Exogenous leukocyte and endogenous elastases can mediate mitogenic activity in pulmonary artery smooth muscle cells by release of extracellular-matrix bound basic fibroblast growth factor. *J Cell Physiol.* (1996) 166:495–505. doi: 10.1002/(SICI)1097-4652(199603)166:3<495::AID-JCP4>3.0.CO;2-K
46. Tu L, Dewachter L, Gore B, Fadel E, Dartevelle P, Simonneau G, et al. Autocrine fibroblast growth factor-2 signaling contributes to altered endothelial phenotype in pulmonary hypertension. *Am J Respir Cell Mol Biol.* (2011) 45:311–22. doi: 10.1165/rcmb.2010-0317OC
47. Chen PY, Qin L, Li G, Tellides G, Simons M. Fibroblast growth factor (FGF) signaling regulates transforming growth factor beta (TGFbeta)-dependent smooth muscle cell phenotype modulation. *Sci Rep.* (2016) 6:33407. doi: 10.1038/srep33407
48. Jones PL, Cowan KN, Rabinovitch M. Tenascin-C, proliferation and subendothelial fibronectin in progressive pulmonary vascular disease. *Am J Pathol.* (1997) 150:1349–60.
49. Cowan KN, Jones PL, Rabinovitch M. Elastase and matrix metalloproteinase inhibitors induce regression, and tenascin-C antisense prevents progression, of vascular disease. *J Clin Invest.* (2000) 105:21–34. doi: 10.1172/JCI6539
50. Alfaidi M, Wilson H, Daigneault M, Burnett A, Ridger V, Chamberlain J, et al. Neutrophil elastase promotes interleukin-1beta secretion from human coronary endothelium. *J Biol Chem.* (2015) 290:24067–78. doi: 10.1074/jbc.M115.659029
51. Prince LR, Allen L, Jones EC, Hellewell PG, Dower SK, Whyte MK, et al. The role of interleukin-1beta in direct and toll-like receptor 4-mediated neutrophil activation and survival. *Am J Pathol.* (2004) 165:1819–26. doi: 10.1016/S0002-9440(10)63437-2
52. Valenzuela-Fernandez A, Planchenault T, Baleux F, Staropoli I, Le-Barillec K, Leduc D, et al. Leukocyte elastase negatively regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding and functions by amino-terminal processing of SDF-1 and CXCR4. *J Biol Chem.* (2002) 277:15677–89. doi: 10.1074/jbc.M111388200
53. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest.* (2010) 120:2423–31. doi: 10.1172/JCI41649
54. Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM. Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* (2003) 19:583–93. doi: 10.1016/S1074-7613(03)00263-2
55. Scuderi P, Nez PA, Duerr ML, Wong BJ, Valdez CM. Cathepsin-G and leukocyte elastase inactivate human tumor necrosis factor and lymphotoxin. *Cell Immunol.* (1991) 135:299–313.
56. Bank U, Kupper B, Reinhold D, Hoffmann T, Ansorge S. Evidence for a crucial role of neutrophil-derived serine proteases in the inactivation of interleukin-6 at sites of inflammation. *FEBS Lett.* (1999) 461:235–40.
57. Sanchez O, Marcos E, Perros F, Fadel E, Tu L, Humbert M, et al. Role of endothelium-derived CC chemokine ligand 2 in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2007) 176:1041–7. doi: 10.1164/rccm.200610-1559OC
58. Cracowski JL, Chabot F, Labarere J, Faure P, Degano B, Schwebel C, et al. Proinflammatory cytokine levels are linked to death in pulmonary arterial hypertension. *Eur Respir J.* (2014) 43:915–7. doi: 10.1183/09031936.00151313
59. Humbert M, Monti G, Brenot F, Sitbon O, Portier A, Grangeot-Keros L, et al. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. *Am J Respir Crit Care Med.* (1995) 151:1628–31. doi: 10.1164/ajrccm.151.5.7735624
60. Heresi GA, Aytekin M, Hammel JP, Wang S, Chatterjee S, Dweik RA. Plasma interleukin-6 adds prognostic information in pulmonary arterial hypertension. *Eur Respir J.* (2014) 43:912–4. doi: 10.1183/09031936.00164713
61. Jonigk D, Golpon H, Bockmeyer CL, Maegel L, Hoeper MM, Gottlieb J, et al. Plexiform lesions in pulmonary arterial hypertension composition, architecture, and microenvironment. *Am J Pathol.* (2011) 179:167–79. doi: 10.1016/j.ajpath.2011.03.040
62. Schermuly RT, Yilmaz H, Ghofrani HA, Woyda K, Pullamsetti S, Schulz A, et al. Inhaled iloprost reverses vascular remodeling in chronic experimental pulmonary hypertension. *Am J Respir Crit Care Med.* (2005) 172:358–63. doi: 10.1164/rccm.200502-296OC
63. George J, D'Armiento J. Transgenic expression of human matrix metalloproteinase-9 augments monocrotaline-induced pulmonary arterial hypertension in mice. *J Hypertens.* (2011) 29:299–308. doi: 10.1097/HJH.0b013e328340a0e4
64. Chelladurai P, Seeger W, Pullamsetti SS. Matrix metalloproteinases and their inhibitors in pulmonary hypertension. *Eur Respir J.* (2012) 40:766–82. doi: 10.1183/09031936.00209911
65. Ormiston ML, Chang C, Long LL, Soon E, Jones D, Machado R, et al. Impaired natural killer cell phenotype and function in idiopathic and heritable pulmonary arterial hypertension. *Circulation* (2012) 126:1099–109. doi: 10.1161/CIRCULATIONAHA.112.110619
66. Goetzl EJ, Banda MJ, Leppert D. Matrix metalloproteinases in immunity. *J Immunol.* (1996) 156:1–4.

67. Heath D, Yacoub M. Lung mast cells in plexogenic pulmonary arteriopathy. *J Clin Pathol.* (1991) 44:1003–6.
68. Farha S, Sharp J, Asosingh K, Park M, Comhair SA, Tang WH, et al. Mast cell number, phenotype, and function in human pulmonary arterial hypertension. *Pulm Circ.* (2012) 2:220–8. doi: 10.4103/2045-8932.97609
69. Kosanovic D, Dahal BK, Peters DM, Seimetz M, Wygrecka M, Hoffmann K, et al. Histological characterization of mast cell chymase in patients with pulmonary hypertension and chronic obstructive pulmonary disease. *Pulm Circ.* (2014) 4:128–36. doi: 10.1086/675642
70. Carter RI, Mumford RA, Treonze KM, Finke PE, Davies P, Si Q, et al. The fibrinogen cleavage product Aalpha-Val360, a specific marker of neutrophil elastase activity *in vivo*. *Thorax* (2011) 66:686–91. doi: 10.1136/thx.2010.154690
71. Russell D, Genschmer KR, Szul T, Noerager B, Xu X, Viera L, et al. Neutrophil-derived exosomes purified from COPD patient bronchoalveolar fluid cause a COPD like phenotype in a mouse model via a neutrophil elastase dependent mechanism. *Am J Respir Crit Care Med.* (2018) 197:A2701-A. doi: 10.1164/ajrccm-conference.2018.197.1_MeetingAbstracts.A2701.
72. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet.* (2000) 67:737–44. doi: 10.1086/303059
73. Atkinson C, Stewart S, Upton PD, Machado R, Thomson JR, Trembath RC, et al. Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation* (2002) 105:1672–8. doi: 10.1161/01.Cir.0000012754.72951.3d
74. Cogan JD, Pauciulo MW, Batchman AP, Prince MA, Robbins IM, Hedges LK, et al. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2006) 174:590–8. doi: 10.1164/rccm.200602-165OC
75. Hamid R, Cogan JD, Hedges LK, Austin E, Phillips JA, 3rd, Newman JH, et al. Penetrance of pulmonary arterial hypertension is modulated by the expression of normal BMPR2 allele. *Hum Mutat.* (2009) 30:649–54. doi: 10.1002/humu.20922
76. Morrell NW, Yang X, Upton PD, Jourdan KB, Morgan N, Sheares KK, et al. Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-beta(1) and bone morphogenetic proteins. *Circulation* (2001) 104:790–5. doi: 10.1161/hc3201.094152
77. 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) 98:209–17. doi: 10.1161/01.RES.0000200180.01710.e6
78. Burton VJ, Ciuclan LI, Holmes AM, Rodman DM, Walker C, Budd DC. Bone morphogenetic protein receptor II regulates pulmonary artery endothelial cell barrier function. *Blood* (2011) 117:333–41. doi: 10.1182/blood-2010-05-285973
79. Steiner MK, Syrkina OL, Kolliputi N, Mark EJ, Hales CA, Waxman AB. Interleukin-6 overexpression induces pulmonary hypertension. *Circ Res.* (2009) 104:236–44. doi: 10.1161/CIRCRESAHA.108.182014
80. Li W, Hoenderdos K, Salmon RM, Upton PD, Condliffe AM, Chilvers ER, et al. Neutrophil and redox dependent proteolysis of bone morphogenetic protein 9: potential role in the pathogenesis of pulmonary arterial hypertension. *Thorax* (2013) 68:A146-A. doi: 10.1136/thoraxjnl-2013-204457.307
81. David L, Mallet C, Keramidas M, Lamande N, Gasc JM, Dupuis-Girod S, et al. Bone morphogenetic protein-9 is a circulating vascular quiescence factor. *Circ Res.* (2008) 102:914–22. doi: 10.1161/CIRCRESAHA.107.165530
82. Long L, Ormiston ML, Yang X, Southwood M, Graf S, Machado RD, et al. Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat Med.* (2015) 21:777–85. doi: 10.1038/nm.3877
83. Appleby SL, Mitrofan CG, Crosby A, Hoenderdos K, Lodge K, Upton PD, et al. Bone morphogenetic protein 9 enhances lipopolysaccharide-induced leukocyte recruitment to the vascular endothelium. *J Immunol.* (2016) 197:3302–14. doi: 10.4049/jimmunol.1601219
84. Alastalo TP, Li M, Perez Vde J, Pham D, Sawada H, Wang JK, et al. Disruption of PPARgamma/beta-catenin-mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. *J Clin Invest.* (2011) 121:3735–46. doi: 10.1172/JCI43382
85. Tojais NF, Cao A, Lai YJ, Wang L, Chen PI, Alcazar MAA, et al. Codependence of bone morphogenetic protein receptor 2 and transforming growth factor-beta in elastic fiber assembly and its perturbation in pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol.* (2017) 37:1559–69. doi: 10.1161/ATVBAHA.117.309696

Conflict of Interest Statement: AT has received funds to attend educational events from Actelion. RZ has a patent for use of FK506 to treat pulmonary hypertension. RZ has performed consultancy work for Actelion and Vivus and has stock options with Selten.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JS and handling Editor declared their shared affiliation.

frontiers in Medicine

MINI REVIEW
published: 01 May 2019
doi: 10.3389/fmed.2019.00093

Thin Air, Thick Vessels: Historical and Current Perspectives on Hypoxic Pulmonary Hypertension

Jason M. Young[1,2], *David R. Williams*[2] *and A. A. Roger Thompson*[2,3]*

[1] Edinburgh Medical School, University of Edinburgh, Edinburgh, United Kingdom, [2] Apex (Altitude Physiology Expeditions), Edinburgh, United Kingdom, [3] Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, United Kingdom

The association between pulmonary hypertension (PH) and hypoxia is well-established, with two key mechanistic processes, hypoxic pulmonary vasoconstriction and hypoxia-induced vascular remodeling, driving changes in pulmonary arterial pressure. In contrast to other forms of pulmonary hypertension, the vascular changes induced by hypoxia are reversible, both in humans returning to sea-level from high altitude and in animal models. This raises the intriguing possibility that the molecular drivers of these hypoxic processes could be targeted to modify pulmonary vascular remodeling in other contexts. In this review, we outline the history of research into PH and hypoxia, before discussing recent advances in our understanding of this relationship at the molecular level, focussing on the role of the oxygen-sensing transcription factors, hypoxia inducible factors (HIFs). Emerging links between HIF and vascular remodeling highlight the potential utility in inhibiting this pathway in pulmonary hypertension and raise possible risks of activating this pathway using HIF-stabilizing medications.

Keywords: hypoxia, pulmonary hypertension, altitude, vascular remodeling, hypoxic pulmonary vasoconstriction (HPV)

OPEN ACCESS

Edited by:
Claudio Sartori,
Université de Lausanne, Switzerland

Reviewed by:
Erik Richard Swenson,
University of Washington,
United States
Robert Naeije,
Free University of Brussels, Belgium

***Correspondence:**
A. A. Roger Thompson
r.thompson@sheffield.ac.uk

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *10 January 2019*
Accepted: *16 April 2019*
Published: *01 May 2019*

Citation:
Young JM, Williams DR and Thompson AAR (2019) Thin Air, Thick Vessels: Historical and Current Perspectives on Hypoxic Pulmonary Hypertension. Front. Med. 6:93. doi: 10.3389/fmed.2019.00093

INTRODUCTION

Pulmonary hypertension (PH) is a feature of several distinct clinical phenotypes which, by differing means, result in increased pressure within the pulmonary vasculature. Despite some advancements in treatment over recent years (1), most forms of PH are progressive and life-limiting. In the current classification of PH etiology, Group III (PH due to lung diseases and/or hypoxia) is the second commonest cause of elevated pulmonary artery pressure, behind heart disease (2). Group III encompasses a broad range of conditions such as chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD) and sleep apnoea (3). Alongside parenchymal changes, two key pathological process, pulmonary vascular remodeling and vasoconstriction, contribute to PH in this group of patients but treatment with pulmonary vasodilators has, to date, been disappointing. New approaches to the management of these patients are thus urgently required to improve outcomes as 3 year survival remains as low as 33% for COPD patients with mean pulmonary artery pressures >40 mmHg (1, 2, 4). While pathologic mechanisms might vary depending on the underlying disease or phenotype, a better understanding of the defining component of Group III disorders, hypoxia, may help provide new targets for therapies.

A causal relationship between hypoxia and PH is well established; hypoxia is frequently used to both precipitate PH in animal models (5) and to induce aberrant cell phenotypes *in vitro* (6). These

approaches have greatly improved our understanding of the underlying physiological mechanisms that drive the pathology. In humans, compelling evidence of the effects of hypoxia on pulmonary vascular tone and remodeling derives from studies performed at altitude, where the inherent reduction in barometric pressure results in hypobaric hypoxia. This approach is advantageous for evaluation of the effects of hypoxia on the pulmonary vasculature in relative isolation, without the complicating factors of disease. In this review, we outline the historical context of research into PH and hypoxia and discuss emerging molecular mechanisms for this relationship. We focus on the role of the oxygen-sensing transcription factors, hypoxia inducible factors (HIFs), and links between HIFs and vascular remodeling.

IMPORTANT DEFINITIONS

Before embarking on this review, it is important to consider the definitions of PH used within this manuscript and others. The term PH is used to describe elevation in mean pulmonary artery pressure (mPAP) from any cause. PH was first classified as a mPAP exceeding 25 mmHg at the 1st World Symposium on Pulmonary Hypertension (WSPH) in 1973 (7). Notably, at the recent 6th WSPH, the upper limit of normal for mPAP was set at 20 mmHg, argued in part due to emerging evidence of poorer survival in patients with mPAPs of 21–24 mmHg and in part based on the distribution of values in healthy population data (8). For a diagnosis of pre-capillary pulmonary hypertension, of any cause, an increased pulmonary vascular resistance (PVR $>$ 3 WU) is also required (8). Pre-capillary hemodynamics that meet the above definition, are not uncommon in patients with lung disease (4, 9), but the prevalence of increased PVR in healthy individuals who are hypoxic without lung disease, for example altitude residents and those with sleep apnoea, is less clear and will be discussed later (10). To avoid confusion we have, where possible, included values ($\pm$SD) from the cited literature indicating recorded pulmonary artery pressures and/or PVR.

PULMONARY HYPERTENSION: A HISTORY

Pathological changes in the pulmonary arteries co-existing with right ventricular hypertrophy (RVH) were first observed by the German physician Ernst von Romberg toward the end of the nineteenth century, which he coined "pulmonary vascular sclerosis" (11). However, the etiology of PH remained elusive at this time and was wrongly attributed to syphilis for many years (12, 13). Whilst the British cardiologist Oscar Brenner eventually disproved this link in 1935, he could not provide an explanation for pulmonary vascular changes coinciding with RVH (14). It was only with the advent of right heart catheterization in the mid-twentieth century that these observations were intrinsically linked by raised pulmonary artery pressure (PAP). Despite extensive use in animals in the early twentieth century, cardiac catheterization in humans was widely considered unsafe until Werner Forssman's gallant self-catheterization of his right heart in 1929 (15, 16). Whilst this act of bravery was initially poorly received and widely ignored by the medical community, American physicians Dickinson Richards and Andrew Cournard would recognize the importance of Forssman's work in the 1940s. Their pioneering research characterized mPAP in cardiac and pulmonary diseases for the first time, a feat for which they were awarded a Nobel Prize, together with Forssman, in 1956 (17, 18).

Further work in the 1950s began to establish the clinical and pathological features of PH. In 1951, one of the first detailed descriptions of the haemodynamic profiles of the disease was provided by David Dresdale who also observed cyanosis, orthopnoea and haemoptysis amongst patients with idiopathic PH. Dresdale and others termed their findings "primary pulmonary hypertension" (19, 20); this terminology provided important nomenclature for the emerging research community. Additionally, an extensive characterization of histological changes in PH was described by Donald Heath who, in collaboration with William Whitaker, first detailed extensive thickening of the pulmonary arterial wall associated with fibrosis in 1953, amongst individuals with congenital heart disease, mitral stenosis and idiopathic PH (21, 22). Heath and Jesse Edwards subsequently produced a detailed histological classification system correlated to PH severity in Eisenmenger's syndrome, which ranged from early vascular medial hypertrophy in mild PH to late intimal fibrosis in severe disease (23).

EARLY LINKS BETWEEN ACUTE HYPOXIA AND PULMONARY HYPERTENSION

Despite elevated PAP being first associated with ventilatory failure in 1852 (24), a causal relationship between hypoxia and PH only became established in 1946 when von Euler and colleagues demonstrated increased mPAP on exposing cats to both hypoxia and hypercapnia (25); in 1947, Dresdale reported similar findings in humans (26). These reports constituted the first measurements of pulmonary arteriole constriction to hypoxia, or hypoxic pulmonary vasoconstriction (HPV), a phenotype which contrasts the vasodilating properties of hypoxia on the systemic circulation (27). At the time, von Euler correctly hypothesized that this physiological response is beneficial in order to shunt blood from areas of regional lung hypoxia that stems from reduced ventilation, thus maintaining blood oxygenation (a concept now termed ventilation-perfusion matching).

However, the adverse effects of this response in the context of more global alveolar hypoxia soon became apparent, particularly in relation to high-altitude pulmonary oedema (HAPE). Whilst a syndrome of cough, blood-stained sputum and severe breathlessness was previously recognized in high altitude sojourners, Hurtado was the first to attribute this to pulmonary oedema in 1937 (28). PH was first identified as co-existing with HAPE in 1962 by Fred et al. (29) in one patient with a mPAP of 46 mmHg, although Hultgren and Spickard had proposed this association in 1960, providing clinical descriptions of a loud second heart sound and electrographic changes consistent with PH in 41 cases of HAPE in Peru (30). Hultgren et al. subsequently confirmed this in seven individuals following acute exposure to

high altitude in 1964, in whom mPAP ranged from 33 to 117 mmHg (PVR reported in 2 patients; 8 and 36 WU). Importantly, the authors could also demonstrate a degree of reversibility of pulmonary oedema and elevated mPAP on administration of 100% oxygen (31). Further work from this group, along with others (32), identified a predisposition to pulmonary oedema amongst five individuals with mPAPs of 38.8 ± 10.3 mmHg on ascent to 3,100 m (33).

Despite the early identification of PH as a factor in the pathogenesis of HAPE, how this results in oedema formation remains unclear. Hultgren proposed that because HPV is heterogeneous, areas of the lung are over-perfused leading to pulmonary capillary stress failure in HAPE (34). Indeed, subsequent studies in HAPE-susceptible individuals have provided evidence of exaggerated heterogeneity of perfusion (35), whilst haemodynamic studies have also demonstrated elevated pulmonary capillary pressures (19 ± 1 mmHg vs. 13 ± 1 mmHg in controls) and arterial pressures (mPAP 37 ± 2 mmHg vs. 26 ± 1 mmHg in controls) amongst such individuals at high altitude (36). Other factors in HAPE pathogenesis include impaired nitric oxide (NO) biosynthesis and reduced alveolar fluid reabsorption, as reviewed here (37, 38).

CHRONIC HYPOXIA AND REMODELING OF THE PULMONARY VASCULATURE

Concurrently, research began to investigate the effects of chronic hypoxia on the pulmonary vasculature of high-altitude populations. This initially began in cattle which often developed significant oedema around the lower chest at high altitude, dubbed "brisket disease," a condition that caused significant mortality upon ascent. In the 1940s, Rue Jensen first identified right ventricular dilatation and failure co-existing with brisket disease amongst the high-altitude cattle populations in Colorado (39), with further work with Grover, Reeves and Will identifying a positive correlation between the severity of RVH and the degree of raised PAP (40). Further breeding experiments led by Grover and Reeves suggested an autosomal dominant inheritance of HAPH among these cattle (41, 42). In contrast to Hultgren's later findings amongst patients with HAPE (31), 100% oxygen did not fully reverse PH in cattle (40), indicating a lesser role of HPV in PH pathogenesis in the setting of chronic hypoxia. Interestingly, similar findings were documented by Anand et al. amongst a human population, detailing evidence of peripheral oedema and shortness of breath amongst Indian soldiers who had sojourned at altitudes above 5,800 m for 18 weeks. While no measurements were made at altitude, shortly after return to sea level right heart catheter studies on these patients provided evidence of mild pre-capillary pulmonary hypertension, with mPAP and PVR measured as 26.1 ± 4.5 mmHg and 3.41 ± 2.46 WU, respectively (43).

Elevated PAP in human populations at high altitude was first reported in 1956 by Canepa in one of the first reports of human right heart catheterization in Peruvians from Morochoca (4,540 m), recorded as 25 (range 18–29) mmHg amongst 7 highlanders and 34 and 35 mmHg amongst two chronic mountain sickness patients; however, these findings were initially attributed to polycythaemia, abnormal ventilation and increased cardiac output (44). It would take the work of fellow Peruvians Dante Peñaloza and Javier Arias-Stella in the 1960s to demonstrate that PH amongst high altitude populations was associated with remodeling of the pulmonary vasculature (45–47). Earlier work from Peñaloza confirmed elevated mPAP (23 ± 5.1 mmHg) associated with RVH in Peruvians at high altitude (48, 49) and interestingly, also identified PH amongst new born children both at sea level and altitude, with a swift resolution at sea level that was not recognized amongst Peruvian infants (50). Importantly, the authors found no difference in PAWP and CO between residents at sea level and altitude, with PVR elevated at 4.15 ± 2.66 WU in high altitude dwellers (46, 49). Oxygen administration to Peruvian adults resulted in minor reductions in mPAP of 15–20% (45, 47), mirroring prior results in brisket disease (40) and indicating that pulmonary vascular remodeling was primarily responsible for PH in chronic hypoxia. Further weight to this hypothesis was added by Jensen and Alexander, who later demonstrated a linear relationship between medial hypertrophy of the pulmonary arteries and PAP amongst cattle (51). Notably, despite a failure of immediate resolution with oxygen, Peñaloza identified a normalization of mPAP (12 ± 1.9 mmHg) and PVR (1.81 ± 0.44 WU) amongst high altitude populations following 2 years spent at sea level, demonstrating that changes as a result of chronic hypoxic exposure are not permanent (52). Complementing this finding, the Indian soldiers studied by Anand et al., who had developed signs of right heart failure during their altitude sojourn, made a full recovery, with reversal of cardiomegaly and normalization of mPAP (16.3 ± 2.9 mmHg) and PVR pulmonary vascular resistance (1.34 ± 0.48 WU) 12–16 weeks after descent from high altitude (43).

While the above studies in healthy individuals imply that elevated pulmonary artery pressures are found ubiquitously at altitude, whether the magnitude of elevation in healthy altitude residents reaches that which would define pre-capillary PH remains unclear. A recent meta-analysis by Soria et al. revealed an average systolic PAP of 25.3 mmHg across high altitude populations with a wider distribution than amongst lowlanders implying a low prevalence of PH even by the new WHO criteria (8, 10). Furthermore, PVR is seldom reported and a notable limitation of reported PVRs among historical catheterization studies at altitude, is the lack of correction for hematocrit. Resistance to blood flow is dependent upon viscosity as well as vessel dimensions [reviewed by Vanderpool and Naeije (53)], with equations describing the relationship derived from isolated perfused lung experiments involving alterations in haematocrit (54). Thus, reporting of haematocrit is important in determining true PVR (53, 55) and may lead to false assumptions regarding the extent of vascular remodeling in healthy individuals following hypoxic exposure.

Nonetheless, similar to observations in patients with lung disease, there is a sub-population of altitude residents who develop more severe PH. A consensus definition for high altitude PH (HAPH) was reported in 2005, to encompass those at altitude with exaggerated elevation in PAP and signs of RVH and right heart failure (56). HAPH was defined as a mean PAP of >30

mmHg (or systolic PAP > 50 mmHg) in the absence of excessive erythrocytosis (hemoglobin concentration > 19 g/dl for women, > 21 g/dl for men). This definition allowed discrimination between HAPH and chronic mountain sickness (CMS), in which there is excessive erythrocytosis (57, 58). Despite aforementioned epidemiological studies indicating the rarity of HAPH by the above definition amongst high altitude dwellers (10), the study of such individuals may provide important insights into molecular pathways that drive vasoconstrictive and remodeling processes in both hypoxic PH and, potentially, other forms of PAH. However, it could be argued that a revision of the current definition of HAPH, to include haematocrit-corrected PVR, would facilitate this research.

INTER-SPECIES VARIATION AT HIGH ALTITUDE

Following these results in both humans and cattle, Donald Heath became interested in inter-species variability in the pulmonary vasculature of high-altitude populations. Heath traveled to Cerro de Pasco, Peru (4,330 m) alongside Peter Harris in 1965, in what became the first of many high-altitude research expeditions dedicated to PH research. A descriptive overview of this work is provided by one of this article's authors in **Box 1**. In 1974, Heath published their research in llamas (*Lama glama*) demonstrating a lack of pulmonary arteriole muscularisation or RVH at altitude, contrasting previous findings in humans and cattle (*Bos taurus*) (59). A similarly thin walled pulmonary vasculature was also identified in the Himalayan yak (*Bos grunniens*) (60), indicating a role of natural selection in the loss of the thick-walled, reactive pulmonary arteries typically characteristic of the Bos genus.

An interesting biological issue arises when species from within the same genus interbreed; one such example is the interbreeding of cattle giving rise to species such as the dzo (cow x yak) and stol (dzo x bull) (61). In 1986, work from Peter Harris' group identified that protection from PH correlated with the degree of yak heritage; whilst dzos and yaks demonstrated minimal PH, half of the stols had significantly raised PAPs similar to that of cattle (62), indicating a degree of inheritance. These observations lend support to the concept that animals indigenous to high altitude have become genetically adapted to their hypoxic environment, vs. acclimatization as seen in other species.

MOLECULAR MECHANISMS OF PH IN ACUTE AND CHRONIC HYPOXIC EXPOSURE

While the evidence above clearly illustrates connections between hypoxic exposure and pulmonary hypertension, the underlying genetic, molecular and cellular mechanisms that regulate these phenotypes remain unclear and in part, controversial. Nonetheless, basic science work over the last 25 years has advanced our understanding of common pathways that govern both adaptation to altitude and hypoxia-induced PH. Reviewed extensively elsewhere (63–65), pulmonary vasoconstriction in acute hypoxia comprises at least two phases involving distinct mechanisms. Initially, changes in redox status within smooth muscle cell mitochondria mediate alterations in potassium and voltage-gated calcium channel flux, promoting contraction (63). Subsequently, vasoconstriction is maintained by mechanisms that include reduced bioavailability of NO (66), release of endothelial-derived vasoconstrictors (67) and increases in myofilament calcium sensitivity (68). The focus of this article will be on the role of the hypoxia-inducible factors (HIFs) in vascular remodeling due to chronic hypoxic exposure.

Box 1 | Adaptation to chronic hypoxia in the andes.

The recognition of the different biological classes of man and mammals at high altitude is best illustrated by taking a mental stroll around the streets and surrounding countryside of any small town in the high Andes. The studies undertaken demonstrated that there was no single stereotypical man or mammal at high altitude.

Cerro de Pasco is a mining community with a population of 70,000 people, situated at an attitude of 4330 m in the central Andes of Peru. In the streets will be a number of lowlanders who may have arrived at high altitude in a matter of hours from Lima on the coast. Approximately 50% will suffer from benign acute mountain sickness mainly characterized by headache, insomnia, anorexia, nausea and dizziness. These symptoms are the consequence of hypobaric hypoxia and may be regarded as the physiological components of early acclimatization.

In contrast, most people are native Quechua Indians born and bred in the high Andes. These descendants of the Inca people have very characteristic physical features of skin color with deeply polycythaemic and suffused conjunctiva and lips. Many will have a capacious chest which looks prominent and out of proportion to their short and stocky physique. These native highlanders lead normal busy lives at high altitude. They participate in vigorous games of football at altitudes exceeding the summit of the Matterhorn in the Swiss Alps.

Living on the pastures surrounding Cerro de Pasco are examples of indigenous mountain animals such as the llama, alpaca, vicuna and guanaco. These animals have been living on the Andean altiplano for many thousands of years. One cannot help but be impressed by the vigor and activity of these animals in an atmosphere characterized by severe hypobaric hypoxia.

HYPOXIA-INDUCIBLE FACTORS (HIFs) AND PULMONARY VASCULAR REMODELING

HIFs are a family of heterodimeric transcription factors, discovered in 1995 (69), whose alpha subunits are stabilized in hypoxia by the inhibition of oxygen-dependent prolyl hydroxylase (PHD) enzyme activity (70); under normoxic conditions, hydroxylation of HIF-alpha by PHDs targets them for ubiquitination by the VHL complex, resulting in subsequent proteasomal degradation (**Figure 1**) (71). Whilst HIF-1α is expressed ubiquitously throughout body tissues (72), HIF-2α expression is tissue specific with an endothelial bias (73, 74). In hypoxia, stabilization of HIF-α subunits induces transcription of targets with a wide range of functions.

Perhaps unsurprisingly, genetic variation in the HIF pathway has been identified amongst indigenous altitude dwellers. Notably, genome wide association studies in the Tibetan

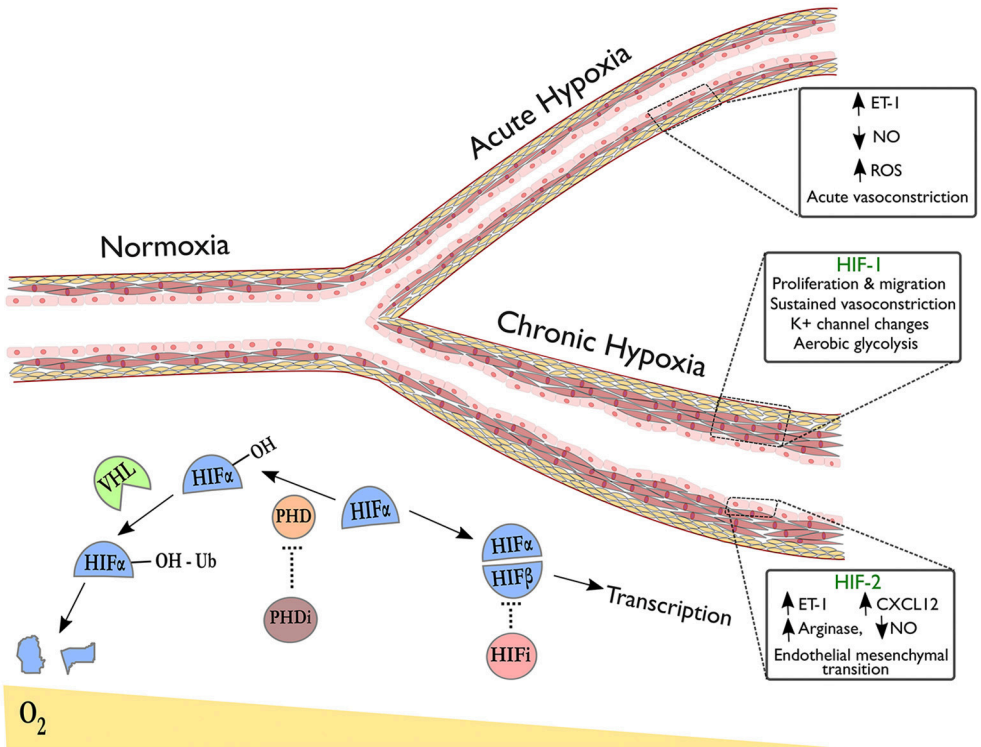


FIGURE 1 | Pulmonary vascular responses to hypoxia with emphasis on the role of HIF isoforms in remodeling. The right upper branch of this vessel depicts vasoconstriction in acute hypoxia, occurring due to alterations in redox and NO signaling and release of vasoactive mediators. The lower branch indicates remodeling in the context of sustained hypoxic exposure and illustrates HIF-dependent processes revealed by tissue-specific deletion of HIF-isoforms in endothelial cells (HIF-2α) or smooth muscle cells (HIF-1α). Below the vessel, a schematic shows degradation of hydroxylated HIF-α subunits in normoxia via the von-Hippel Lindau (VHL) pathway. In hypoxia or following treatment with prolyl hydroxylase inhibitors (PHDi), HIF-α stabilization and dimerization with HIF-β occurs, leading to transcription of target genes. HIF inhibitors (HIFi) with specific activity against HIF-2α are in clinical development.

population have identified single nucleotide polymorphisms (SNPs) in *EPAS1* (encoding HIF-2α) and *ELGN1* (encoding PHD2) that were not enriched in lowlanders (75–77). *EPAS1* variants were associated with lower PAP (78) and a high frequency *ELGN1* mutation has been linked to reduced proliferation of erythroid progenitors in response to EPO, thus dampening hypoxia-induced erythrocytosis (79). These findings demonstrate a selection pressure for specific HIF pathway polymorphisms over 25,000 years at altitude that has aided adaptation for the Tibetan population. Interestingly, such variation is not observed in Andean counterparts, a population that has resided at altitude for 15,000 years and who are more susceptible to PH (80, 81) and erythrocytosis (82).

However, the evidence for HIF pathway polymorphisms influencing remodeling processes is weakened by the observation that correction for erythrocytosis reduces mPAP amongst Andean populations to values near those of Tibetans (57). Thus, correcting for erythrocytosis argues against a susceptibility of Andean populations to HIF-mediated remodeling processes. In light of the new PH definition (8), however, Andean corrected mPAP remains consistently above 20 mmHg at rest and the slope of rise in mPAP with cardiac output is steeper than that of lowlanders (57).

Furthermore, evidence from murine models strongly implicates the HIF pathway in hypoxia-induced vascular remodeling. Soon after the discovery of the pathway, early work in both *Hif1a* and *Hif2a* heterozygotes revealed a marked reduction in PH and vascular remodeling following chronic exposure to 10% oxygen (83, 84). Conversely, *HIF2A* (*EPAS1*) gain-of-function mutations can predispose to PH; a *Hif2a* variant in high altitude cattle increases susceptibility to brisket disease (85), whilst a *HIF2A* mutation causing familial erythrocytosis is also associated with elevated systolic PAP in humans (86). A mouse generated to have the same G536W gain-of-function mutation in the *Hif2a* gene also developed erythrocytosis and PH, providing further evidence of cross-species conservation of this HIF-2α role (87). Additionally, both animal models and patients with Chuvash polycythaemia (CP), characterized by a VHL mutation, exhibit marked erythrocytosis and elevated PAP that could be rescued in mice by *Hif2a* but not *Hif1a* deletion (88–90). While the descriptions of elevated PAP in humans with CP did not include right heart catheter data or haematocrit-corrected PVR, it is worth noting that elevations in systolic PAP and vessel muscularisation in young mice with homozygous VHL mutations, preceded the onset of polycythaemia (90).

TISSUE-SPECIFIC MANIPULATION OF HIF EXPRESSION REVEALS DISTINCT ROLES FOR HIF ISOFORMS IN PULMONARY VASCULAR REMODELING

Evidence is now emerging as to how the HIF isoforms regulate pulmonary vascular cell function, with advances gained through use of murine tissue-specific deletion models, see **Figure 1**. For example, HIF-1α has been implicated in both vasoconstriction and vascular cell proliferation, the two key components of hypoxic pulmonary hypertension. Ball et al. demonstrated that inducible *Hif1a* deletion in PASMCs reduced right ventricular systolic pressure, arterial wall thickness and vessel muscularisation in chronic hypoxia (91), whilst Shiekh et al. reported a dependence on *Hif1a* in PASMC progenitors in order to drive distal migration and expansion (92). Proposed mechanisms that could explain these findings include enhanced intracellular calcium via *Hif1a* dependent downregulation of K^+ channels (93) and upregulation of transient receptor potential calcium channels (94), recognized to enhance vasoconstriction, PASMC proliferation and migration (95). HIF-1α also mediates pro-proliferative metabolic changes in PASMCs and fibroblasts that could contribute to hypoxia-induced remodeling. One widely recognized consequence of HIF signaling amongst cancer cells is the favoring of glycolysis over oxidative phosphorylation in aerobic conditions, known as the "Warburg effect," inducing glycolytic enzymes to enhance ATP production and promote tumor growth (96, 97). Interestingly, HIF signaling amongst pulmonary arterial smooth muscle cells (PASMCs) and fibroblasts results in a similar shift to aerobic glycolysis as seen in tumors (98–100), with increased glucose uptake observed in the lungs of rats with hypoxia-induced PH and in PAH patients (101, 102). This metabolic reprogramming of pulmonary vascular cells has proven stable *ex vivo* with evidence of underlying epigenetic regulation (103, 104). Targeting these mechanisms may limit hypoxia-induced PASMC proliferation in the pulmonary vasculature.

Consistent with its predominantly endothelial expression profile, a growing body of evidence implicates endothelial cell (EC) HIF-2α expression as essential for pulmonary vascular remodeling through varied biological mechanisms, see **Figure 1**. Two studies have demonstrated severe and spontaneous PH following *Phd2* knockdown in murine ECs (105, 106). Double knockouts of *Phd2* and either HIF isoform revealed that this was a *Hif2a*-mediated phenotype (105, 106) but the studies highlighted different mechanisms: one associating HIF-2α expression with reduced expression of the potent vasoconstrictor endothelin-1 (ET-1) (106) and the other demonstrating HIF-2α involvement in CXCL12-mediated PASMC proliferation (105). Reduced EC *Phd2* expression was also observed amongst occlusive vessels in IPAH (105), implying relevance to human pathology. Notably, these *Phd2* knockout mice did not develop polycythaemia prior to the development of PH.

The NO synthesis pathway has also been implicated in EC HIF-2α-mediated remodeling. Cowburn et al. observed a similar level of protection from hypoxia-induced PH as *Hif2a* knockdown following EC-specific deletion of arginase-1 (*Arg1*), a downstream HIF-2α target and negative regulator of NO synthesis (107). Additionally, ECs from PH patients demonstrated impaired NO production *in vitro*, restored on arginase inhibition (107). A further mechanism by which HIF-2α could contribute to remodeling is through regulation of endothelial-mesenchymal transition (EMT), a process implicated in pathogenic remodeling (108). Tang et al. showed that markers of EMT were regulated by HIF-2α in ECs and that while endothelial-specific deletion of *Hif2a* protected mice from hypoxia-induced PH, deletion of *Hif2a* in vascular smooth muscle cells did not (109).

There remain notable controversies in the literature surrounding HIF-mediated regulation of remodeling. Whilst Ball et al. demonstrated a role for PASMC *Hif1a* in chronic hypoxic remodeling using a tamoxifen-inducible conditional deletion (91), Kim et al. reported enhanced pulmonary arterial tone in the absence of arterial muscularisation following constitutive PASMC-specific *Hif1a* deletion (110). Similarly, constitutive EC *Hif1a* deletion was found to confer no protection to PH by three authors (105–107), whilst Shiekh et al. could ameliorate PH following tamoxifen-inducible conditional EC *Hif1a* deletion, which prevented PASMC expansion and distal migration (92). Alongside evidence detailing the importance of embryonic HIF signaling for the developing vasculature (111), these observed differences imply a role of early HIF-1α signaling in pulmonary vessel development.

The crucial role of HIF isoforms in hypoxia-induced PH has identified the inhibition of these molecules as an important strategy for targeting remodeling processes. Whilst efforts to develop HIF pathway inhibitors have previously proven challenging due to poor efficacy, HIF isoform specificity and adverse effects (112, 113), a specific HIF-2α small molecule inhibitor developed to treat renal cancer has demonstrated a favorable safety profile in a recent Phase I trial (114). Encouragingly, the use of another HIF-2α inhibitor, C76, has recently been demonstrated to attenuate remodeling in three murine models of PH, with no notable inhibition of HIF-1α (115).

The pleiotropic nature of HIF signaling has identified several other pathways as possible therapeutic targets. Using congenic linkage analysis, Zhao et al. discovered a dependence on intracellular zinc in hypoxia-induced remodeling. Homozygous deletion of the zinc transporter ZIP12, a target of both HIF-1α and HIF-2α, was found to attenuate PAP, RVH and vascular remodeling in chronic hypoxia (116). Additionally, induction of ZIP12 was also reported in the pulmonary tissue in Brisket disease and highland human populations (116). How intracellular zinc influences hypoxia-induced remodeling remains unclear; however, targeting intracellular zinc homeostasis may represent a further therapeutic strategy.

CONCLUSIONS

This article has reviewed historical observations connecting hypoxia and pulmonary hypertension and described more

recent insights into the molecular mechanisms involved in hypoxia-induced remodeling. Notably, the evidence linking HIF expression to processes involved in vascular remodeling strongly raises the prospect of HIF inhibition, and in particular HIF-2α inhibition, as a strategy in order to ameliorate vascular pathology in the context of chronic hypoxia. The recent success of a HIF-2α inhibitor in several murine models is supportive of such a strategy and may lead to the consideration of clinical trials amongst PH patients in the future. However, the mechanistic links between HIF-pathway activation and PH, notably the development of spontaneous PH following *Phd2* deletion, should also raise a note of caution for use of PHD inhibitors which are currently undergoing Phase II/III clinical trials in renal anemia (117).

Genetic insights gained through study of high-altitude populations suggests that a greater appreciation of factors underlying altitude adaptation may highlight further mechanisms involved in the regulation of vascular remodeling. However, while similarities exist between the pathological features of hypoxia-induced PH and other forms of the disease, the extent of overlap in the pathological mechanisms, even for patients with chronic respiratory disease, remains unclear. Furthermore, the notable lack of correction for haematocrit in previous work reporting PVR at altitude casts some doubt over some of the apparent differences between altitude populations, which may in fact be due to differences in haematocrit. Nonetheless, a reversal of PH on return to sea level provides the tantalizing possibility that exploiting endogenous mechanisms might provide agents that reverse vascular remodeling in hypoxic disease. Therefore, there is still hope that lessons learned from studying hypoxia-induced disease could impact on the search for agents that target pervasive vascular remodeling in other forms of PH.

AUTHOR CONTRIBUTIONS

JY wrote the manuscript. DW and AART drafted additional text and edited the manuscript. All authors approved the final version.

FUNDING

AART is supported by a British Heart Foundation Intermediate Clinical Fellowship (FS/18/13/33281).

REFERENCES

1. Gall H, Felix JF, Schneck FK, Milger K, Sommer N, Voswinckel R, et al. The giessen pulmonary hypertension registry: survival in pulmonary hypertension subgroups. *J Hear Lung Transplant*. (2017) 36:957–67. doi: 10.1016/j.healun.2017.02.016
2. Strange G, Playford D, Stewart S, Deague JA, Nelson H, Kent A, et al. Pulmonary hypertension: prevalence and mortality in the Armadale echocardiography cohort. *Heart*. (2012) 98:1805–11. doi: 10.1136/heartjnl-2012-301992
3. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. (2013) 62:D34–41. doi: 10.1016/j.jacc.2013.10.029
4. Hurdman J, Condliffe R, Elliot CA, Swift A, Rajaram S, Davies C, et al. Pulmonary hypertension in COPD: results from the ASPIRE registry. *Eur Respir J*. (2013) 41:1292–301. doi: 10.1183/09031936.00079512
5. Maarman G, Lecour S, Butrous G, Thienemann F, Sliwa K. A comprehensive review: the evolution of animal models in pulmonary hypertension research; are we there yet? *Pulm Circ*. (2013) 3:739–56. doi: 10.1086/674770
6. Pugliese SC, Poth JM, Fini MA, Olschewski A, El Kasmi KC, Stenmark KR. The role of inflammation in hypoxic pulmonary hypertension: from cellular mechanisms to clinical phenotypes. *Am J Physiol Lung Cell Mol Physiol*. (2015) 308:L229–52. doi: 10.1152/ajplung.00238.2014
7. Hatano S, Strasser T, World Health Organization. *Primary Pulmonary Hypertension: Report on a WHO Meeting, Geneva, 15-17 October 1973*. Geneva: World Health Organization (1975).
8. Galiè N, McLaughlin V V, Rubin LJ, Simonneau G. An overview of the 6th World Symposium on Pulmonary Hypertension. *Eur Respir J*. (2019) 53:1802148. doi: 10.1183/13993003.02148-2018
9. Seeger W, Adir Y, Barberà JA, Champion H, Coghlan JG, Cottin V, et al. Pulmonary hypertension in chronic lung diseases. *J Am Coll Cardiol*. (2013) 62:D109–16. doi: 10.1016/j.jacc.2013.10.036
10. Soria R, Egger M, Scherrer U, Bender N, Rimoldi SF, Rimoldi SF. Pulmonary artery pressure and arterial oxygen saturation in people living at high or low altitude: systematic review and meta-analysis. *J Appl Physiol*. (2016) 121:1151–9. doi: 10.1152/japplphysiol.00394.2016.-More
11. Park MH. Historical perspective on the classification and nomenclature of pulmonary hypertension. In: Maron B, Zamanian R, Waxman A, editors. *Pulmonary Hypertension*. Cham: Springer International Publishing (2016). p. 3–15.
12. Escudero P. The Black Cardiacs and the Ayerza's disease. *Rev Crit*. (1911).
13. Mazzei JA, Mazzei ME. A tribute: Abel Ayerza and pulmonary hypertension. *Eur Respir Rev*. (2011) 20:220–1. doi: 10.1183/09059180.00006811
14. Brenner O. Pathology of the vessels of the pulmonary circulation. *Arch Intern Med*. (1935) 56:211. doi: 10.1001/archinte.1935.03920020003001
15. Forssmann W. Die Sondierung des Rechten Herzens. *Klin Wochenschr*. (1929) 8:2085–7. doi: 10.1007/BF01875120
16. van Wolferen SA, Grünberg K, Vonk Noordegraaf A. Diagnosis and management of pulmonary hypertension over the past 100 years. *Respir Med*. (2007) 101:389–98. doi: 10.1016/J.RMED.2006.11.022
17. Cournand A. Control of the pulmonary circulation in man with some remarks on methodology. In: *Nobel Lectures: Physiology or Medicine*. Amsterdam: Elsevier Publishing (1964). p. 529–42.
18. Richards DW. The contributions of right heart catheterization to physiology and medicine, with some observations on the physiopathology of pulmonary heart disease. *Am Heart J*. (1957) 54:161–71. doi: 10.1016/0002-8703(57)90143-6
19. Dresdale DT, Schultz M, Michtom RJ. Primary pulmonary hypertension. *Am J Med*. (1951) 11:686–705. doi: 10.1016/0002-9343(51)90020-4
20. Gilmour JR, Evans W. Primary pulmonary hypertension. *J Pathol Bacteriol*. (1946) 58:687–97. doi: 10.1002/path.1700580410
21. Heath D, Whitaker W. Hypertensive pulmonary vascular disease. *Circulation*. (1956) 14:323–43. doi: 10.1161/circ.14.3.323
22. Whitaker W. The initiation of an interest in the pulmonary circulation. *Thorax*. (1994) 49:S2–4. doi: 10.1136/thx.49.Suppl.S2
23. Heath D, Edwards J. The pathology of hypertensive pulmonary vascular disease. *Circulation*. (1958) 18:533–47. doi: 10.1161/circ.18.4.533
24. Beutner CA. Ueber die Strom-und Druckkrafte des Blutes in der Arteria pulmonalis. *Z Ration Med*. (1852) 2:97–138.
25. Euler USV, Liljestrand G. Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol Scand*. (1946) 12:301–20. doi: 10.1111/j.1748-1716.1946.tb00389.x
26. Motley HL, Cournand A, Werko L, Himmelstein A, Dresdale D. The influence of short periods of induced acute anoxia upon pulmonary artery pressures in man. *Am J Physiol Content*. (1947) 150:315–20. doi: 10.1152/ajplegacy.1947.150.2.315
27. Kulandavelu S, Balkan W, Hare JM. Regulation of oxygen delivery to the body via hypoxic vasodilation. *Proc Natl Acad Sci USA*. (2015) 112:6254–5. doi: 10.1073/pnas.1506523112

28. Hurtado A. Aspectos Fisiologicos y Patologicos de la Vida en la Altura. *Rev Med Peru*. (1937) 9:3–52.
29. Fred HL, Schmidt AM, Bates T, Hecht HH. Acute pulmonary edema of altitude: clinical and physiologic observations. *Circulation*. (1962) 25:929–37. doi: 10.1161/01.CIR.25.6.929
30. West JB. *High Life : A History of High-Altitude Physiology and Medicine*. New York: Springer (1998).
31. Hultgren HN, Lopez CE, Lundberg E, Miller H. Physiologic studies of pulmonary edema at high altitude. *Circulation*. (1964) 29:393–408. doi: 10.1161/01.CIR.29.3.393
32. Viswanathan R, Jain SK, Subramanian S, Subramanian TAV, Dua GL, Giri J. Pulmonary Edema of High Altitude. *Am Rev Respir Dis*. (1969) 100:334–41. doi: 10.1164/arrd.1969.100.3.334
33. Hultgren HN, Grover RF, Hartley LH. Abnormal circulatory responses to high altitude in subjects with a previous history of high-altitude pulmonary edema. *Circulation*. (1971) 44:759–70. doi: 10.1161/01.CIR.44.5.759
34. Hultgren HN. High-altitude pulmonary edema: current concepts. *Annu Rev Med*. (1996) 47:267–84. doi: 10.1146/annurev.med.47.1.267
35. Dehnert C, Risse F, Ley S, Kuder TA, Buhmann R, Puderbach M, et al. Magnetic resonance imaging of uneven pulmonary perfusion in hypoxia in humans. *Am J Respir Crit Care Med*. (2006) 174:1132–8. doi: 10.1164/rccm.200606-780OC
36. Maggiorini M, Mélot C, Pierre S, Pfeiffer F, Greve I, Sartori C, et al. High-altitude pulmonary edema is initially caused by an increase in capillary pressure. *Circulation*. (2001) 103:2078–83. doi: 10.1161/01.CIR.103.16.2078
37. Scherrer U, Rexhaj E, Jayet PY, Allemann Y, Sartori C. New insights in the pathogenesis of high-altitude pulmonary edema. *Prog Cardiovasc Dis*. (2010) 52:485–92. doi: 10.1016/j.pcad.2010.02.004
38. Swenson ER, Bärtsch P. High-altitude pulmonary edema. *Comprehen Physiol*. (2012) 2: 2753–73. doi: 10.1002/cphy.c100029
39. Rhodes J. Comparative physiology of hypoxic pulmonary hypertension: historical clues from brisket disease. *J Appl Physiol*. (2005) 98:1092–100. doi: 10.1152/japplphysiol.01017.2004
40. Alexander AF, Will DH, Grover RF, Reeves JT. Pulmonary hypertention and right ventricular hypertrophy in catle at high altitude. *Am J Vet Res*. (1960) 21:199–204.
41. Will DH, Hicks JL, Card CS, Alexander AF. Inherited susceptibility of cattle to high-altitude pulmonary hypertension. *J Appl Physiol*. (1975) 38:491–4. doi: 10.1152/jappl.1975.38.3.491
42. Weir EK, Tucker A, Reeves JT, Will DH, Grover RF. The genetic factor influencing pulmonary hypertension in cattle at high altitude. *Cardiovasc Res*. (1974) 8:745–9.
43. Anand IS, Chandrashekhar Y, Bali HK, Wahi PL, Jindal SK, Malhotra RM, et al. Adult subacute mountain sickness—a syndrome of congestive heart failure in man at very high altitude. *Lancet*. (1990) 335:561–5. doi: 10.1016/0140-6736(90)90348-9
44. Rotta A, Cánepa A, Hurtado A, Velásquez T, Chávez R. Pulmonary circulation at sea level and at high altitudes. *J Appl Physiol*. (1956) 9:328–36. doi: 10.1152/jappl.1956.9.3.328
45. Arias-Stella J, Saldana M. The terminal portion of the pulmonary arterial tree in people native to high altitudes. *Circulation*. (1963) 28:915–25. doi: 10.1161/01.CIR.28.5.915
46. Penaloza D, Arias-Stella J. The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. *Circulation*. (2007) 115:1132–46. doi: 10.1161/CIRCULATIONAHA.106.624544
47. Arias-Stella J, Castillo Y. The muscular pulmonary arterial branches in stillborn natives of high altitude. *Lab Invest*. (1966) 15:1951–9.
48. Peñaloza D, Gamboa R, Marticorena E, Echevarría M, Dyer J, Gutierrez E. The influence of high altitudes on the electrical activity of the heart. *Am Heart J*. (1961) 61:101–15. doi: 10.1016/0002-8703(61)90522-1
49. Peñaloza D, Sime F, Banchero N, Gamboa R, Cruz J, Marticorena E. Pulmonary hypertension in healthy men born and living at high altitudes. *Am J Cardiol*. (1963) 11:150–7. doi: 10.1016/0002-9149(63)90055-9
50. Peñaloza D, Gamboa R, Dyer J, Echevarría M, Marticorena E. The influence of high altitudes on the electrical activity of the heart. I. Electrocardiographic and vectocardiographic observations in the newborn, infants, and children. *Am Heart J*. (1960) 59:111–28. doi: 10.1016/0002-8703(60)90390-2
51. Alexander AF, Jensen R. Pulmonary vascular pathology of high-altitude induced pulmonary hypertension in cattle. *Am J Vet Res*. (1963) 24:1112–22.
52. Sime F, Peñaloza D, Ruiz L. Bradycardia, increased cardiac output, and reversal of pulmonary hypertension in altitude natives living at sea level. *Br Heart J*. (1971) 33:647–57. doi: 10.1136/hrt.33.5.647
53. Vanderpool RR, Naeije R. Hematocrit-corrected pulmonary vascular resistance. *Am J Respir Crit Care Med*. (2018) 198:305–9. doi: 10.1164/rccm.201801-0081PP
54. Linehan JH, Haworth ST, Nelin LD, Krenz GS, Dawson CA. A simple distensible vessel model for interpreting pulmonary vascular pressure-flow curves. *J Appl Physiol*. (1992) 73:987–94. doi: 10.1152/jappl.1992.73.3.987
55. Hoffman JIE. Pulmonary vascular resistance and viscosity: the forgotten factor. *Pediatr Cardiol*. (2011) 32:557–61. doi: 10.1007/s00246-011-9954-3
56. León-Velarde F, Maggiorini M, Reeves JT, Aldashev A, Asmus I, Bernardi L, et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt Med Biol*. (2005) 6:147–57. doi: 10.1089/ham.2005.6.147
57. Naeije R, Vanderpool R. Pulmonary hypertension and chronic mountain sickness. *High Alt Med Biol*. (2013) 14:117–25. doi: 10.1089/ham.2012.1124
58. Villafuerte FC, Corante N. Chronic mountain sickness: clinical aspects, etiology, management, and treatment. *High Alt Med Biol*. (2016) 17:61–9. doi: 10.1089/ham.2016.0031
59. Heath D, Smith P, Williams D, Harris P, Arias-Stella J, Krüger H. The heart and pulmonary vasculature of the llama (Lama glama). *Thorax*. (1974) 29:463–71. doi: 10.1136/thx.29.4.463
60. Heath D, Williams D, Dickinson J. The pulmonary arteries of the yak. *Cardiovasc Res*. (1984) 18:133–139. doi: 10.1093/cvr/18.3.133
61. Porter V, Alderson L, Hall S, Sponenberg P. *Mason's World Encyclopedia of Livestock Breeds and Breeding: 2 Volume Pack*. Wallington: CAB International (2016).
62. Anand IS, Harris E, Ferrari R, Pearce P, Harris P. Pulmonary haemodynamics of the yak, cattle, and cross breeds at high altitude. *Thorax*. (1986) 41:696–700. doi: 10.1136/thx.41.9.696
63. Dunham-Snary KJ, Wu D, Sykes EA, Thakrar A, Parlow LRG, Mewburn JD, et al. Hypoxic pulmonary vasoconstriction: from molecular mechanisms to medicine. *Chest*. (2017) 151:181–92 doi: 10.1016/j.chest.2016.09.001
64. Wilkins MR, Ghofrani H-A, Weissmann N, Aldashev A, Zhao L. Pathophysiology and treatment of high-altitude pulmonary vascular disease. *Circulation*. (2015) 131:582–90. doi: 10.1161/CIRCULATIONAHA.114.006977
65. Siques P, Brito J, Pena E. Reactive oxygen species and pulmonary vasculature during hypobaric hypoxia. *Front Physiol*. (2018) 9:865. doi: 10.3389/fphys.2018.00865
66. Bailey DM, Dehnert C, Luks AM, Menold E, Castell C, Schendler G, et al. High-altitude pulmonary hypertension is associated with a free radical-mediated reduction in pulmonary nitric oxide bioavailability. *J Physiol*. (2010) 588:4837–47. doi: 10.1113/jphysiol.2010.194704
67. Kourembanas S, Marsden PA, McQuillan LP, Faller DV. Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. *J Clin Invest*. (1991) 88:1054–7. doi: 10.1172/JCI115367
68. Weigand L, Shimoda LA, Sylvester JT. Enhancement of myofilament calcium sensitivity by acute hypoxia in rat distal pulmonary arteries. *Am J Physiol Lung Cell Mol Physiol*. (2011) 301:L380–7. doi: 10.1152/ajplung.00068.2011
69. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci USA*. (1995) 92:5510–4. doi: 10.1073/pnas.92.12.5510
70. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science*. (2001) 292:468–72. doi: 10.1126/science.1059796
71. Maxwell PH, Wiesener MS, Chang G-W, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. (1999) 399:271–5. doi: 10.1038/20459
72. Semenza GL. Hypoxia-inducible factor 1: master regulator of O2 homeostasis. *Curr Opin Genet Dev*. (1998) 8:588–94.
73. Patel SA, Simon MC. Biology of hypoxia-inducible factor-2alpha in development and disease. *Cell Death Differ*. (2008) 15:628–34. doi: 10.1038/cdd.2008.17

74. Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev*. (1997) 11:72–82. doi: 10.1101/gad.11.1.72
75. Beall CM, Cavalleri GL, Deng L, Elston RC, Gao Y, Knight J, et al. Natural selection on EPAS1 (HIF2) associated with low hemoglobin concentration in Tibetan highlanders. *Proc Natl Acad Sci USA*. (2010) 107:11459–64. doi: 10.1073/pnas.1002443107
76. Yi X, Liang Y, Huerta-Sanchez E, Jin X, Cuo ZXP, Pool JE, et al. Sequencing of 50 human exomes reveals adaptation to high altitude. *Science*. (2010) 329:75–8. doi: 10.1126/science.1190371
77. Xiang K, Ouzhuluobu, Peng Y, Yang Z, Zhang X, Cui C, et al. Identification of a Tibetan-specific mutation in the hypoxic gene EGLN1 and its contribution to high-altitude adaptation. *Mol Biol Evol*. (2013) 30:1889–98. doi: 10.1093/molbev/mst090
78. Peng Y, Cui C, He Y, Ouzhuluobu, Zhang H, Yang D, et al. Down-regulation of EPAS1 transcription and genetic adaptation of tibetans to high-altitude hypoxia. *Mol Biol Evol*. (2017) 34:818–30. doi: 10.1093/molbev/msw280
79. Lorenzo FR, Huff C, Myllymäki M, Olenchock B, Swierczek S, Tashi T, et al. A genetic mechanism for Tibetan high-altitude adaptation. *Nat Genet*. (2014) 46:951–6. doi: 10.1038/ng.3067
80. Groves BM, Sutton J, Droma T, McCullough RG, McCullough RE, Zhuang J, et al. Minimal hypoxic pulmonary hypertension in normal Tibetans at 3,658 m. *J Appl Physiol*. (1993) 74:312–8. doi: 10.1152/jappl.1993.74.1.312
81. Gupta ML, Rao KS, Anand IS, Banerjee AK, Boparai MS. Lack of smooth muscle in the small pulmonary arteries of the native Ladakhi: is the Himalayan highlander adapted? *Am Rev Respir Dis*. (1992) 145:1201–4. doi: 10.1164/ajrccm/145.5.1201
82. Beall CM. Two routes to functional adaptation: Tibetan and Andean high-altitude natives. *Proc Natl Acad Sci USA*. (2007) 104:8655–60. doi: 10.1073/pnas.0701985104
83. Yu AY, Shimoda LA, Iyer N V, Huso DL, Sun X, McWilliams R, et al. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. *J Clin Invest*. (1999) 103:691–6. doi: 10.1172/JCI5912
84. Brusselmans K, Compernolle V, Tjwa M, Wiesener MS, Maxwell PH, Collen D, et al. Heterozygous deficiency of hypoxia-inducible factor−2α protects mice against pulmonary hypertension and right ventricular dysfunction during prolonged hypoxia. *J Clin Invest*. (2003) 111:1519–27. doi: 10.1172/JCI200315496
85. Newman JH, Holt TN, Cogan JD, Womack B, Phillips JA, Li C, et al. Increased prevalence of EPAS1 variant in cattle with high-altitude pulmonary hypertension. *Nat Commun*. (2015) 6:6863. doi: 10.1038/ncomms7863
86. Tan Q, Kerestes H, Percy MJ, Pietrofesa R, Chen L, Khurana TS, et al. Erythrocytosis and pulmonary hypertension in a mouse model of human HIF2A gain of function mutation. *J Biol Chem*. (2013) 288:17134–44. doi: 10.1074/jbc.M112.444059
87. Gale DP, Harten SK, Reid CDL, Tuddenham EGD, Maxwell PH. Autosomal dominant erythrocytosis and pulmonary arterial hypertension associated with an activating HIF2 alpha mutation. *Blood*. (2008) 112:919–21. doi: 10.1182/blood-2008-04-153718
88. Bushuev VI, Miasnikova GY, Sergueeva AI, Polyakova LA, Okhotin D, Gaskin PR, et al. Endothelin-1, vascular endothelial growth factor and systolic pulmonary artery pressure in patients with Chuvash polycythemia. *Haematologica*. (2006) 91:744–9. Available online at: http://www.haematologica.org/content/91/6/744.full.pdf+html
89. Bond J, Gale DP, Connor T, Adams S, de Boer J, Gascoyne DM, et al. Dysregulation of the HIF pathway due to VHL mutation causing severe erythrocytosis and pulmonary arterial hypertension. *Blood*. (2011) 117:3699–701. doi: 10.1182/blood-2010-12-327569
90. Hickey MM, Richardson T, Wang T, Mosqueira M, Arguiri E, Yu H, et al. The von Hippel–Lindau Chuvash mutation promotes pulmonary hypertension and fibrosis in mice. *J Clin Invest*. (2010) 120:827–39. doi: 10.1172/JCI36362
91. Ball MK, Waypa GB, Mungai PT, Nielsen JM, Czech L, Dudley VJ, et al. Regulation of hypoxia-induced pulmonary hypertension by vascular smooth muscle hypoxia-inducible factor-1alpha. *Am J Respir Crit Care Med*. (2014) 189:314–24. doi: 10.1164/rccm.201302-0302OC
92. Sheikh AQ, Saddouk FZ, Ntokou A, Mazurek R, Greif DM. Cell autonomous and non-cell autonomous regulation of SMC progenitors in pulmonary hypertension. *Cell Rep*. (2018) 23:1152–65. doi: 10.1016/j.celrep.2018.03.043
93. Shimoda LA, Manalo DJ, Sham JS, Semenza GL, Sylvester JT. Partial HIF-1alpha deficiency impairs pulmonary arterial myocyte electrophysiological responses to hypoxia. *Am J Physiol Lung Cell Mol Physiol*. (2001) 281:L202–8. doi: 10.1152/ajplung.2001.281.1.L202
94. Wang J, Weigand L, Lu W, Sylvester JT, Semenza GL, Shimoda LA. Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca2+ in pulmonary arterial smooth muscle cells. *Circ Res*. (2006) 98:1528–37. doi: 10.1161/01.RES.0000227551.68124.98
95. Martin E, Dahan D, Cardouat G, Gillibert-Duplantier J, Marthan R, Savineau JP, et al. Involvement of TRPV1 and TRPV4 channels in migration of rat pulmonary arterial smooth muscle cells. *Pflugers Arch Eur J Physiol*. (2012) 464:261–72. doi: 10.1007/s00424-012-1136-5
96. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. (2009) 324:1029–33. doi: 10.1126/science.1160809
97. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci*. (2016) 41:211–8. doi: 10.1016/j.tibs.2015.12.001
98. Fijalkowska I, Xu W, Comhair SAA, Janocha AJ, Mavrakis LA, Krishnamachary B, et al. Hypoxia inducible-factor1α regulates the metabolic shift of pulmonary hypertensive endothelial cells. *Am J Pathol*. (2010) 176:1130–8. doi: 10.2353/ajpath.2010.090832
99. Plecitá-Hlavatá L, Tauber J, Li M, Zhang H, Flockton AR, Pullamsetti SS, et al. Constitutive reprogramming of fibroblast mitochondrial metabolism in pulmonary hypertension. *Am J Respir Cell Mol Biol*. (2016) 55:47–57. doi: 10.1165/rcmb.2015-0142OC
100. Stenmark KR, Tuder RM, El Kasmi KC. Metabolic reprogramming and inflammation act in concert to control vascular remodeling in hypoxic pulmonary hypertension. *J Appl Physiol*. (2015) 119:1164–72. doi: 10.1152/japplphysiol.00283.2015
101. Marsboom G, Wietholt C, Haney CR, Toth PT, Ryan JJ, Morrow E, et al. Lung ^{18}F-fluorodeoxyglucose positron emission tomography for diagnosis and monitoring of pulmonary arterial hypertension. *Am J Respir Crit Care Med*. (2012) 185:670–9. doi: 10.1164/rccm.201108-1562OC
102. Zhao L, Ashek A, Wang L, Fang W, Dabral S, Dubois O, et al. Heterogeneity in lung 18FDG uptake in PAH: potential of dynamic 18FDG-PET with kinetic analysis as a bridging biomarker for pulmonary remodeling targeted treatments. *Circulation*. (2013) 128:1214–24. doi: 10.1161/CIRCULATIONAHA.113.004136
103. Li M, Riddle SR, Frid MG, El Kasmi KC, McKinsey TA, Sokol RJ, et al. Emergence of fibroblasts with a proinflammatory epigenetically altered phenotype in severe hypoxic pulmonary hypertension. *J Immunol*. (2011) 187:2711–22. doi: 10.4049/jimmunol.1100479
104. Zhao L, Chen C-N, Hajji N, Oliver E, Cotroneo E, Wharton J, et al. Histone deacetylation inhibition in pulmonary hypertension: therapeutic potential of valproic acid and suberoylanilide hydroxamic acid. *Circulation*. (2012) 126:455–67. doi: 10.1161/CIRCULATIONAHA.112.103176
105. Dai Z, Li M, Wharton J, Zhu MM, Zhao YY. Prolyl-4 Hydroxylase 2 (PHD2) deficiency in endothelial cells and hematopoietic cells induces obliterative vascular remodeling and severe pulmonary arterial hypertension in mice and humans through hypoxia-inducible factor-2α. *Circulation*. (2016) 133:2447–58. doi: 10.1161/CIRCULATIONAHA.116.021494
106. Kapitsinou PP, Rajendran G, Astleford L, Michael M, Schonfeld MP, Fields T, et al. The endothelial prolyl-4-hydroxylase domain 2/hypoxia-inducible factor 2 axis regulates pulmonary artery pressure in mice. *Mol Cell Biol*. (2016) 36:1584–94. doi: 10.1128/MCB.01055-15
107. Cowburn AS, Crosby A, Macias D, Branco C, Colaço RDDR, Southwood M, et al. HIF2α-arginase axis is essential for the development of pulmonary hypertension. *Proc Natl Acad Sci USA*. (2016) 113:8801–6. doi: 10.1073/pnas.1602978113
108. Good RB, Gilbane AJ, Trinder SL, Denton CP, Coghlan G, Abraham DJ, et al. Endothelial to mesenchymal transition contributes to endothelial dysfunction in pulmonary arterial hypertension. *Am J Pathol*. (2015) 185:1850–8. doi: 10.1016/j.ajpath.2015.03.019
109. Tang H, Babicheva A, McDermott KM, Gu Y, Ayon RJ, Song S, et al. Endothelial HIF-2α contributes to severe pulmonary hypertension

by inducing endothelial-to-mesenchymal transition. *Am J Physiol Cell Mol Physiol*. (2017) 314:ajplung.00096.2017. doi: 10.1152/ajplung.00096.2017

110. Kim Y-M, Barnes EA, Alvira CM, Ying L, Reddy S, Cornfield DN. Hypoxia-inducible factor-1 in pulmonary artery smooth muscle cells lowers vascular tone by decreasing myosin light chain phosphorylation. *Circ Res*. (2013) 112:1230–3. doi: 10.1161/CIRCRESAHA.112.300646
111. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, et al. Cellular and developmental control of O2 homeostasis by hypoxia- inducible factor 1α. *Genes Dev*. (1998) 12:149–62. doi: 10.1101/gad.12.2.149
112. Wigerup C, Påhlman S, Bexell D. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. *Pharmacol Ther*. (2016) 164:152–69. doi: 10.1016/j.pharmthera.2016.04.009
113. Yu T, Tang B, Sun X. Development of inhibitors targeting hypoxia-inducible factor 1 and 2 for cancer therapy. *Yonsei Med J*. (2017) 58:489–96. doi: 10.3349/ymj.2017.58.3.489
114. Courtney KD, Infante JR, Lam ET, Figlin RA, Rini BI, Brugarolas J, et al. Phase I dose-escalation trial of PT2385, a first-in-class hypoxia-inducible factor-2α antagonist in patients with previously treated advanced clear cell renal cell carcinoma. *J Clin Oncol*. (2017) 36:JCO.2017.74.2627. doi: 10.1200/JCO.2017.74.2627
115. Dai Z, Zhu MM, Peng Y, Machireddy N, Evans CE, Machado R, et al. Therapeutic targeting of vascular remodeling and right heart failure in PAH with HIF-2α inhibitor. *Am J Respir Crit Care Med*. (2018) 198:1423–34. doi: 10.1164/rccm.201710-2079OC
116. Zhao L, Oliver E, Maratou K, Atanur SS, Dubois OD, Cotroneo E, et al. The zinc transporter ZIP12 regulates the pulmonary vascular response to chronic hypoxia. *Nature*. (2015) 524:356–60. doi: 10.1038/nature14620
117. Gupta N, Wish JB. Hypoxia-inducible factor prolyl hydroxylase inhibitors: a potential new treatment for anemia in patients with CKD. *Am J Kidney Dis*. (2017) 69:815–26. doi: 10.1053/j.ajkd.2016.12.011

Conflict of Interest Statement: AART has received funds to attend educational events from Actelion.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 23 July 2019
doi: 10.3389/fmed.2019.00169

Arrhythmic Burden and Outcomes in Pulmonary Arterial Hypertension

Jennifer T. Middleton[1,2,3], *Angshuman Maulik*[2,3], *Robert Lewis*[1,2], *David G. Kiely*[1], *Mark Toshner*[4,5], *Athanasios Charalampopoulos*[1], *Andreas Kyriacou*[3] *and Alexander Rothman*[1,2,3]*

[1] Sheffield Pulmonary Vascular Disease Unit, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom, [2] Department of Infection, Immunity and Cardiovascular Disease, Medical School, University of Sheffield, Sheffield, United Kingdom, [3] Department of Cardiology, Northern General Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom, [4] Department of Medicine, Addenbrooke's Hospital, University of Cambridge, Cambridge, United Kingdom, [5] Royal Papworth Hospital NHS Foundation Trust, Cambridgeshire, United Kingdom

OPEN ACCESS

Edited by:
Argyrios Tzouvelekis, Alexander Fleming Biomedical Sciences Research Center, Greece

Reviewed by:
Iraklis M. Tsangaris, National and Kapodistrian University of Athens, Greece
Stylianos Orfanos, National and Kapodistrian University of Athens, Greece

***Correspondence:**
Alexander Rothman
a.rothman@sheffield.ac.uk

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *03 April 2019*
Accepted: *08 July 2019*
Published: *23 July 2019*

Citation:
Middleton JT, Maulik A, Lewis R, Kiely DG, Toshner M, Charalampopoulos A, Kyriacou A and Rothman A (2019) Arrhythmic Burden and Outcomes in Pulmonary Arterial Hypertension. Front. Med. 6:169. doi: 10.3389/fmed.2019.00169

Pulmonary arterial hypertension (PAH) is a devastating, life-limiting disease driven by small vessel vascular remodeling leading to a rise in pulmonary vascular resistance (PVR). Patients present with a range of symptoms including shortness of breath, exercise intolerance, palpitations or syncope. Symptoms may be related to vascular disease progression or arrhythmia secondary to the adaptation of the right heart to pressure overload. Arrhythmic burden is high in patients with left heart disease and guideline-based treatment of arrhythmias improves quality of life and prognosis. In PAH the incidence and prevalence of arrhythmias is less well-defined and there are no PAH-specific guidelines for arrhythmia management. We undertook a literature search identifying 13 relevant papers; detection of arrhythmias was acquired from 12-lead electrocardiogram (ECG) or Holter monitors. In all forms of pulmonary hypertension (PH) the prevalence of supraventricular arrhythmias (SVA) was 26–31%, ventricular arrhythmias (VA) 24% and a 5-year incidence of SVA ∼13.2–25.1%. Prevalence and incidence of arrhythmias in PAH is less clear due to limited study numbers and the heterogenous nature of the patient population studied. For arrhythmia treatment, only single-arm studies of therapeutic strategies were reported using antiarrhythmic drugs (AAD), direct current cardioversion (DCCV) and ablation. Periods between ECG or Holter have not been investigated, highlighting the possibility that significant arrhythmias may be undetected. Advances in monitoring allow long-term surveillance via implanted/non-invasive monitors. Use of such technologies may provide an accurate estimate of incidence and prevalence of arrhythmias in patients with PAH, further defining relationships to adverse outcomes, and therapeutic options.

Keywords: arrhythmia, pulmonary arterial hypertension, right heart failure, atrial fibrillation, atrial flutter, ventricular tachycardia

INTRODUCTION

The recent World Symposium classifies pulmonary hypertension into five groups based on underlying cause (1). Group one incorporates pulmonary arterial hypertension including familial and idiopathic causes; group two is secondary to left sided heart disease; group three to lung disease and hypoxia; group four is secondary to chronic thromboembolic disease (CTEPH) and group five includes miscellaneous causes including sarcoidosis and hematological disease (1). Increased right ventricular pressure and volume overload of the right heart leads to structural changes that impair left ventricular filling (2) and function which may predispose to the development of arrhythmias. In this review we will discuss the prevalence and incidence of arrhythmia in PAH and the potential treatment options.

CARDIAC ARRHYTHMIAS: CLASSIFICATION AND MECHANISM

Cardiac arrhythmias are caused by either disruption of the cardiac action potential or structural/functional changes to the heart that result in abnormalities of electrical conduction. Classification is made based on heart rate (bradycardic or tachycardic) and sub-divided based on site of origin (supraventricular or ventricular arrhythmia). In the left and right atrium, dilatation and stretch results in structural, and ion channel adaptations resulting in an increased development of SVA's including atrial fibrillation (AF) and atrial flutter (see **Figures 1A,B**). Subsequent loss of atrial contraction leads to underfilling of the ventricle and reduction in cardiac output (CO). VA's can be precipitated by a dilated, dysfunctional ventricle, or altered ventricular substrate in the form of scar (3), giving rise to pro-arrhythmic myocardium (see **Figures 2A,B**). In left sided heart failure there is an increased risk of arrhythmia, which when present often exacerbates left ventricular failure (3, 4). Impaired left ventricular function results in reduced CO due to inadequate filling and contraction leading to compensatory hypertrophy and dilatation of the left ventricle. These changes increase sarcomere length and alter sensitivity to calcium leading to more forceful ventricular contraction (5), the resultant ion channel remodeling (6) can make myocardium pro-arrhythmic (3). Bradyarrhythmia presents often secondary to changes in heart structure, nodal fibrosis, or rate-limiting medication used to treat heart failure.

Current guidelines for the treatment of arrhythmia specifically relate to patients with left sided heart disease (4–9). Management strategies include AAD's that alter the action of specific ion channels to stabilize myocardium; anticoagulation to reduce stroke risk in AF (6); synchronized DCCV to revert to normal sinus rhythm (NSR); electrophysiology studies (EPS); and/or ablation to identify abnormal heart rhythms using intracardiac catheters and ablation to disrupt abnormal conduction pathways and finally permanent pacemakers or internal defibrillator devices (7, 10). Guidance for the treatment of arrhythmias in patients with PAH is lacking despite the potential harmful/unknown effects of AAD's and or therapies in this cohort (7).

Arrhythmias in PAH

Patients with PAH often describe palpitations and/or syncope. The incidence and prevalence of cardiac arrhythmias in PAH is not well-described, however a number of recent studies describe a significant arrhythmic burden in all forms of PH, and a relationship to clinical outcome. These studies are based on "snapshots" in time using 12 lead ECGs or short-term Holter monitors. We undertook a literature-based review searching PubMed with the search terms "pulmonary arterial hypertension, supraventricular, and ventricular arrhythmias, atrial fibrillation/flutter, ventricular tachycardia, and mode of death," 13 relevant papers were reviewed as well as PAH registry data. A review of the REVEAL PAH registry data demonstrated a relationship between increased heart rate and adverse outcomes (11, 12) and during follow up, Burger et al. found 5.1% of patients were admitted secondary to an arrhythmia, however further details were not provided (13).

Supraventricular Arrhythmias

Prevalence

In a multi-center, observational study (14), patients with PAH/inoperable CTEPH attending routine outpatients had their notes reviewed retrospectively for evidence of AF or atrial flutter. 297 patients were identified as fulfilling criteria (PAH 266/CTEPH 31) with 90% on PAH specific medication. An ECG diagnosis of AF or atrial flutter was noted in 79 (26.5%), approximately 50% of those were deemed to be paroxysmal in nature. Patients with AF were as a group, of increased age, male gender with a higher systolic blood pressure (BP), a reduced left ventricular ejection fraction (LVEF), and had a higher rate of chronic obstructive lung disease (COPD). At right heart catheterization (RHC), the AF group had a higher pulmonary capillary wedge pressure (PCWP), and lower pulmonary vascular resistance, potentially suggesting a more elderly population with a greater burden of co-morbid condition. There was no difference found in mean pulmonary artery pressure (PAP) or CO. Results suggest that a diagnosis of permanent or paroxysmal AF/atrial flutter in PAH confers an increased mortality. The use of 12-lead ECGs alone and the unbalanced co-morbidities of patients with and without AF/atrial flutter masked the true burden of arrhythmias in patients with PAH (14).

Rottlaender et al. (15), investigated patients with all forms of PH who developed SVA in a retrospective study of 225 patients. Thirty one percentage of patients recruited were found to have AF (41% paroxysmal) and it was apparent that AF was more likely if secondary to PH-left heart disease and less common in CTEPH. All patients identified as having AF deteriorated clinically at the time of arrhythmia. The true prevalence of SVA was again difficult to determine due to the inclusion of all types of PH. In contrast Ruiz-Cano et al. retrospectively reviewed 282 patients with PAH. In total 28 SVA episodes were detected: AF 12 patients (42.8%); atrial flutter 12 (42.8%); other SVA 4 (14.2%), 82% of SVA episodes resulted in symptoms and clinical deterioration / worsening right heart failure (RHF).

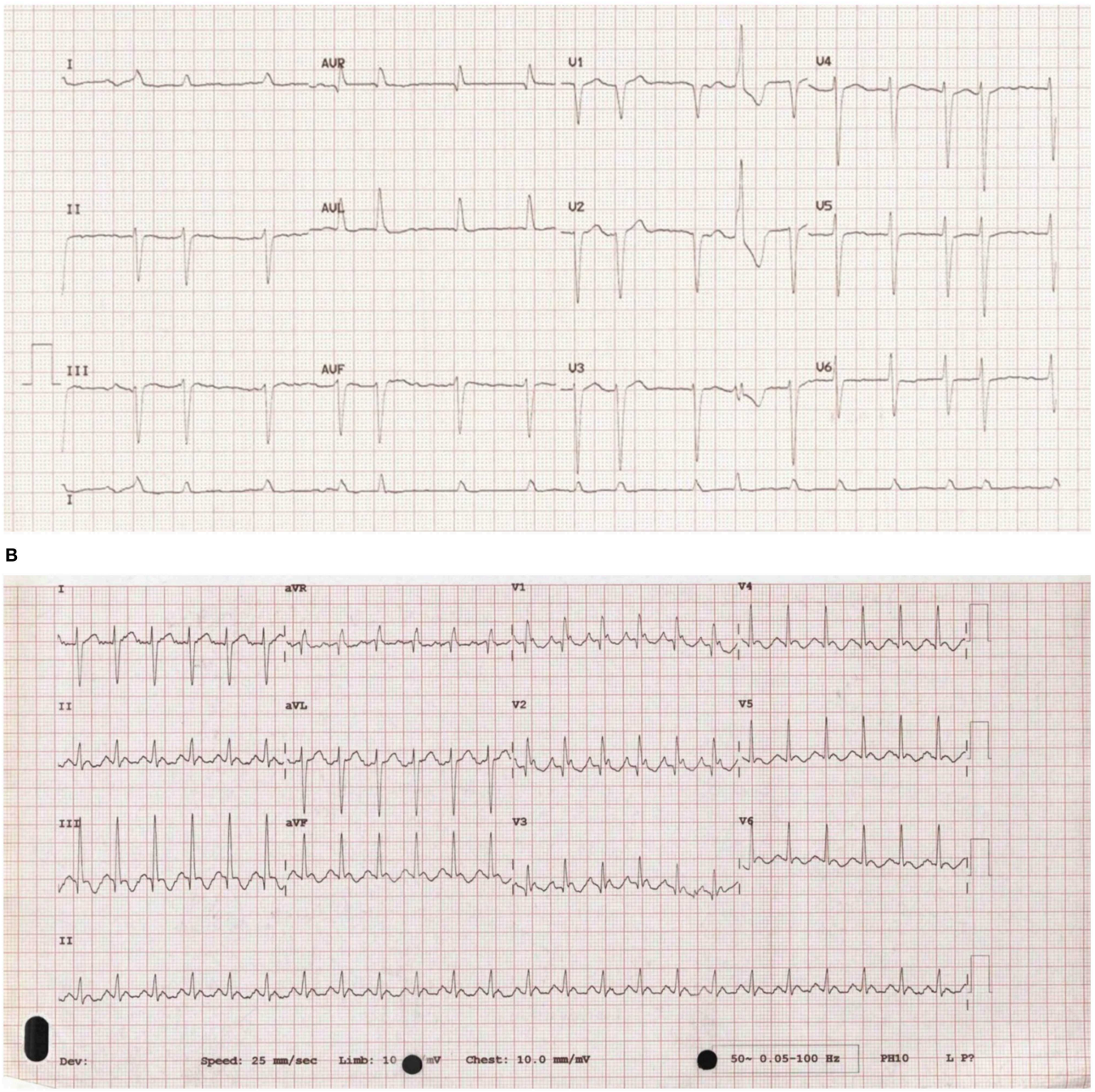


FIGURE 1 | (A) An ECG of atrial fibrillation. An example of an ECG taken from a patient with atrial fibrillation showing uncoordinated atrial activity resulting in an irregular ventricular rate. **(B)** An ECG of atrial flutter. An example of an ECG taken from a patient with atrial flutter. It is characterized by rapid, regular atrial depolarizations (typically, but not always at a rate of 300 bpm) resulting in a ventricular rate of 150 bpm.

Time from PAH diagnosis to SVA was approximately 60 months and time from SVA diagnosis to death/transplant was 17.8 months suggesting increased morbidity and mortality with the development of SVA.

Incidence

A number of studies investigated incidence through prospective/retrospective cohort studies (summarized in **Table 1**), with 6-year incidence of SVA in PAH approximately 15.8% and in all forms of PH a 5-year incidence of between 13.2 and 25.1% (17–21). In a retrospective, cohort study, Cannillo et al. examined patients with PAH, lung disease-associated PH, and CTEPH who had NSR at baseline on a 12-lead ECG, patients with previous SVA diagnosis were excluded (18). Retrospective analysis of ECG's was undertaken during follow-up, if admitted or if they developed palpitations. Holter monitors were only

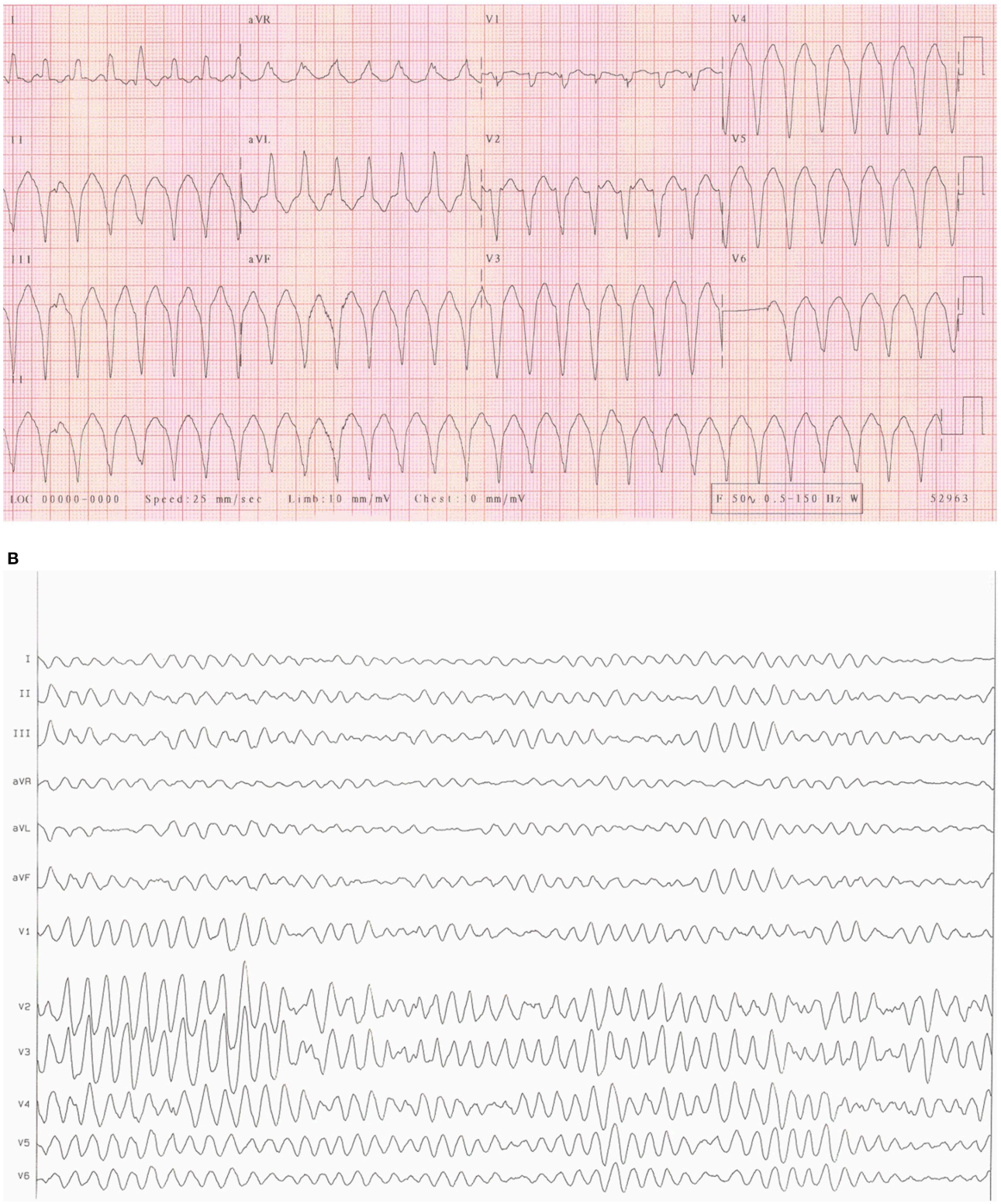


FIGURE 2 | (A) An ECG of ventricular tachycardia. An example of an ECG from a patient with sustained monomorphic ventricular tachycardia. It is characterized by regular, broad QRS complexes of similar morphology often over 100 bpm. **(B)** An ECG of Ventricular fibrillation. An example of an ECG from a patient with ventricular fibrillation. It is characterized by rapid, erratic ventricular activity as the heart fibrillates rather than pumps effectively. This rhythm quickly degenerates into cardiac arrest.

fitted in patients reporting palpitations, not as a screen for asymptomatic SVA's. 77 patients met criteria, 17 patients (22%) were newly diagnosed with SVA during the follow-up period (35 months). Patients were found to have persistent AF in 8 patients (47%), permanent AF 3 patients (17%), paroxysmal SVA 3 patients (17%), atrial ectopic tachycardia 2 patients (12%), right atrial flutter 2 patients (12%) and paroxysmal AF (PAF) in 1 patient (6%), other SVA 1 patient (6%). Diagnosis of SVA's occurred on average 15.1 months after PH diagnosis and was associated with worsening parameters (World health organization (WHO)-functional class/6-min walk test (6MWT)/brain natriuretic protein (BNP), and increased mortality. This suggests SVA to be a manifestation of more severe PAH [as per Tongers (21) and Ruiz-Cano (16)]. Severe PAH resulted in more hospital admissions and closer monitoring by default, potentially introducing detection bias of SVA in these patients. Consistent with these findings, Mercurio et al. (17) again looked at PAH only and specifically idiopathic PAH and systemic sclerosis-associated pulmonary hypertension (SSc-PH). A prospective, single center study recruited 317 patients in total (116 idiopathic PAH) and of these 42 developed SVA. In keeping with Smith et al. (14) these patients had higher baseline PCWP and in this case right atrial pressure (RAP). In 90.1% cases the onset of SVA resulted in clinical worsening and RHF. A 5-year incidence of SVA in idiopathic PAH and SSc-PAH was found to be 13.2%, lower than previously documented by Olsson et al. in a similar PH group (20). Olsson et al. described a 5-year incidence of 25.1% of SVA in PAH and inoperable CTEPH. Patients again had NSR at baseline and diagnosis of arrhythmia was based on 12-lead ECG's only. Forty-Eight patients (20%) had at least one episode of SVA. They also showed patients with persistent AF to have a worse prognosis than those with PAF or NSR (20). This followed on from a previous study by Tongers et al. (21), that retrospectively recruited 231 patients under follow-up for PAH and inoperable CTEPH. Thirty-one episodes of SVA were noted on 12-lead ECG in 27 patients (Atrial flutter 15, AF 13, and other SVA 3). Episodes were again associated with marked clinical deterioration and RHF (84% SVA episodes), improving if NSR was restored. Interestingly this study appears to show an increased mortality in AF patients where NSR could not be restored. All 15 patients with atrial flutter had NSR, restored with medication (1), DCCV (6), overdrive pacing (15) or ablation (5), with one death during follow-up. In the AF group only 2 patients had NSR restored by DCCV. NSR in the remaining 9 was not achieved despite multiple medications and DCCV, with 8 deaths during follow-up. This suggests an increased mortality in AF and PAH if NSR is not achieved. It would also appear that treatment measures in the AF group were less aggressive (ablation was not attempted) but the study was from 2007 so potentially now outdated as ablation is more common place today.

In patients with idiopathic PAH on disease specific therapies Wen et al. (19) undertook a prospective, cohort study with 280 patients recruited specifically with NSR at baseline. Forty patients developed SVA at least once during follow-up, 5-year incidence was calculated as 15.8%, potentially a more realistic reflection in PAH alone. Patients as with previous studies did not tolerate SVA and restoration of NSR correlated with clinical recovery. Studies again only diagnosed SVA on 12-lead ECG, potentially failing to diagnose asymptomatic SVA.

Treatment

AAD's, overdrive pacing, DCCV, and radiofrequency ablation are all guideline based therapies for SVA (6, 7), (although not PAH-specific). Existing data showed that rhythm control has been the most popular first line strategy and restoration of NSR demonstrated clinical improvement. Cannillo et al. (18) attempted rhythm control in 76% of patients diagnosed with SVA, NSR was restored in 11 (65%) cases but there was a high recurrence rate (∼80%). Olsson et al. (20) diagnosed patients with SVA on 12-lead ECG and largely from patients presenting with deterioration of PAH/RHF symptoms rather than symptoms of arrhythmia [as per Cannillo et al. (18)]. Those with atrial flutter received ablation earlier with the concurrent use of AAD's with or without synchronized DCCV. For stable AF 10–14 days of oral amiodarone was prescribed pre DCCV and continued thereafter. If there were signs of RHF, amiodarone was given intravenously, and DCCV was performed. NSR was restored in 21/24 patients with atrial flutter and 16/24 patients with AF, resulting in clinical improvement. As such this observational study demonstrates that amiodarone was well-tolerated in PAH. Five-year survival in PAH/inoperable CTEPH was 68% with a fall to 58% if the patient developed a transient SVA and reduced further to 47% in permanent SVA. They also found that AF was more resistant to treatment than atrial flutter (similar to non-PAH disease). Consistent with these findings, Ruiz-Cano et al. (16) found that after the first episode of SVA (despite restoration of NSR/adequate rate control) 46.4% patients required an increase in PAH-specific medications secondary to progressive RHF, an interesting observation but not guideline directed. This data may suggest that the onset of SVA can be a prelude to RHF and/or clinical deterioration and that restoration of NSR or escalating PAH-specific therapy should be considered. Zhang et al. (22) specifically reviewed the efficacy and safety of electrophysiology studies and ablation in SVA and PAH. A retrospective study it reviewed 300 patients over 3 years [PAH 100 (observation) and non-PAH 200 (control)] with PAF undergoing ablation for the first time. All patients had a 24 h Holter monitor fitted immediately post ablation and AAD's were stopped 2 months later. EPS and ablation were found to be safe and reasonably effective in this cohort of patients. Bandorski et al. (23) agreed and found that 12/14 patients (all PH types) with a diagnosis of atrial flutter during EPS subsequently had successful ablation. Zhang et al. (22) noted that 11.3% of patients had an early recurrence PAF and 7.3% a late recurrence, with some correlation between a higher RAP and the incidence of late PAF recurrence.

In conclusion, incidence of SVA in PH (including PAH) ranges from 13.2 to 25.1% with a relationship to clinical worsening. As such this may be an indicator for restoration of NSR or alteration of PAH specific therapy. A range of therapeutic strategies have been investigated, however conclusions on efficacy are challenging to substantiate as the majority of studies were single arm. Little evidence of harm was identified. As in left heart disease NSR was easier to restore in atrial flutter vs. AF and all

TABLE 1 | Summary of literature reviewing SVA in PH.

Reference	Type of study	Pathology	Number of patients	Demographics in arrhythmia group	Prevalence/ incidence	Investigation	Rhythm identified	NSR restored?	Treatment given	Outcome
Smith et al. (14)	Retrospective 4-year study	PAH CTEPH	PAH 266 CTEPH 31	F:M 53:14% Age 61.8±14 MeanPAP 44±11	Prevalence	12-Lead ECG	AF/atrial flutter 50% paroxysmal	31 patients	86% AAD's 14% Not specified	
Rottlaender et al. (15)	Retrospective 4-year study	All types of PH	Total 225	F:M 36:64% Age 71.2±1.1 MeanPAP 40.8±1.6	Prevalence	12 Lead ECG	AF 41% paroxysmal	Not discussed	Not discussed	Permanent AF = Clinical deterioration.
Ruiz- Cano et al. (16)	Retrospective, 4-year, single center study	PAH	Total 282	F:M 61:39% Age 47.3±4.3	Prevalence	Medical notes/12-lead ECG	AF/atrial flutter /atrioventricular node re-entry tachycardia (AVNRT)	Attempted in all	All underwent EPS +/− ablation	Restoration of NSR = clinical improvement 4 SVA recurrence
Mercurio et al. (17)	Prospective, single center study	Idiopathic PAH PAH-SSc	116 IPAH 201 SSc-PH	F:M 71-29% Age 59+-12.1 MeanPAP 47.3+-14.3	Incidence at 5 years 13.2%		AF/atrial flutter/atrial tachycardia	Attempted in all	90.1% SVA = clinical worsening/RHF.	Restoration of NSR = clinical improvement
Cannillo et al. (18)	Retrospective, single center study? type of PH experienced SVA	PAH PH secondary to lung disease CTEPH (inoperable)	Total 77	F:M 55:45% Mean age 63 MeanPAP 43	Incidence	12 Lead ECG Holter if symptomatic	AF/atrial flutter 4 paroxysmal (23%).	13 patients	AAD's/DCCV/ablation SVA = worsening prognostic parameters and RHF. NSR restored in 11 cases	Recurrent SVA in 9 patients
Li-Wen et al. (19)	Prospective, cohort 6-year study	Idiopathic PAH (all taking PH meds)	Total 280	F:M 72.5:27.5% Age 39+-15 MeanPAP 64+-18	Incidence at 6 years (15.8%)		AF/atrial flutter /atrial tachycardia	21 patients	AAD's/DCCV/ablation SVA = clinical deterioration	NSR restored = clinical improvement 6 x recurrence
Olsson et al. (20)	Prospective 5-year, single center study	PAH or CTEPH (inoperable) NSR at baseline, all receiving PH meds	157 PAH 82 Inoperable CTEPH	F:M 65:35% Age 58+-9 MeanPAP 52+-8	Incidence at 5 years (25.1%)	12-Lead ECG	AF/atrial flutter	21/24 atrial flutter 16/24 AF	AAD's/overdrive pacing /DCCV/ablation	NSR = clinical improvement
Tongers et al. (21)	Retrospective 6-year study	PAH CTEPH (inoperable)	Total 231	F:M 65:35% Age 49+-13 MeanPAP 50+-10	Cumulative incidence of 11.7% and annual risk of 2.8%/ patient.	12 Lead ECG AVNRT diagnosed on EPS	AF/atrial flutter /AVNRT 4 SVA recurrence	15/15 atrial flutter 2/13 AF	AAD's/overdrive pacing /DCCV/ablation	Restoration of NSR = clinical improvement. Increased mortality in AF group
Zhang et al. (22)	Retrospective, 3-year, single center study	PAH Non- PAH PAF	PAH 100 Non-PAH 200	F:M 54.4:45.6% Age 62.9 + 6.8 MeanPAP 31.9+-6.2	X	Holter post ablation + 24-h Holter 3 monthly	11.3% early recurrence of PAF 7.3% Late recurrence of PAF	Attempted in all	All patients underwent radiofrequency ablation AAD's stopped 2 months post ablation	Suggests raised PAP increases chance of late recurrence PAF.

studies suggested that restoration of NSR resulted in improved clinical outcomes.

Ventricular Arrhythmias

The impact of ventricular arrhythmias in patients with PH is examined in a series of papers by Bandorski et al. An initial retrospective, two-center study (23) analyzed data from 55 PH patients presenting with indications for EPS (14 with group I PH). Fifteen had non-sustained ventricular tachycardia (NSVT) on a Holter monitor, however the prognostic relevance is unclear although likely to infer increased risk and extrapolation of prevalence from such a study is challenging due to small numbers and the large contribution of patients (23 in this study) with left heart disease-associated PH. There were no evidence suggesting EPS or ablation to be unsafe or ineffective in PH patients.

In a larger study, Bandorski et al. (24) sought to determine the incidence of VA in PH. Ninety two patients were enrolled in total (54 Group I, 10 Group 3, 26 Group 4, 2 Group 5), all of whom were on PAH-specific medication and in NSR at the time of enrolment. During 72-h Holter monitoring, 17 patients (18.5%) had a detectable arrhythmia [NSVT (12 patients), second degree atrioventricular block (1), intermittent complete heart block (1), and atrial flutter (1)]. Although small and including patients with all PH types, they undertook Holter monitoring and found arrhythmia in asymptomatic patients highlighting the potential inaccuracy of determining the incidence or prevalence of asymptomatic arrhythmia from 12-lead ECG. It highlighted that the use of Holter monitoring in this cohort was beneficial for arrhythmia diagnosis and this may be a useful tool in PAH patients also. To determine the prognostic significance of NSVT Bandorski et al. (25) examined 78 patients with PAH or inoperable CTEPH. Fifty-Five patients with PAH and 23 CTEPH underwent a clinical review, bloods, Holter monitoring, 6 MWT, echocardiography and RHC (25), of whom 12 had newly detected NSVT.

Relatively little evidence exists defining the prevalence and incidence of VA in PAH. Prevalence is estimated to be ∼27% in all PH types with a considerable proportion of whom have co-existing left heart disease. Longer studies utilizing Holter monitoring rather than 12-lead ECG show higher rates of VA suggesting a bias in methods. The most reliable incidence of VA in all PH was ∼15–18.5% based on Holter monitoring (25) suggesting a significant burden. Therapeutic options are unclear with only single arm studies available.

Bradyarrhythmia's

Limited data is available. Whilst determining the incidence of VA in PH Bandorski et al. (24), identified that 4/17 patients with newly diagnosed arrhythmias had intermittent heart block. Two patients, despite not taking rate limiting medication, progressed to complete heart block, and required pacemaker implantation. Studies are small and the relationship to poor outcome is uncertain.

Mode of Death

The predominant cause of death in patients with PAH is thought to be RHF or sudden cardiac death (SCD) (26). Hoeper et al. (27) undertook a retrospective, multi-center (17 referral centers in Europe and the United States) looking at patients with PAH who had developed cardiac arrest. 3130 patients with PAH were treated over 3 years and 513 had circulatory arrest. Cardiopulmonary resuscitation (CPR) was attempted in 132 (26%) but despite the majority occurring in a hospital setting only 8 patients (6%) survived to > 90 days. No apparent differences were found preceding cardiac arrest accounting for why a patient did or did not survive. It is difficult to fully assess cause of death, this data showed that 54% of patients were admitted with intercurrent illness; 49% died from progressive RHF; 18% respiratory failure, and 8% from other causes. Seventeen percent died from SCD and it is unclear as to whether this was secondary to PAH progression or potentially treatable arrhythmia.

CONCLUSION

Present data suggest that patients with PH have increased risk of arrhythmia, however accurate estimates of incidence and prevalence in patients with PAH remain elusive. PAH is a rare disease and as such patient numbers are often limited and the studies undertaken have grouped patients with PAH with other forms of related disease. As such, longitudinal studies with defined enrolment criteria are required to determine the arrhythmic burden of patients with PAH. The enrolment of patients with pre-existing symptoms is a potential source of bias exemplified by the increased rates of asymptomatic SVA and VA (24) identified with prolonged monitoring. Arrhythmias have been demonstrated to precede adverse clinical events and it is therefore of clinical importance to accurately define prevalence and incidence and examine potential therapeutic options. Current evidence highlights gaps in our knowledge as we only have "snapshots" of data from a 12-Lead ECG or Holter monitoring. Advances in technology now allow for long term monitoring of cardiac rhythm and as such a prospective study with continuous monitoring may further inform incidence, prevalence and relationships to adverse outcomes, prior to studies of therapeutic strategies.

AUTHOR CONTRIBUTIONS

JM and AR wrote the manuscript draft. All authors critically reviewed the paper and approved the final manuscript for submission.

FUNDING

JM is funded by a Donald Heath research Fellowship. AR is supported by a Wellcome Trust Clinical Research Career Development Fellowship (206632/Z/17/Z).

REFERENCES

1. Galiè N, McLaughlin VV, Rubin LJ, Simonneau G. An overview of the 6th world symposium on pulmonary hypertension. *Europ Respirat J.* (2019) 53:3. doi: 10.1183/13993003.02148-2018
2. Peacock AJ, Naeije R, Rubin LJ. *Pulmonary Circulation: Diseases and Their Treatment. 3rd Ed*. Boca Raton, FL: CRC Press (2011). doi: 10.1201/b13219
3. Masarone D, Limongelli G, Rubino M, Valente F, Vastarella R, Ammendola E, et al. Management of arrhythmias in heart failure. *Cardiovas Develop Dis.* (2017) 4:E3. doi: 10.3390/jcdd4010003
4. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Europ Heart J.* (2016) 18:891–975. doi: 10.1002/ejhf.592
5. Braveny P. Editorial: heart, calcium and time. *J Exp Clin Cardiol.* (2002) 7:3–6.
6. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur J Cardiothorac Surg.* (2017) 50:e1–e88. doi: 10.1016/j.rec.2016.11.033
7. Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Bloma N, Borggrefe M, Camm J, et al. 2015 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the european society of cardiology (ESC). Endorsed by: association for european paediatric and congenital cardiology (AEPC). *Europ Heart J.* (2015) 36:2793–867l. doi: 10.1093/eurheartj/ehv316
8. Adler A. *Implantable Cardioverter Defibrillators and Cardiac Resynchronisation Therapy for Arrhythmias and Heart Failure.* London: NICE Guidance (2014).
9. Cowan C. *Atrial Fibrillation: Management Atrial Fibrillation: Management Clinical Guideline*. NICE Guidance (2014).
10. Brignole, M, Aurricchio A, Baron-Esquivias G, Bordachar P, Boriani G, Breithardt O-A, et al. ESC Guidelines on cardiac pacing and cardiac resynchronisation therapy. *Eur Heart J.* (2013) 34:2281–329. doi: 10.1093/eurheart/eht150
11. Malcolm MB. Systemic BP and heart rate as prognostic indicators in pulmonary arterial hypertension. *Chest.* (2013) 144:959–65. doi: 10.1378/chest.12-2572
12. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS. Predicting survival in pulmonary arterial hypertension insights from the registry to evaluate early and long-term pulmonary arterial hypertension disease management (REVEAL). *Circulation.* (2010) 122:164–72. doi: 10.1161/CIRCULATIONAHA.109.898122
13. Burger CD, Long PK, Shah MR, McGoon MD, Miller DP, Romero AJ, et al. Characterization of first-time hospitalizations in patients with newly diagnosed pulmonary arterial hypertension in the reveal registry. *J Public Chestnet Org.* (2014) 146:1263–73. doi: 10.1378/chest.14-0193
14. Smith B, Genuardi MV, Koczo A, Zou RH, Thoma FW, Handen A, et al. EXPRESS: atrial arrhythmias are associated with increased mortality in pulmonary arterial hypertension. *Pulm Circ.* (2018) 8:204589401879031. doi: 10.1177/2045894018790316
15. Rottlaender D, Motloch LJ, Schmidt D, Reda S, Larbig R, Wolny M, et al. Clinical impact of atrial fibrillation in patients with pulmonary hypertension. *PLoS ONE.* (2012) 7:e33902. doi: 10.1371/journal.pone.0033902
16. Ruiz-Cano MJ, Gonzalez-Mansilla A, Escribano P, Delgado J, Arribas F, Torres J, et al. Clinical implications of supraventricular arrhythmias in patients with severe pulmonary arterial hypertension. *Int J Cardiol.* (2011) 146:105–6. doi: 10.1016/j.ijcard.2010.09.065
17. Mercurio V, Peloquin G, Bourji KI, Diab N, Sato T, Enobun B, et al. Pulmonary arterial hypertension and atrial arrhythmias: incidence, risk factors, and clinical impact. *Pulmonary Circulat.* (2018) 8:4–11. doi: 10.1177/2045894018769874
18. Cannillo M, Grosso Marra W, Gili S, D'Ascenzo F, Morello M, Mercante L, et al. Supraventricular arrhythmias in patients with pulmonary arterial hypertension. *Am J Cardiol.* (2015) 116:1883–9. doi: 10.1016/j.amjcard.2015.09.039
19. Wen L, Sun ML, An P, Jiang X, Sun K, Zheng L, et al. Frequency of supraventricular arrhythmias in patients with idiopathic pulmonary arterial hypertension. *Am J Cardiol.* (2014) 114:1420–5. doi: 10.1016/j.amjcard.2014.07.079
20. Olsson KM, Nickel NP, Tongers J, Hoeper MM. Atrial flutter and fibrillation in patients with pulmonary hypertension. *Int J Cardiol.* (2013) 167:2300–5. doi: 10.1016/j.ijcard.2012.06.024
21. Tongers J, Schwerdtfeger B, Klein G, Kempf T, Schaefer A, Knapp JM, et al. Incidence and clinical relevance of supraventricular tachyarrhythmias in pulmonary hypertension. *Am Heart J.* (2007) 153:127–32. doi: 10.1016/j.ahj.2006.09.008
22. Zhang Y-Q, Zhang F-L, Wang W-W, Chen X-H, Chen J-H, Chen LL. The correlation of pulmonary arterial hypertension with late recurrence of paroxysmal atrial fibrillation after catheter ablation. *J Thoracic Dis.* (2018) 10:2789–94. doi: 10.21037/jtd.2018.04.92
23. Bandorski D, Schmitt J, Kurzlechner C, Erkapic D, Hamm CW, Seeger W, et al. Electrophysiological studies in patients with pulmonary hypertension: a retrospective investigation. *Biomed Res Int.* (2014) 2014:617565. doi: 10.1155/2014/617565
24. Bandorski D, Erkapic D, Stempfl J, Höltgen R, Grünig E, Schmitt J, et al. Ventricular tachycardias in patients with pulmonary hypertension: an underestimated prevalence? *A Prospective Clinical Study. Herzschrittmachertherapie Elektrophysiologie.* (2015) 26:155–62. doi: 10.1007/s00399-015-0364-8
25. Bandorski D, Bogossian H, Stempfl J, Seeger W, Hecker M, Ghofrani A, et al. Prognostic relevance of nonsustained ventricular tachycardia in patients with pulmonary hypertension. *BioMed Res Int.* (2016) 2016:1327265. doi: 10.1155/2016/1327265
26. Galiè N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera J, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the task force for the diagnosis and treatment of pulmonary hypertension of the european society of cardiology (ESC) and the european respiratory society (ERS), endorsed by the international Society of Heart and Lung Transplantation (ISHLT). *Euro Heart J.* (2009) 30:2493–537. doi: 10.1093/eurheartj/ehp297
27. Hoeper MM, Galié N, Murali S, Olschewski H, Rubenfire M, Robbins IM, et al. Outcome after cardiopulmonary resuscitation in patients with pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2002) 165:341–4. doi: 10.1164/ajrccm.165.3.200109-0130c

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

NOMENCLATURE

6MWT: 6-minute walk test
AAD: Antiarrhythmic drugs
AF: Atrial fibrillation
AVNRT: Atrioventricular node re-entry tachycardia
BP: Blood pressure
BNP: Brain natriuretic protein
CO: Cardiac output
COPD: Chronic obstructive pulmonary disease
CPR: Cardiopulmonary resuscitation
CTEPH: Chronic thromboembolic pulmonary hypertension
DCCV: Direct current cardioversion
ECG: Electrocardiogram
EPS: Electrophysiology study
LVEF: Left ventricular ejection fraction
NSR: Normal sinus rhythm
NSVT: Non-sustained ventricular tachycardia
PAF: Paroxysmal atrial fibrillation
PAH: Pulmonary arterial hypertension
PAP: Pulmonary arterial pressure
PCWP: Pulmonary capillary wedge pressure
PH: Pulmonary hypertension
PVR: Pulmonary vascular resistance
RAP: Right atrial pressure
RHC: Right heart catheter
RHF: Right heart failure
SCD: Sudden cardiac death
SSc-PH: Systemic sclerosis-associated pulmonary hypertension
SVA: Supraventricular arrhythmia
VA: Ventricular arrhythmia
WHO: World health organization

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership