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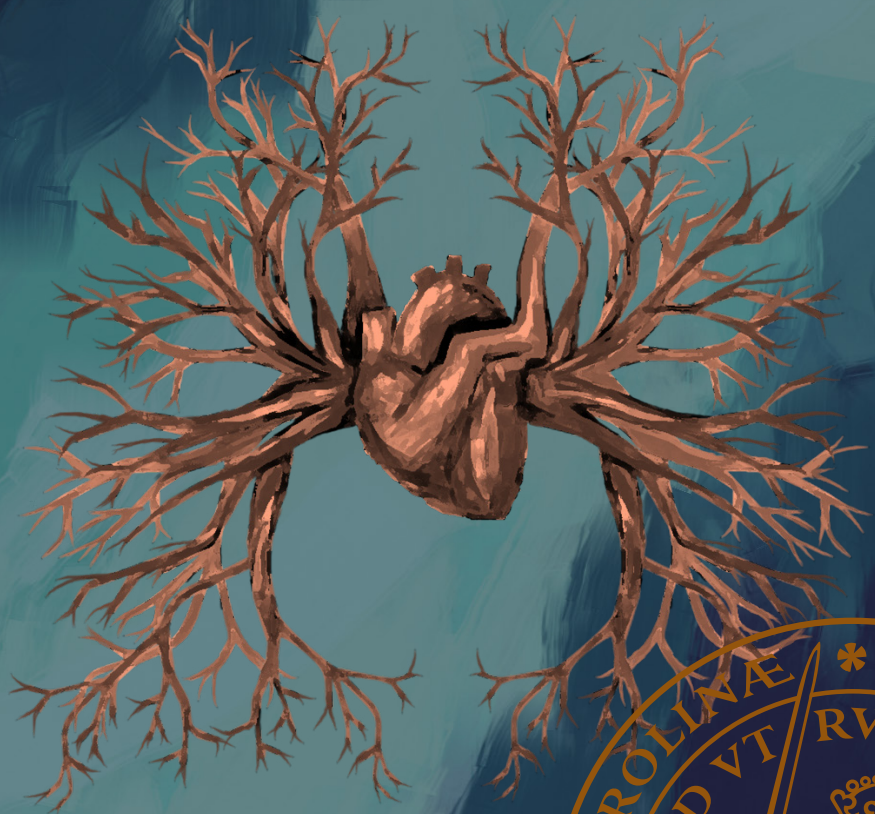

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Abdulla Ahmed, MD

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Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on the 12th of December 2025 at 09.00 in Segerfalk Lecture Hall, BMC, Lund University, Lund, Sweden

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TITLE AND SUBTITLE

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ABSTRACT

Pulmonary hypertension (PH) affects approximately 1 % of the global population and 10 % of individuals aged > 65 years. Most commonly, PH is associated with left heart disease. Pulmonary arterial hypertension (PAH) is a rare form of PH, characterized by endothelial dysfunction with excessive vasoconstriction and vascular remodeling, leading to right heart failure and ultimately death. The underlying disease mechanisms are incompletely understood but involve deranged metabolic, extracellular matrix, and inflammatory signaling.

Papers I – II investigated the plasma level dynamics of extracellular matrix and metabolic proteins, in relation to hemodynamics pre and 1-year post heart transplantation (HT). Paper III investigated the plasma levels of cardiovascular disease-associated proteins in relation to hemodynamics and prognosis in advanced heart failure and PH. Paper IV explored the diagnostic and prognostic potential of plasma proteins involved in coagulation, inflammation, and metabolism in PAH. Paper V evaluated the European Society of Cardiology/European Respiratory Society (ESC/ERS) guidelines-derived three- and four-strata risk stratification strategies and established a comprehensive web-based risk calculator to facilitate risk assessment in PAH. Papers I – IV were based on the Lund Cardio Pulmonary Registry, and paper V was based on a PAH cohort from Skåne University Hospital in Lund.

In Papers I – III, plasma prolargin, matrix metalloproteinase-2 (MMP-2), soluble receptor for advanced glycation end products (sRAGE), insulin-like growth factor binding protein 7 (IGFBP7), and adrenomedullin peptides and precursor levels (ADM) were higher in advanced heart failure compared to healthy participants. The high levels pre-HT decreased at the 1-year follow-up post-HT. The five proteins were associated with several invasively measured hemodynamic variables, and baseline plasma ADM was associated with survival. In paper IV, patients with PAH at diagnosis had the lowest levels of plasma a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13) compared with healthy participants, chronic thromboembolic PH, heart failure with reduced or preserved ejection fraction and PH, as well as heart failure without PH. Plasma von Willebrand Factor (vWF) at diagnosis was associated with survival in PAH. Paper V demonstrated that at baseline, the Swedish Pulmonary Arterial Hypertension Registry (SPAHR) three-strata and the Updated SPAHR strategies for risk calculation, both based on 3 – 6 variables, had the highest accuracies in predicting mortality in PAH. At follow-ups, the Updated SPAHR risk strategy based on 7 – 11 variables had the highest prognostic accuracy in predicting mortality in PAH. Also, a comprehensive web-based risk calculator was developed.

The present thesis highlights several plasma proteins related to pathways recognized in the pathophysiology of heart failure and PH, as well as PAH. Further research on these proteins may further advance our understanding of these conditions. The established risk stratification webpage and the evaluation of the Updated SPAHR can facilitate clinical implementation of the 2022 ESC/ERS PH guidelines.

Key words: ADAMTS-13, adrenomedullin, IGFBP-7, plasma biomarkers, risk assessment, risk stratification, sRAGE.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

In the name of Allah, the Most Gracious, the Most Merciful

﴿اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿3﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿4﴾ عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ ﴿5﴾﴾

Qur´an, 96: 3 – 5

Read: And thy Lord is the Most Bounteous (3) Who teacheth by the pen (4)
Teacheth man that which he knew not (5)

Pickthall M. The Meaning Of The Glorious Koran
An Explanatory Translation (1948)

Table of contents

List of papers

The thesis is based on the following papers, referred to in the text by their respective Roman numerals.

I. **Ahmed A**, Ahmed S, Arvidsson M, Bouzina H, Lundgren J, Rådegran G. Prolargin and matrix metalloproteinase-2 in heart failure after heart transplantation and their association with haemodynamics. *ESC Heart Failure* 2020; 7:224-235.

II. **Ahmed A**, Ahmed S, Arvidsson M, Bouzina H, Lundgren J, Rådegran G. Elevated plasma sRAGE and IGFBP7 in heart failure decrease after heart transplantation in association with haemodynamics. *ESC Heart Failure* 2020; 7:2340-2353.

III. **Ahmed A**, Kania K, Abdul Rahim H, Ahmed S, Rådegran G. Adrenomedullin peptides and precursor levels in relation to haemodynamics and prognosis after heart transplantation. *ESC Heart Failure* 2023; 10:2427-2437.

IV. **Ahmed A**, Ahmed S, Rådegran G. Plasma ADAMTS13 and von Willebrand Factor in diagnosis and prediction of prognosis in pulmonary arterial hypertension. *Pulmonary Circulation* 2021; 11:1-15 20458940211041500.

V. **Ahmed A**, Ahmed S, Kempe D, Rådegran G. Evaluation of the European Society of cardiology/European Respiratory Society derived three- and four-strata risk stratification models in pulmonary arterial hypertension: Introducing an internet-based risk stratification calculator. *European Heart Journal Open* 2023; 3:1-14.

Scientific contributions related to but not included in the present doctoral dissertation. The publications are arranged in chronological order using Arabic numerals, with 1 representing the earliest and the highest number denoting the most recent.

1. Arvidsson M, **Ahmed A**, Bouzina H, Rådegran G. Matrix metalloproteinase 7 in diagnosis and differentiation of pulmonary arterial hypertension. *Pulmonary Circulation* 2019; 9:2045894019895414.
2. Ahmed S, **Ahmed A**, Säleby J, Bouzina H, Lundgren J, Rådegran G. Elevated plasma tyrosine kinases VEGF-D and HER4 in heart failure patients decrease after heart transplantation in association with improved haemodynamics. *Heart and Vessels* 2020; 35:786-799.
3. Ahmed S, **Ahmed A**, Bouzina H, Lundgren J, Rådegran G. Elevated plasma endocan and BOC in heart failure patients decrease after heart transplantation in association with improved hemodynamics. *Heart and Vessels* 2020; 35:1614-1628.
4. Arvidsson M, **Ahmed A**, Bouzina H, Rådegran G. Plasma proteoglycan prolargin in diagnosis and differentiation of pulmonary arterial hypertension. *ESC Heart Failure* 2021; 8:1230-1243.
5. Ahmed S, **Ahmed A**, Rådegran G. Plasma tumour and metabolism related biomarkers AMBP, LPL and Glyoxalase I differentiate heart failure with preserved ejection fraction with pulmonary hypertension from pulmonary arterial hypertension. *International Journal of Cardiology* 2021; 345:68-76.
6. Ahmed S, **Ahmed A**, Rådegran G. Data on plasma tumour and metabolism related proteins' potential in differentiation of HFpEF-PH from PAH and in prognosis of left heart failure patients with pulmonary hypertension. *Data in Brief* 2022; 40:107747.
7. Arvidsson M, **Ahmed A**, Säleby J, Hesselstrand R, Rådegran G. Plasma matrix metalloproteinase 2 is associated with severity and mortality in pulmonary arterial hypertension. *Pulmonary Circulation* 2022; 12:e12041.
8. Ahmed S, **Ahmed A**, Rådegran G. The authors' reply to the letter "plasma tumour and metabolism related biomarkers differentiate PAH from HFpEF-PH may improve long-term prognosis". *International Journal of Cardiology* 2022; 348:108.
9. Löfdahl E, Ahmed S, **Ahmed A**, Rådegran G. Plasma biomarkers for clinical assessment of bone mineral density in heart transplanted patients-a single-center study at Skåne University Hospital in Lund. *Transplant International* 2022; 35:10161.

10. Helleberg S, Engel A, Ahmed S, **Ahmed A**, Rådegran G. Higher plasma il-6 and ptx3 are associated with worse survival in left heart failure with pulmonary hypertension. *American Heart Journal Plus: Cardiology Research and Practice* 2022; 20:100190.

11. Ahmed S, **Ahmed A**, Rådegran G. Structured evaluation of unclear dyspnea - of great importance to early identify patients with PAH and CTEPH and improve prognosis. *Lakartidningen* 2022; 119.

12. Kania K, **Ahmed A**, Ahmed S, Rådegran G. Elevated plasma wif-1 levels are associated with worse prognosis in heart failure with pulmonary hypertension. *ESC Heart Failure* 2022; 9:4139-4149.

13. **Ahmed A**, Ahmed S, Rådegran G. Risk assessment in pulmonary arterial hypertension: A step towards clinical implementation based on the 2022 ESC/ERS pulmonary hypertension guidelines. *Pulmonary Circulation* 2023; 13:e12253.

14. Arvidsson M, **Ahmed A**, Säleby J, Ahmed S, Hesselstrand R, Rådegran G. Plasma TRAIL and ANXA1 in diagnosis and prognostication of pulmonary arterial hypertension. *Pulmonary Circulation* 2023; 13:e12269.

15. Engel Sällberg A, Helleberg S, Ahmed S, **Ahmed A**, Rådegran G. Plasma tumour necrosis factor-alpha-related proteins in prognosis of heart failure with pulmonary hypertension. *ESC Heart Failure* 2023; 10:3582-3591.

16. **Ahmed A**, Ahmed S, Rådegran G. Risk stratification in pulmonary arterial hypertension - implementation of an internet-based risk calculator to guide treatment. *Lakartidningen* 2023; 120:1254-1257.

17. **Ahmed A**, Ahmed S, Rådegran G. Risk assessment in pulmonary arterial hypertension patients with multiple comorbidities and/or advanced age–where do we stand and what's next? *Pulmonary Circulation* 2023; 13:e12314.

18. Ahmed S, Lundgren J, **Ahmed A**, Rådegran G. Plasma VEGF-D and sFLT-1 are potential biomarkers of hemodynamics and congestion in heart failure and following heart transplantation. *JHLT Open* 2023; 2:1-12 100013.

19. Ahmed S, **Ahmed A**, Rådegran G. Structured evaluation of unclear dyspnea–an attempt to shorten the diagnostic delay in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. *Pulmonary Circulation* 2024; 14:e12340.

20. Ahmed S, **Ahmed A**, Rådegran G. Circulating biomarkers in pulmonary arterial hypertension: State-of-the-art review and future directions. *JHLT Open* 2024; 6:1-16 100152.

Summary in Swedish
Sammanfattning på svenska

Högt tryck i lungkretsloppet, så kallad pulmonell hypertension (PH), är ett tillstånd som förekommer hos 1 procent av världens befolkning och cirka 10 procent hos personer över 65 år. PH är oftast kopplat till vänstersidig hjärtsjukdom, såsom hjärtsvikt. Pulmonell arteriell hypertension (PAH) är däremot en ovanlig form av PH, där blodkärlen i lungorna drar ihop sig och blir trånga, styva, och mindre elastiska. Detta kan leda till högersidig hjärtsvikt och död. De bakomliggande mekanismerna vid såväl hjärtsvikt och PH som PAH är fortsatt ofullständigt kartlagda, men inbegriper störningar i ämnesomsättning (metabolismen), förändringar i vävnadens byggnadsställning (extracellulärmatrix), och rubbad inflammatorisk signalering.

I denna avhandling var syftet i delarbete I och II att undersöka dynamiken av plasmaproteiner som är relaterade till metabolism och extracellulärmatrix, i relation till invasivt mätta tryck i hjärtat och lungkärlen (hemodynamik), före och ett år efter hjärttransplantation. Delarbete III syftade till att undersöka olika plasmaproteiner kopplade till hjärt-kärlsjukdom i relation till hemodynamik och överlevnad hos individer med svår hjärtsvikt och PH. I delarbete IV var syftet att utforska om plasmaproteiner kopplade till inflammation, blodets förmåga att levra sig (koagulation), och metabolism hade potential att förbättra diagnostik och förutsäga överlevnad hos individer med PAH. Delarbete V syftade till att utvärdera en riskskattningsstrategi för PAH, kallad *uppdaterade SPAHR,* samt att underlätta implementeringen bland kliniker genom att etablera en internetbaserad webbplats för riskskattning.

Delarbete I – IV baserades på data från Lund Cardio Pulmonary Registry, medan delarbete V byggde på data från Skånes Universitetssjukhus i Lund. I delarbete I – III var plasmaproteinerna prolargin, MMP-2, sRAGE, IGFBP7, och ADM högre hos individer med svår hjärtsvikt jämfört med friska individer. De förhöjda nivåerna före hjärttransplantation minskade efter ingreppet och närmade sig de nivåer som ses hos friska individer. Dessa fem proteiner var även kopplade till hemodynamik. I delarbete IV hade individer med PAH vid diagnos de lägsta nivåerna av plasmaproteinet ADAMTS13 jämfört med friska individer, individer med kronisk tromboembolisk PH, hjärtsvikt med reducerad eller bevarad sammandragningsförmåga, samt hjärtsvikt utan PH. Vidare var nivåerna av plasmaproteinet von Willebrand faktor vid diagnos av PAH kopplad till patienternas överlevnad.

Delarbete V visade att vid diagnos av PAH hade riskskattningsstrategierna SPAHR och *uppdaterade* SPAHR, båda baserade på 3 – 6 riskvariabler, den högsta träffsäkerheten för att förutsäga överlevnad. Vid uppföljningar av PAH hade riskskattningsstrategin *uppdaterade* SPAHR, baserad på 7 – 11 riskvariabler, den högsta träffsäkerheten för att förutsäga överlevnad vid PAH. Dessutom etablerades en webbaserad riskkalkylator som samlar flera riskskattningsstrategier, i syfte att underlätta för kliniker att bedömda PAH-sjukdomens svårighetsgrad och därigenom få stöd i val av behandlingsstrategi.

Denna avhandling belyser flera proteiner i blodplasman som är kopplade till signalvägar som har betydelse för hur hjärtsvikt och PH, samt PAH utvecklas i kroppen. Ytterligare forskning om dessa proteiner kan bidra till en djupare förståelse av dessa sjukdomstillstånd. Den etablerade webbaserade riskkalkylatorn, och utvärderingen av riskskattningsstrategin *uppdaterade* SPAHR kan underlätta den kliniska implementeringen av 2022 års europeiska PH-riktlinjer.

Summary in Arabic ملخص باللغة العربية

إن ارتفاع أو فَرْط ضغط الدم في الدورة الدموية الصغرى، ما يُعرف بفَرْط ضغط الدم الرئوي، هي حالة تُصيب نحو 1 بالمئة من سكان العالم، وحوالي 10 بالمئة من الأشخاص الذين تزيد أعمارهم عن 65 عاماً. يرتبط فَرْط ضغط الدم الرئوي غالباً بأمراض القلب في الجانب الأيسر، على سبيل المثال فشل القلب. من ناحيةٍ أخرى يُعد فَرْط ضغط الدم الشرياني الرئوي شكلًا نادراً من فَرْط ضغط الدم الرئوي. يتميّز بانقباض الأوعية الدموية في الرئتين، مما يؤدي إلى تضييقها وتصلبها وفقدانها للمرونة وهذا قد يؤدي إلى فشل القلب الأيمن والموت. لا تزال الآليات الكامنة وراء كل من فَرْط ضغط الدم الرئوي مقترن بفشل القلب الأيسر، وفَرْط ضغط الدم الشرياني الرئوي غير مفهومة بالكامل، لكنها تشمل اضطرابات في الاستقلاب، وتغيرات في بنية الأنسجة (المصفوفة) خارج الخلية، واختلالات في الإشارات الالتهابية.

تضمنت هذه الأطروحة خمسة بحوث. كان هدف البحث الأول والثاني هو دراسة التغيرات في بروتينات البلازما المرتبطة بالاستقلاب والمصفوفة خارج الخلية، فيما يتعلق بالضغط المقاس بشكل غازي في القلب والأوعية الدموية الرئوية (الديناميكية الدموية)، قبل وبعد عام من عملية زراعة القلب. أما البحث الثالث فكان يهدف إلى دراسة بروتينات البلازما المختلفة المرتبطة بأمراض القلب والأوعية الدموية وعلاقتها بالديناميكية الدموية والبقاء على قيد الحياة لدى الأفراد المصابين بفشل القلب الشديد وفَرْط ضغط الدم الرئوي. أما في البحث الرابع فكان الهدف منه استكشاف ما إذا كانت بروتينات البلازما المرتبطة بالالتهاب أو بالتجلط أو بالاستقلاب لديها القدرة على تحسين التشخيص والتقدير الإنذاري لدى الأفراد المصابين بفرط ضغط الدم الشرياني الرئوي. أما البحث الخامس ولأخير فكان يهدف إلى دراسة وتحليل أداة لتقييم المخاطر في حالات فرط ضغط الدم الشرياني الرئوي، المسماة *SPAHR المحدثة*، بالإضافة إلى إنشاء موقع إلكتروني قائم على الإنترنت لتقييم المخاطر. استندت الأبحاث من الأول إلى الرابع إلى بيانات من سجل لوند القلبي الرئوي، وأما البحث الخامس فاستند على بيانات من مستشفى جامعة إقليم سكونه في لوند.

أظهرت الأبحاث الثلاثة الأولى أنَّ مستويات بروتينات البلازما prolargin وMMP-2 وsRAGE وIGFBP7 وADM كانت أعلى لدى الأفراد المصابين بالفشل القلبي الحاد قبل زراعة القلب مقارنةً بالأفراد الأصحاء. وبعد عام من أجراء العملية، انخفضت المستويات المرتفعة مقترنةً من المستويات لدى الأفراد الأصحاء. كما كانت هذه البروتينات الخمسة مرتبطة بالديناميكية الدموية. أظهر البحث الرابع أنَّ مستويات بروتين البلازما ADAMTS13 عند التشخيص أدنى لدى الأفراد المصابين بفرط ضغط الدم الرئوي الشرياني، مقارنةً بالأفراد الأصحاء، والمصابين بفرط ضغط الدم الرئوي بسبب أمراض تخثرية مزمنة، والمصابين بفرط ضغط الدم الرئوي المرتبط بفشل القلب نتيجةً لخلل في الانقباض أو الانبساط، والمصابين بفشل القلب دون وجود فرط ضغط الدم الرئوي. إضافةً إلى ذلك، كانت مستويات بروتين von Willebrand factor عند التشخيص مرتبطةً بالتقدير الإنذاري لدى المصابين بفرط ضغط الدم الرئوي الشرياني.

وبين البحث الخامس أنّ استراتيجيتا تقييم المخاطر *SPAHR* و *SPAHR المُحدَّثة*، وكلتاهما مبنيتان على 3 إلى 6 عوامل خطورة، تمتعان بأعلى دقة في التقدير الإنذاري عند تشخيص فرط ضغط الدم الرئوي الشرياني. أما في المتابعات اللاحقة لحالات فرط ضغط الدم الرئوي الشرياني، فقد أظهرت الاستراتيجية *SPAHR المُحدَّثة* المعتمدة على 7 إلى 11 عوامل خطورة، أعلى دقة في التقدير الإنذاري. علاوةً على ذلك تم إنشاء موقع إلكتروني قائم على الإنترنت لتقييم المخاطر بهدف تسهيل مهمة الكوادر الطبية في تقييم شدة فرط ضغط الدم الرئوي الشرياني وبالتالي دعمهم في اختيار الاستراتيجية العلاجية المناسبة.

سلطت هذه الأطروحة الضوء على عدد من بروتينات البلازما في الدم، والتي ترتبط بمسارات إشارات خلوية لها دور مهم في تطوّر كل من فرط ضغط الدم الرئوي المرتبط بفشل القلب، و فرط ضغط الدم الشرياني الرئوي. وقد تُسهم الأبحاث المستقبلية حول هذه البروتينات في تعزيز الفهم لهذه الحالات المرضية. كما أن إنشاء أداة تقييم المخاطر الإلكترونية وتقييم استراتيجية *SPAHR المُحدَّثة* قد تُساهم في تسهيل التطبيق السريري للإرشادات الأوروبية الخاصة بفرط ضغط الدم الرئوي لعام 2022.

Abbreviations

6MWD	Six-minute walk distance
ADAMTS13	A disintegrin and metalloproteinase with thrombospondin motifs 13
ADM	Adrenomedullin peptides and precursor levels
APAH	Associated pulmonary arterial hypertension
BMP	Bone morphogenic protein
CHD	Congenital heart disease
CI	Cardiac index
CTD	Connective tissue disease
COMPERA	Comparative, Prospective Registry of Newly Initiated Therapies for pulmonary hypertension
CTEPH	Chronic thromboembolic pulmonary hypertension
ESC/ERS	European Society of Cardiology / European Respiratory Society
FC	Functional class
HT	Heart transplantation
IGFBP7	Insulin-like growth factor binding protein 7
LCPR	Lund Cardio Pulmonary Registry
LHD	Left heart disease
MMP-2	Matrix metalloproteinase-2
mPAP	mean pulmonary arterial pressure
mRAP	mean right atrial pressure
NT-proBNP	N-terminal pro-brain natriuretic peptide
PAH	Pulmonary arterial hypertension
PAWP	Pulmonary arterial wedge pressure
PH	Pulmonary hypertension
PVR	Pulmonary vascular resistance

REVEAL	Registry to Evaluate Early and Long-term Pulmonary Arterial Hypertension Disease Management
RHC	Right heart catheterization
SPAHR	Swedish Pulmonary Arterial Hypertension Registry
sRAGE	Soluble receptor for advanced glycation end products
SUS-Lund	Skåne University Hospital in Lund
SvO_2	Mixed venous oxygen saturation
TGF-β	Transforming growth factor β
WHO	World Health Organization

Introduction

A tale of the heart and lungs – from the porous heart to microscopic details of pulmonary capillaries

In the 2nd century, the ancient Greek physician Claudius Galenus, or Galen, contributed significantly to the anatomical and physiological knowledge of the cardiovascular system. His theory of blood circulation, however, included the inaccurate conception of "the porous heart", where venous blood flows from the right heart to the left via pores in the septum, and subsequently mixed with inhaled air from the lungs.[1, 2] Galens teachings prevailed for more than a millennium, influencing Ibn Sina's (Latin: Avicenna's) "Canon of Medicine", published in the 11th century.[1, 3] During the Islamic Golden Age (~8th – 13th century), the Arab physician Ibn al-Nafis (1213 – 1288), made great contributions to the understanding of the pulmonary circulation in his work "Commentary on Anatomy in Avicenna's Canon", (figure 1, right panel) where he shared that the intraventricular septum is certainly not porous, the output of the right ventricle can only reach the left ventricle through the pulmonary circulation, and that there must be small communications (pronounced *manafidh* in Arabic, i.e. pores/permeabilities) between the pulmonary artery and vein.[2, 4] The third statement, which preceded the discovery of pulmonary capillaries, was also supported ~300 years later by European Renaissance scholars including Michael Servetus (1511 – 1553) and William Harvey (1578 – 1657).[2] In 1661, the Italian physician Marcello Malpighi (1628 – 1694), considered the father of modern pathology and physiopathology, described groundbreaking findings regarding the microscopic details of the pulmonary capillaries in his most famous work "De Pulmonibus Observationes Anatomicae" (figure 1, left panel).[5]

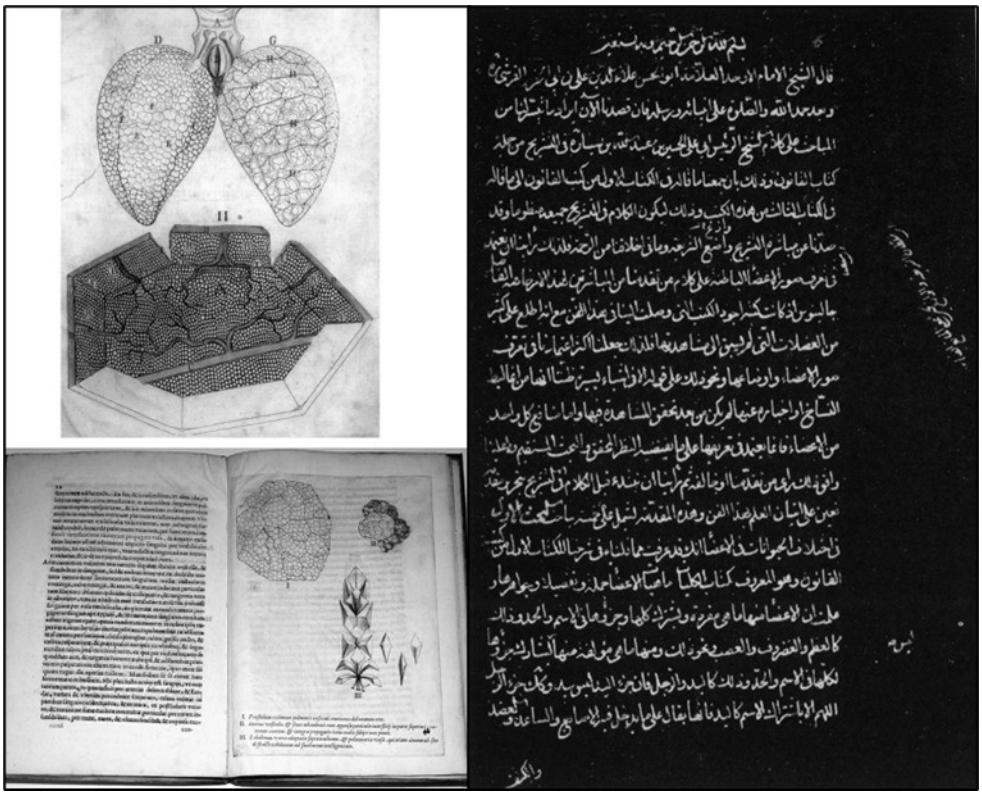

Figure 1.
Photostatic reproduction (hence inverted colors) of the introductory page (Arabic, right to left) of Ibn al-Nafis's work "Commentary on Anatomy in Avicenna's Canon" (right panel). Malpighi's "De Pulmonibus Observationes Anatomicae" (left panel). In the upper part of the left panel, the microscopic structure of the frog lungs is shown. In (II), the connections between venous and arterial capillaries inside an opened alveolus are drawn. In the lower part of the left panel the appearance of the lung, composed by many lobules (left) as well as the alveoli are drawn (right). Reproduced with permission (right panel license: 6018851289706,[4] left panel license: 6018870873903).[5]

Pulmonary hypertension

In 1891, the German physician Ernst von Romberg described pulmonary vascular lesions as "sclerosis of the pulmonary artery" – the earliest pathological description in perhaps the first documented case with pulmonary hypertension (PH).[6-8] Over a century later, pulmonary vascular remodeling, viewed as a hallmark shared by all forms of PH, is still incompletely understood.[9] In recent years, however, new data has advanced our understanding of this enigmatic entity,[9, 10] raising more questions than answers.

The present introduction reviews key components within the literature in the field of PH with relation to the papers I – V. Management of PH requires a broad

understanding and a multidisciplinary approach, particularly the rare form pulmonary arterial hypertension (PAH).[11, 12] Accordingly, the initial papers in the present thesis focus on the most common form of PH and its advanced treatment, subsequently addressing PAH, which also requires highly specialized care. The importance of a broad understanding is highlighted by the 2022 European Society of Cardiology (ESC)/European Respiratory Society (ERS) PH guidelines, which recommend proper training in core competencies, such as those outlined by the ERS[13] and the ESC core curriculum,[14] for management of PH.[11, 12]

PH is a pathophysiological hemodynamic state that may involve a multitude of clinical conditions and can be associated with cardiovascular, respiratory, or other multifactorial disorders.[11, 12, 15] It is a major global health issue affecting all age groups. The prevalence of PH is estimated to be around 1 % of the global population and up to 10 % in those > 65 years of age. Irrespective of underlying cause, developing PH is associated with aggravation of symptoms and increased mortality.[11, 12, 16]

Based on pathophysiological mechanisms, clinical presentation, hemodynamic characteristics, and therapeutic management, PH is clinically divided into five groups according to the World Health Organization (WHO).[11, 12, 16] PH associated with left heart disease (PH-LHD, WHO group II PH) is the leading cause globally, followed by PH associated with lung diseases and/or hypoxia (WHO group III PH).[11, 12, 16] PAH (WHO group I PH), and chronic thromboembolic PH (CTEPH, WHO group IV PH) are rare and severe types of PH, that have been the subject of intense inquiry among researchers, clinicians, and the pharmacological industry during the four last decades.[16] As a result, substantial therapeutic progress has been made, alleviating to some extent the deleterious disease course.[16, 17] PH with unclear and/or multifactorial mechanisms (WHO group V PH), is a less common and studied form of PH, which, however, contributes significantly to the global burden of PH.[11, 12, 16]

Epidemiologically, it is estimated that ~80 % of patients with PH live in developing countries, where congenital heart disease (CHD), schistosomiasis, human immunodeficiency virus, rheumatic heart disease, sickle cell disease, and high altitude are important but understudied causes of PH.[11, 12, 16]

Symptoms and signs of PH are not specific and mostly related to right ventricular (RV) dysfunction. Initial symptoms include dyspnea upon minor exertion, fatigue, and palpitations, making early detection, as well as timely management and treatment a significant challenge.[18] Managing PH requires a holistic and broad understanding as well as a multidisciplinary approach, where the patient with PH is actively involved.[11, 12, 19]

Pulmonary hypertension associated with left heart disease

According to the 2022 ESC/ERS PH guidelines, PH-LHD is characterized hemodynamically as a post-capillary PH, defined by a mean pulmonary arterial pressure (mPAP) > 20 mmHg at rest at a pulmonary arterial wedge pressure (PAWP) > 15 mmHg. Post-capillary PH is further subclassified by pulmonary vascular resistance (PVR), where PVR ≤ 2 Wood units (WU), defines isolated post-capillary PH (Ipc-PH), and PVR > 2 WU defines combined post- and pre-capillary PH (Cpc-PH). The hemodynamic definitions were revised compared to the 2015 ESC/ERS PH guideliens,[11, 12, 20, 21] (table 1).

PH-LHD is clinically classified based on the 2022 ESC/ERS guidelines into several subgroups depending on the underlying condition including heart failure with reduced/mildly reduced-, and preserved ejection fraction (HFrEF/HFmrEF, and HFpEF, respectively), left-sided valvular disease, and congenital or acquired cardiovascular conditions leading to post-capillary PH. However, in the proceedings of the 7th World Symposium on PH (WSPH), the clinical classification was disaggregated to improve the alignment between the underlying LHD and clinical decision making with respect to clinical profile, pathophysiology, prognosis, and treatment approach. The subgroup "valvular heart disease" was proposed to be replaced by aortic-, mitral-, and mixed valvular disease. Furthermore, a new subgroup, "cardiomyopathies with specific etiologies", was added, encompassing hypertrophic-, amyloid-, Fabry disease- and Chagas disease related cardiomyopathy.[15, 22]

PH is estimated to affect at least 50 % of patients with HFpEF or HFrEF, and around 20 – 30 % have Cpc-PH based on PVR.[11, 12, 16, 23] Data from echocardiographic studies indicate that up to 65 % of patients with symptomatic aortic valve stenosis are affected by PH, whereas the majority of patients with severe mitral stenosis, or with significant degenerative or functional mitral regurgitation have PH.[11, 12, 24]

Several mechanisms are involved in the pathophysiology of PH-LHD, i.e. increased left-sided filling pressures that transmit backwards into the pulmonary circulation; endothelial dysfunction of the pulmonary arteries favoring vasoconstriction; vascular remodeling of the pulmonary arterioles and/or venules; RV dysfunction/dilatation and functional tricuspid valve regurgitation; as well as deranged RV – pulmonary arterial (PA) coupling. RV – PA coupling is becoming the subject of increasing interest in the field of PH.[11, 12, 22, 25] It is described as the relationship between PA afterload and RV contractility, defined as the ratio of PA elastance (a comprehensive measure accounting for the resistive and pulsatile component of afterload) and RV end-systolic elastance (a measure of contractility).[26]

Table 1. Hemodynamic definitions of pulmonary hypertension (PH) according to the 2015 and 2022 European Society of Cardiology/European Respiratory Society (ESC/ERS) PH guidelines

CO, cardiac output; DPG, diastolic pulmonary pressure gradient (diastolic pulmonary arterial pressure – pulmonary arterial wedge pressure(PAWP)); mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; and WU, Wood units. Adapted from the 2015 and 2022 ESC/ERS PH guidelines,[11, 20] (license: 6024890787484, and 6005421432576, respectively).

Definition	Hemodynamic characteristics (right heart catheterization) – ESC/ERS 2015[20, 21]	Hemodynamic characteristics (right heart catheterization) – ESC/ERS 2022[11, 12]
PH	mPAP > 25 mmHg at rest	mPAP > 20 mmHg at rest
Pre-capillary PH	mPAP > 25 mmHg t rest PAWP ≤ 15mmHg PVR > 3 WU	mPAP > 20 mmHg t rest PAWP ≤ 15mmHg PVR > 2 WU
Post-capillary PH	mPAP > 25 mmHg at rest PAWP > 15mmg	mPAP > 20 mmHg at rest PAWP > 15mmg
Isolated post-capillary PH	mPAP > 25 mmHg at rest PAWP > 15mmHg DPG<7 mmHg and/or PVR ≤ 3 WU	mPAP > 20 mmHg at rest PAWP > 15mmHg PVR ≤ 2 WU
Combined post- and pre-capillary PH	mPAP > 25 mmHg at rest PAWP > 15mmHg DPG ≥7 mmHg and/or PVR > 3 WU	mPAP > 20 mmHg at rest PAWP > 15mmHg PVR > 2 WU
Exercise PH	—	mPAP/CO slope between rest and exercise > 3 mmHg/L/min
Unclassified PH	—	mPAP > 20 mmHg at rest PAWP ≤ 15mmHg PVR ≤ 2 WU

Management of pulmonary hypertension associated with left heart disease

Management of PH-LHD involves treatment of the underlying condition while considering the impact of comorbidities. PAH-targeted therapies are not recommended in patients with PH-LHD.[11, 12, 22] In patients with HFrEF, HFmrEF, and HFpEF, guideline-directed drugs, devices and interventional/surgical therapy are recommended.[11, 12, 27, 28]

Advanced heart failure may present as the end-stage phase for several etiologies, characterized by severe and persistent symptoms (New York Heart Association, NYHA, class III (advanced) or IV) despite maximal medical treatment. Prognosis is poor in this patient group with 1-year mortality rates ranging between 25 % and 75%.[27]

The 2018 updated Heart Failure Association of the ESC (HFA-ESC) defines advanced heart failure by certain criteria to facilitate timely referral to advanced centers of heart failure.[29] The HFA-ESC definition provides together with the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) profiles, and the International Society for Heart and Lung Transplantation (ISHLT) guidelines a rigorous framework to identify those with an indication for advanced therapies, including LVAD and heart transplantation (HT).[27, 30, 31]

HT remains the gold standard for treatment of advanced heart failure in the absence of contraindications.[27] The presence of a compromised hemodynamic profile including elevated systolic pulmonary arterial pressure (sPAP) with increased transpulmonary pressure gradient (TPG) or PVR may constitute a contraindication for HT listing.[30] Particularly, elevated pre-transplant PVR, for which the most robust and consistent data exist,[11, 12, 32] is related to increased early post-transplant mortality.[30, 32] In eligible candidates on which vasodilatory testing renders persisting unfavorable hemodynamics, mechanical circulatory support can be considered including left ventricular assist device (LVAD), as a bridge to HT.

Heart transplantation in Sweden

The first HT in Sweden was performed in 1984 at Sahlgrenska University Hospital in Gothenburg. The first HT at Skåne University Hospital in Lund (SUS-Lund) was performed in 1988.[33] To date, more than 1500 heart transplants have been performed in Sweden, of which 638 at SUS-Lund during 1988 – 2024.

Until 2010, HT was performed at three centers in Sweden (Karolinska University Hospital, Sahlgrenska University Hospital and SUS-Lund). In 2005, the Swedish authorities decided to centralize highly specialized medical procedures to increase patient safety and cost-effectiveness. In 2011, the Swedish National Board of Health and Welfare nationally centralized heart transplant procedures to two centers; Sahlgrenska University Hospital, and SUS-Lund. Since then, SUS-Lund has performed on average 30 heart transplants a year. The post-transplant 1-year survival rate in adults (≥ 18 years) in Sweden during 2011 – 2020 was 94.6 % – an increase from 87.9 % during 2001 – 2010 (figure 2, right panel).[33] By contrast, the post-transplant 1-year survival estimates based on the ISHLT registry in adults for the eras 2000 – 2011 and 2012 – 2017 were ~84 % and 86.2 %, respectively (figure 2, left panel).[34]

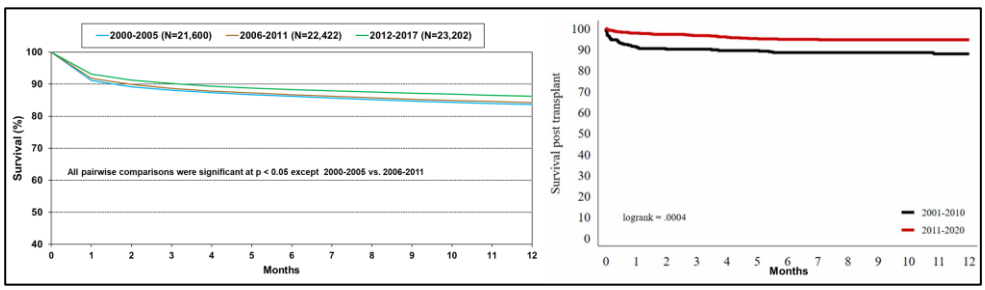

Figure 2. Survival post-heart transplantation

Kaplan-Meier survival estimates in adults within 1-year post-HT based on the International Society for Heart and Lung Transplantation data (left panel) and Swedish data (right panel), stratified by era. Exact survival estimates at 12 months in the left panel for the eras 2000 – 2005, 2006 – 2011, and 2012 – 2017 were 83.6 %, 84.2 %, and 86.2 %, respectively (obtained by hovering over the intersection in the supplementary data slides provided by the International Thoracic Organ Transplant Registry).[34] Reproduced with permission (left panel license: 6001270394231, right panel: CC-BY).

In Sweden, the results of thoracic transplants are reported to the Scandiatransplant registry, which in turn reports to the ISHLT registry.[33, 35] Scandiatransplant is an organ exchange organization, founded in 1969, for Sweden, Denmark, Finland, Iceland, Norway, and Estonia. Scandiatransplant cover a population of around 30 million people and more than 2000 patients are transplanted yearly within the organization.[35]

Pulmonary arterial hypertension

Definition and classification

According to the 2022 ESC/ERS guidelines, PAH is hemodynamically classified as a pre-capillary PH, defined by an mPAP > 20 mmHg at rest, PAWP < 15mmHg, and a PVR > 2 WU (table 1).[11, 12] Epidemiologically, it is a rare form of PH with an incidence ranging between 1.1 and 6.6 cases/million adults and year, and a prevalence between 48 – 55 cases/million adults in the developed world.[11, 12, 16, 36]

PAH was previously believed to primarily affect younger females.[11, 12] However, aside from hereditary PAH (HPAH), contemporary European US registries indicate that PAH is more frequently diagnosed in older populations (≥ 65 years).[11, 12, 37] According to the French PH registry, patients diagnosed with PAH during 2002 – 2003 had a mean age of 53 years.[38] In the Registry to Evaluate Early and Long-term PAH Disease Management (REVEAL), the mean age for the adult PAH population enrolled during 2006 – 2007 was 49 years.[37] REVEAL enrollees matched with the population of the National Institutes of Health (NIH) registry during 1981 – 1985 displayed an increase in age at diagnosis (45 versus 36 years).[37, 39] In Germany, the mean age of adults at PAH diagnosis in 2014 was 64 years,[40] and according to the Comparative, Prospective Registry of Newly Initiated Therapies for PH (COMPERA), the mean age of enrollees with PAH during 2007 – 2014 was 61 years.[40]

The Swedish PAH registry, SPAHR (https://www.ucr.uu.se/spahr/), initiated by the Swedish Society of Pulmonary Hypertension (SveFPH; https://www.svefph.se/pulmonell-hypertension), is a nationwide ongoing registry in which adults diagnosed with PAH are prospectively enrolled since 2008. SPAHR also includes retrospective data on patients with PAH during 2000 – 2007. According to SPAHR, the average and median age at PAH diagnosis during 2000 – 2023 was approximately 65 years, and around 60 % of the diagnosed during 2011 – 2023 were > 65 years of age.[41]

According to the clinical classification of the 2022 ESC/ERS PH guidelines, PAH is divided into idiopathic (IPAH), hereditary (HPAH), familial (FPAH), associated PAH (APAH), PAH with features of venous/capillary involvement, and persistent PH of the newborn. Detailed information on the classification of PAH is available in table 2. The most common type of PAH reported in registries from the developed world are

IPAH, which accounts for 50 – 60 % of PAH cases,[11, 12, 16, 37] followed by PAH associated with connective tissue disease (CTD-APAH), and PAH associated with congenital heart disease (CHD-APAH).[38, 40, 41]

In the proceedings of the 7th WSPH, the subgroup "long-term responders to calcium channel blockers (CCBs)" was reintroduced. Initially a proposed subgroup in the 6th WSPH, the term was later not embraced by the 2022 ESC/ERS PH guidelines. Instead, the 2022 ESC/ERS PH guidelines added "acute vasoresponders at vasoreactivity testing" and "non-responders at vasoreactivity testing" as subgroups within IPAH.[11, 12, 15]

Approximately 12 % of IPAH and drug-associated PAH are acute vasoresponders, whereas the corresponding estimate in HPAH is less than 5 %.[15] Less than two-thirds of those with positive acute vasoreactivity testing are defined as long-term responders to CCBs, i.e., exhibit a sustained improvement clinically and hemodynamically after ≥ 1 year on CCBs.[15, 42] This makes the current classification of vasoreactivity less suitable as positive vasoresponders comprise a heterogenic group of long-term responders to CCBs, and those who will require management with PAH-targeted drugs. The latter group suggests different pathophysiological mechanisms, clinical presentation, and most importantly therapeutic management.[15] Thus, the 7th WSPH proceedings proposed that a more pragmatic approach is to reintroduce long-term responders to CCBs as it aligns better with the aims of the PH clinical classification, i.e., classify according to similar pathophysiological mechanisms, clinical presentation, haemodynamics, and therapeutic management.[15]

Table 2. Clinical classification of pulmonary hypertension (PH) according to the 2022 European Society of Cardiology/European Respiratory Society PH guidelines

HIV, human immunodeficiency virus; PAH, pulmonary arterial hypertension; PCH, pulmonary capillary hemangiomatosis; PVOD, pulmonary veno-occlusive disease. Adapted with permission (license: 6005421432576).[11]

*The 7th WSPH proposed replacing these subgroups with "long-term responders to calcium channel blockers.

PAH (WHO group I PH)	
1.1	Idiopathic
	1.1.1 Non-responders at vasoreactivity testing*
	1.1.2 Acute responders at vasoreactivity testing*
1.2	Heritable
1.3	Associated with drugs and toxins
1.4	Associated with:
	1.4.1 Connective tissue disease
	1.4.2 HIV infection
	1.4.3 Portal hypertension
	1.4.4 Congenital heart disease
	1.4.5 Schistosomiasis
1.5	PAH with features of venous/capillary (PVOD/PCH) involvement
1.6	Persistent PH of the newborn

Hereditary pulmonary arterial hypertension and established genes

In 2000, the first reports of heterozygous germline variants in the gene bone morphogenetic protein receptor type II (*BMPR2*) were identified as the main genetic cause of familial PAH.[43, 44] Since then, more than 300 independent mutations,[45, 46] and at least 500 unique *BMPR2* variants have been reported.[46] Mutations in *BMPR2* account for 75 – 80% of cases with familiar PAH, and 20-25% of sporadic cases.[16, 45]

In the proceedings of the 7th WSPH, the level of evidence associating certain genes with PAH was systematically assessed, using the standardized Clinical Genome Resource (ClinGen) semi-quantitative scoring system. Based on genetic and experimental data, the scoring system classifies genes into having "definitive evidence", "moderate evidence", and "limited evidence" of PAH association. There are currently 12 genes with definitive evidence (*ACVRL1*, *ATP13A3*, *BMPR2; CAV1*, *EIF2AK4*, *ENG*, *GDF2*, *KCNK3*, *KDR*, *SMAD9*, *SOX17*, and *TBX4*), and 3 with moderate evidence (*ABCC8*, *GGCX*, and *TET2*), i.e., a total of 15 genes that are recommended for inclusion in genetic testing panels of PAH.[47]

Pathophysiology

The pathophysiology of PAH is still incompletely understood. Current knowledge pivots around different pathways on which treatments have been directed, including the prostacyclin-, endothelin-, nitric oxide-, CCB related-, and the bone morphogenic protein/transforming growth factor β (BMP/TGF-β) pathways. Keywords include vascular remodeling, endothelial dysfunction, and excessive vasoconstriction.

Pulmonary vascular remodeling

Pulmonary vascular remodeling is characterized by structural and functional changes occurring in the pulmonary circulation. In PAH, pulmonary vascular remodeling affects predominantly the pulmonary arterioles, whereas in other groups such as PH-LHD, the small- to medium-sized pulmonary veins and capillaries are primarily affected.[9] When a substantial portion of the pulmonary circulation is engaged, hemodynamic changes occur including elevations in PVR and pulmonary arterial pressures, impacting on the structure and function of the right ventricle.[9, 10]

Key features of pulmonary vascular remodeling include vasoconstriction, thickening of the pulmonary arterial walls, muscularization of pulmonary arterioles, reduction in capillary density (capillary rarefaction), in-situ thrombosis, and the presence of plexiform lesions in addition to other complex vascular formations.[9, 10, 48] These features involve the accumulation of endothelial cells, vascular smooth muscle cells, myofibroblasts, fibroblasts, and immune cells inside and around the vascular walls.[48, 49] Although incompletely understood, these features of vascular remodeling are driven by three main components, i.e. dysfunction of the pulmonary vascular endothelium; dysfunction of the vascular smooth muscle; as well as dysregulation of the immune system and sustained inflammation (figure 2).[48]

Dysfunction of the pulmonary endothelium

Endothelial dysfunction is one of the hallmarks of the pathophysiology of PAH. It is characterized by altered secretion of vasoconstrictors including endothelin-1, serotonin, and angiotensin II; a decrease in vasodilators including nitric oxide and prostacyclin; secretion of mediators that alter the prothrombotic and antithrombotic activities; imbalances in pro- versus anti-inflammatory signals; as well as altered activators versus inhibitors of vascular smooth muscle cell growth and migration.[48] Other processes recognized as triggers of endothelial dysfunction in PAH include endothelial cell death, as well as excessive production of reactive oxygen species and inflammatory mediators. These processes adversely affect the paracrine (cell communication to surrounding cells) crosstalk between endothelial- and resident cells such as fibroblasts and vascular smooth muscle cells, leading to recruitment of inflammatory and immune cells that amplify these adverse processes sustaining the progression of pulmonary vascular remodeling, (figure 3).

Dysfunction of the vascular smooth muscle

In PAH, vascular smooth muscle cells have abnormal growth, prolonged survival, and abnormalities that amplify ion channel response to certain growth factors such as platelet-derived growth factor, fibroblast growth factor 2, and epidermal growth factor. Additionally, the TGF-β signaling pathway is affected, including a decreased activity of the BMP-Smad1/5/8 signal transduction, and enhanced activin-Smad/2/3, contributing to vascular smooth muscle cell depolarization (increased intracellular Ca^{2+} leading to vasoconstriction), proliferation, resistance to apoptosis, as well as increased production, degradation and organization of the extracellular matrix components in pulmonary vessels (figure 3).[48]

Dysregulation of the immune system and sustained inflammation

Perivascular infiltrates of inflammatory cells have been shown to precede the structural pulmonary vascular remodeling in experimental PH. Different cell types from patients with PAH such as pulmonary artery smooth muscle cells, endothelial cells, fibroblasts, and myofibroblasts express a pro-inflammatory state with elevated cytokines, chemokines, and increased expression of the inflammatory cell adhesion molecule intercellular adhesion molecule 1 (ICAM1).[10] Additionally, excessive local secretion of interleukin (IL)-1, IL-6, leptin, leukotriene B4, and tumor necrosis factor-α (TNF-α) serve an integral part in mediating the changes in the pulmonary vasculature in PAH (figure 3).[10] Changes in the immune system include impaired T-regulatory cell function, T-helper 17 cell polarization,[50] recruitment of dendritic cells, and circulating autoantibodies in patients with PAH without the presence of related autoimmune conditions.[10] Other contributing factors to the dysregulation of the immune system in PAH include shear stress, chronic hypoxia, metabolic derangements, and deranged BMPR2 signaling.[51]

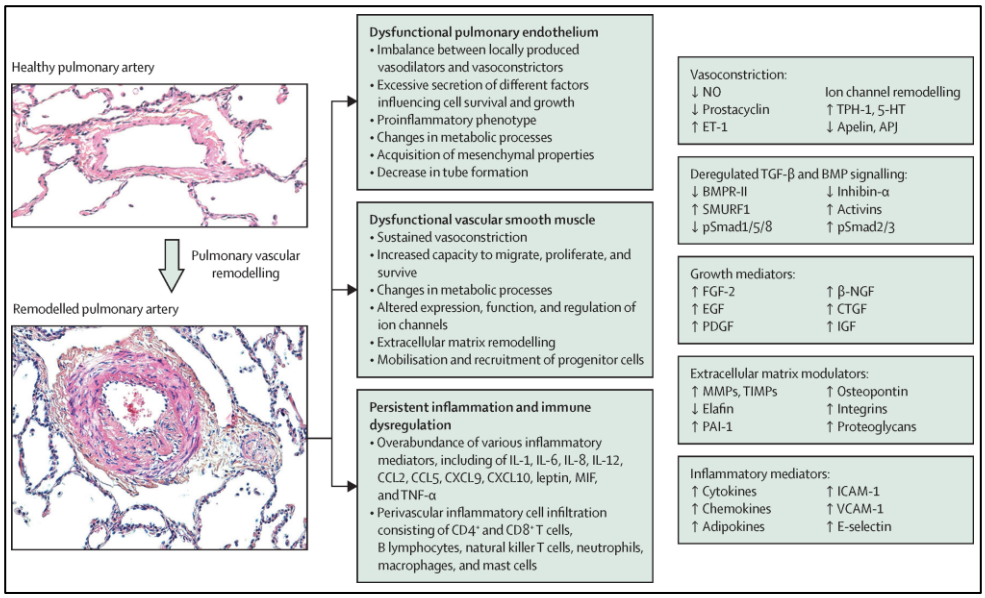

Figure 3. Pulmonary vascular remodeling in pulmonary arterial hypertension
The right part of the figure represent mediators and signaling pathways involved in pulmonary vascular remodeling. 5-HT, serotonin; β-NGF, β-nerve growth factor; APJ, apelin receptor; BMP, bone morphogenetic protein; BMPR-II, BMP receptor type II; CCL, C-C motif chemokine; CTGF, connective tissue growth factor; CXCL, C-X-C motif chemokine; EGF, epidermal growth factor; ET-1, endothelin-1; FGF-2, fibroblast growth factor 2; ICAM-1, intercellular adhesion molecule 1; IGF, insulin-like growth factor; IL, interleukin; MIF, macrophage migration inhibitory factor; MMPs, matrix metalloproteinases; PAH, pulmonary arterial hypertension; PAI-1, plasminogen activator inhibitor 1; PDGF, platelet-derived growth factor; pSmad, phosphorylated Smad; SMURF1, Smad-specific E3 ubiquitin-protein ligase 1; TGF-β, transforming growth factor-β; TIMPs, tissue inhibitors of metalloproteinases; TNF-α, tumour necrosis factor-α; TPH-1, tryptophan hydroxylase 1; VCAM-1, vascular cell adhesion protein 1. Adapted with permission (license: 6018690871976).[48]

Plexiform lesions

Histopathologically, plexiform lesions are complex vascular formations originating from the remodeled pulmonary arteries and are typically present in advanced PAH.[52] At the cellular level, plexiform lesions are composed of a proliferating network of endothelium-lined vascular channels supported by myofibroblasts, smooth muscle cells, and undifferentiated mesenchymal cells.[53]

Recent work from our colleagues at Lund University employing synchrotron-based micro-computed tomography to study the three-dimensional structure of plexiform lesions identified four distinct types; type 1 lesions present in supernumerary arteries and predominantly connect to the vasa vasorum; type 2 lesions originate from 90-degree branches of pulmonary arteries directed toward terminal bronchiole or larger airways, connecting the pulmonary artery with peribronchial systemic vessels. The connections from the pulmonary arteries to the systemic circulation in type 1 and 2 plexiform lesions may serve to relieve suprasystemic pulmonary pressures, leading to a right to left shunting and systemic desaturation.[9, 54] Type 3 lesions present at abrupt

ends of distal pulmonary arteries, flowing to dilated pulmonary vessels; and type 4 lesions are present when two or more lumens form, either through completely occluded pulmonary arteries with recanalization, or through pulmonary arteries with incomplete obstruction (figure 4).[54]

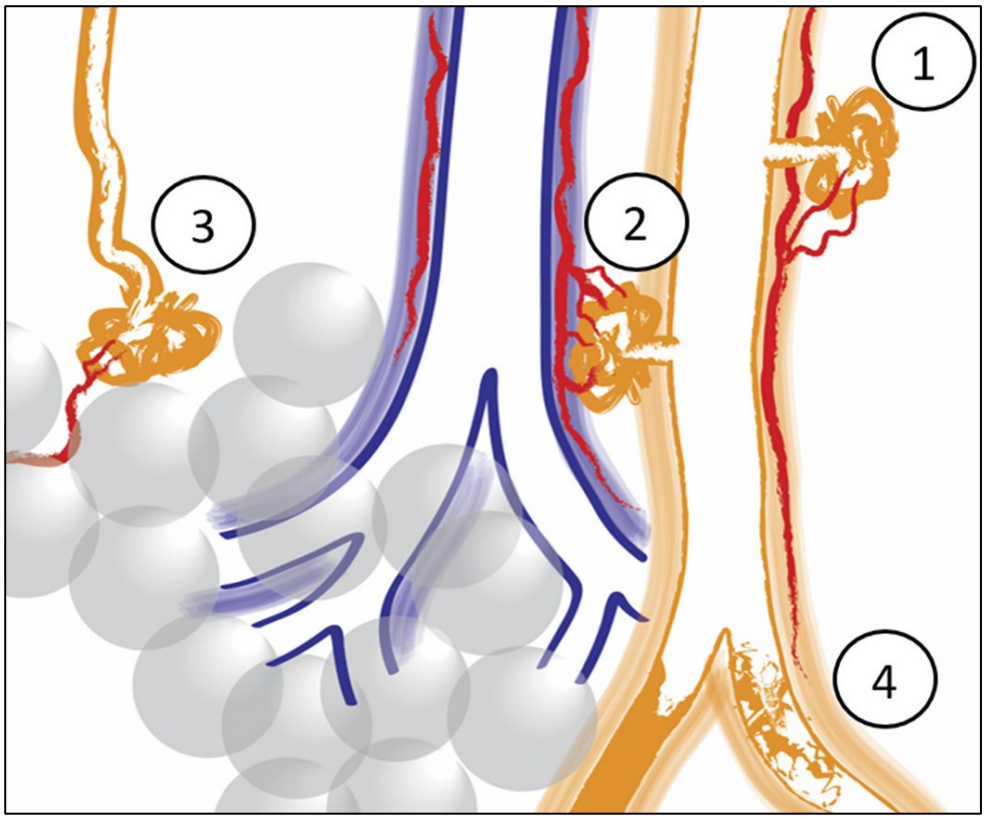

Figure 4. Emerging types of plexiform lesions in pulmonary arterial hypertension
Type 1 plexiform lesions (1) originate from the pulmonary artery (orange), with connections to the vasa vasorum (red). Type 2 lesions (2) form between pulmonary arteries and airways (blue), connecting the pulmonary arteries to peribronchial pulmonary vessels (red). Type 3 plexiform lesions (3) form at abrupt ends of distal pulmonary arteries, flowing into small, dilated vessels (red). Type 4 lesions (4) are present when two or more lumens form in the pulmonary artery either through recanalization of a complete obstruction or as a result incomplete occlusion. Type 1 and 2 are hypothesized to relieve suprasystemic pressure in the pulmonary arteries by right-left shunting and systemic desaturation. Reproduced with permission (license: 6004371302531).[54]

Diagnosis

Diagnostic delay

Despite major advances and the development and approval of several treatments that slow the progression of and improve outcomes in PAH, the diagnostic delay has not

markedly improved.[11, 12] Historically, the NIH registry reported in 1987 a median diagnostic delay of 1.27 years.[39, 55] Results from a study published more than three decades later reported a median diagnostic delay of 1.2 years, based on a PAH cohort spanning between 2004 and 2017 enrolled in the PH Society of Australia and New Zealand (PHSANZ) Registry.[55, 56] The binational study further reported a mean diagnostic delay of 2.5 years, with 35 % having a delay exceeding 2 years, which was associated with increased mortality rates independent of age, sex, and PAH subtype.[56] Further analysis did not demonstrate improvement in the diagnostic delay between 2004 and 2017. In the REVEAL registry, patients diagnosed with PAH in 2001 – 2009 had a median and a mean diagnostic delay of 1.1 and 2.6 years, respectively, with 21 % having a delay exceeding 2 years.[57, 58] Also, registry data from France,[38] Spain,[59] and China[60] have reported mean diagnostic delays of 2.3, ~2.2, and 2.2 years, respectively.[59, 60]

In 2020, the Swedish PAH patient association "PAH Sverige" did a member survey concerning the pre-diagnostic time and found that 25 % of the patients reported at least 10 visits to a primary health care facility before referral to specialized centers in Sweden. Additionally, 40 % of the patients reported waiting after onset of symptoms for more than 1 year before seeking medical attention.[61, 62] In 2023, our colleagues published a study based on the SPAHR and other linked national registries to assess healthcare utilization – defined as hospitalizations, outpatient visits, and treatments; as well as productivity loss – defined as sick leave and disability pension, up to five years before PAH diagnosis compared to controls with matched geographical location, age, and sex. Healthcare utilization and productivity loss were significantly higher in the PAH population compared to controls, starting at 3 and 5 years, respectively, before PAH diagnosis. This suggests that physical impairment precedes the diagnosis of PAH by several years, warranting better strategies for earlier diagnosis.[63]

Diagnostic approach

A holistic view and a multimodal approach are required for the diagnosis of PAH, which is set following a RHC confirming a pre-capillary PH (table 2), in the absence of other causes of PH (WHO groups II – V).[11, 12, 15, 37] In the 2022 ESC/ERS PH guidelines, a multistep approach was proposed to diagnose PH/PAH. As symptoms of PH are non-specific, the 2022 ESC/ERS PH guidelines proposed a stepwise approach for patients with suspected PH or unexplained dyspnea (figure 5).[11, 12, 64]

In step 1, initial evaluation with a comprehensive medical history and a physical assessment to evaluate the probability of PH and other underlying causes. In step 2, non-invasive testing including echocardiography is recommended to further assign the probability of PH and the presence of other disorders. In step 3, referral to a PH centre is performed if the PH probability is intermediate or high, or when there are risk factors or family history of PAH or risk factors for pulmonary embolism. In a PH centre, a comprehensive assessment is performed including RHC depending on the clinical scenario. Another arm in the diagnostic algorithm, "fast-track referral", is recommended for those with warning signs (denoted [a] in figure 5).[11, 12]

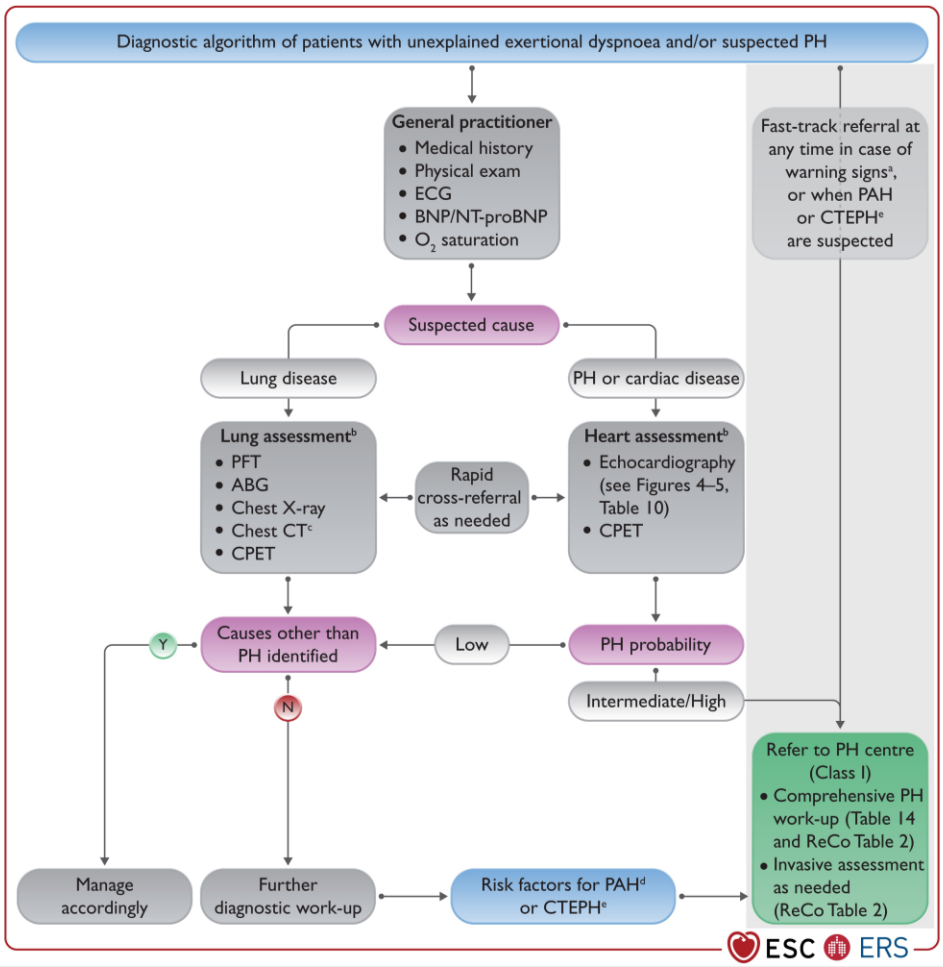

Figure 5. Diagnostic algorithm of PH according to the 2022 ESC/ERS PH guidelines
ABG, arterial blood gas; BNP, brain natriuretic peptide; CPET, cardiopulmonary exercise testing; CT, computed tomography; CTEPH, chronic thromboembolic pulmonary hypertension; ECG, electrocardiogram; HIV, human immunodeficiency virus; NT-proBNP, N-terminal pro-BNP; PAH, pulmonary arterial hypertension; PE, pulmonary embolism; PFT, pulmonary function tests; ReCo, recommendation. [b] Lung and heart assessment by specialist as per local practice. [c] As indicated; CT pulmonary angiography recommended if PH suspected. [d] Includes connective tissue disease (especially systemic sclerosis), portal hypertension, HIV infection, and family history of PAH. [e] History of PE, permanent intravascular devices, inflammatory bowel diseases, essential thrombocythemia, splenectomy, high-dose thyroid hormone replacement, and malignancy.[11, 12] Reused with permission (license: 6005421432576).

In 2022, our group published, at a national level, a complementary structured diagnostic algorithm with the focus to increase awareness, decrease the diagnostic delay, and for timely referral of patients with PAH or CTEPH.[65] Subsequently published internationally, the algorithm proposes a "structured evaluation of unclear dyspnea", rather than initial suspicion of PH as suggested in the 2022 ESC/ERS PH guidelines.[11, 12, 64] Given the low incidence of specifically PAH and CTEPH,[11, 12, 36] it may not be

realistic to expect colleagues in the primary care to suspect PH upon the patient's initial medical contact. Our complementary structured algorithm is based on a multistep approach, aligned with the responsibility and available resources of the respective healthcare facilities,[64] which will be reported more extensively in the PhD thesis of Salaheldin Ahmed in 2026.

Prognosis

Survival of patients with PAH before approval of PAH specific treatments was demonstrated in the NIH registry in 1981 – 1985, with a median time of 2.8 years, and for those with Raynaud's phenomenon a median time of 11.8 months. The 1-, 3- and 5-year survival rates were 68 %, 48 %, and 34 %, respectively.[66] In a cohort of patients with systemic sclerosis (SSc) where a subpopulation was diagnosed with APAH-SSc between 1963 and 1983, the 2-year survival rate was 40 %.[67]

In the treatment era, survival rates have somewhat improved, although not satisfactory. In a study based on the REVEAL registry, the 1-, 3- and 5- year survival estimates for newly diagnosed adults with PAH between 2006 and 2009 were 86.3 %, 69.3 %, and 61.2 %, respectively.[68] In the COMPERA registry, the 1-, 3- and 5-year survival estimates of adults diagnosed with PAH between 2010 and 2019 were 90.0 %, 69.2 %, and 55.3 %, respectively.[69] However, patients without a follow-up were excluded, which may have slightly overestimated survival rates. In the SPAHR registry, the 1-, 3-, and 5-year survival rates of adults diagnosed with PAH between 2008 and 2023 were 87 %, 66 %, and 50 %, respectively. The corresponding rates between 2015 and 2023 were 88 %, 67 %, and 52 %, respectively.

Treatment

The optimal care for patients with PAH according to the 2022 ESC/ERS PH guidelines requires a multidisciplinary strategy embracing both general and directed measures. General measures include symptomatic care and treatment of comorbidities, whereas directed measures include the use of PAH specific therapies.[11, 12, 70]

General measures

For those with a stable clinical condition with optimized pharmacological treatment, physical activity, and supervised rehabilitation have positive effects on 6MWD, quality of life, and WHO-FC.[11, 12, 71] Symptomatic treatment with diuretics (loop diuretics, mineralocorticoid receptor antagonists, and thiazides) is recommended to reduce fluid retention associated with right-sided heart failure. Oxygen administration reduces PVR and improves exercise tolerance, but there are no data supporting benefits on the course of PAH.[11, 12] Based on evidence from studies enrolling patients with chronic obstructive pulmonary disease (COPD), the 2022 ESC/ERS PH guidelines recommend administration of long term oxygen therapy in patients with partial oxygen

pressure < 8 kPa or arterial oxygen saturation (SaO_2) < 92 %.[72] The use of anticoagulation therapy in PAH may be considered on an individual basis in the absence of robust data in relation to PAH. Additionally, immunization against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza, Streptococcus pneumoniae, and respiratory syncytial virus is recommended. Severe iron deficiency, which is common in PAH, is associated with worsening of symptoms and myocardial function, as well as increased risk of mortality.[11, 12, 73] Thus, intravenous iron supplementation is recommended for those with severe iron deficiency anemia (hemoglobin < 70 – 80 g/L). Finally, providing psychosocial support, implementing shared decision making when applicable, monitoring of adherence to medical therapy and identification of barriers for non-adherence are integral components for successfully managing PAH.[11, 12, 70]

Directed measures – pulmonary arterial hypertension specific therapies

Following the approval of epoprostenol sodium for the long-term treatment of primary pulmonary hypertension in 1995,[74, 75] multiple drugs have been developed and approved for PAH.[70, 76] Current PAH-specific drugs are active via four routes of administration (oral, inhaled, subcutaneous, and intravenous), targeting the prostacyclin, endothelin, nitric oxide, and the BMP/TGF-β pathways (figure 6).[9, 76, 77]

Treatments targeting the prostacyclin pathway

The prostacyclin pathway is abnormal in PAH, with less expression of prostacyclin synthase in pulmonary arterial cells. Mainly produced by endothelial cells, prostacyclin induces vasodilation, inhibits platelet aggregation, and has cytoprotective and antiproliferative properties.[11, 12, 78]

Epoprostenol is a synthetic prostacyclin analogue that has a 3 – 5 min half-life, requiring continuous intravenous administration via an infusion pump. Three randomized clinical trials (RCTs) have demonstrated the efficacy of epoprostenol with improvement in symptoms, exercise capacity, hemodynamics, and survival,[75, 79, 80] and long-term efficacy in IPAH and APAH. Serious adverse events include infusion pump malfunction, catheter obstruction, local site infection, and sepsis.[11, 12, 81-83]

Treprostinil, an analogue of epoprostenol, is available for subcutaneous, intravenous, inhaled, and oral administration, and was approved by the United States Food and Drug Administration (FDA) in 2002, 2005, 2009, and 2013, repsectively.[6] Subcutaneous treprostinil improves symptoms, exercise capacity, and hemodynamics in PAH. Infusion site pain was present in 85 % of the treated patients.[84] The TRUST-1 trial (NCT00494533) was performed with intravenous treprostinil and demonstrated improved exercise capacity, dyspnea, and WHO-FC, but enrollment was prematurely terminated due to safety considerations.[85] The TRIUMPH I trial (NCT00147199) was performed with inhaled treprostinil in symptomatic patients with PAH on either sildenafil or bosentan as background therapy. It demonstrated improved 6MWD and quality of life.[86] Oral treprostinil was evaluated in the FREEDOM-M trial (NCT00325403) as monotherapy with improved exercise capacity.[87] In addition, two

RCTs (FREEDOM-C, NCT00325442; and FREEDOM-C2, NCT00887978) on patients with PAH on background therapy with endothelin receptor antagonist (ERA) and/or phosphodiesterase-5 inhibitors (PDE5i) did not reach the primary endpoint of improved 6MWD.[88, 89]

Selexipag, approved by the FDA in 2015,[6] is an oral, selective prostacyclin receptor agonist. It was evaluated in patients with PAH on background ERA and/or PDE5i, with improved PVR.[90] Additionally, the GRIPHON trial (NCT01106014) demonstrated that selexipag alone or in combination with ERA and/or PDE5i reduced the relative risk of the primary end point of mortality or a complication related to PAH by 40 %, and improved 6MWD. Common side effects include headache, nausea, diarrhea, and jaw pain.[91]

Treatments targeting the endothelin pathway

The endothelin receptor types A and B, that are abundant in pulmonary arterial smooth muscle cells, promote vasoconstriction and proliferation upon activation by endothelin-1. On the other hand, activation of endothelin B receptors, primary expressed by pulmonary endothelial cells, mediate vasodilation and antiproliferation through production of nitric oxide and prostacyclin, counterbalancing the effect of endothelin-1.[11, 12, 92] Blocking both A and B receptors versus selective blocking of endothelin A receptors are in general believed to have quite similar effects in PAH.[78]

Bosentan, approved by the FDA in 2001,[6] is an oral dual endothelin-A and endothelin-B receptor antagonist and the first molecule of its class to be synthesized. Bosentan has been evaluated in IPAH, HIV-PAH, CTD-APAH and Eisenmenger's syndrome in several clinical trials including Study 351, BREATHE-1, BREATHE-2, BREATHE-4, BREATHE-5, and EARLY among others, demonstrating positive effects on 6MWD, WHO-FC, hemodynamics, echocardiographic variables, and time to clinical worseneing.[93-98] Dose-dependent elevations in liver transferases can occur in ~10 % of treated patients.[11, 12]

Ambrisentan, approved by the FDA in 2007, is an oral, A-selective ERA, which has been evaluated in the RCTs ARIES-1 and ARIES-2 (NCT00091598).[92] Ambrisentan improved symptoms, exercise capacity, hemodynamics, and time to clinical worsening.[92, 99] While the incidence of liver function test abnormalities was reported to be between 0,8 % and 3 %, an increased incidence of peripheral edema has been reported.[11, 12, 78, 100]

Macitentan, approved by the FDA in 2013, is an oral, dual ERA. Macitentan was evaluated in SERAPHIN (NCT00660179), which demonstrated significantly reduced morbidity and mortality in patients with PAH.[101] Liver test abnormalities were reported in ~3.5 %, and hemoglobin ≤ 80 g/L between 1.7 % and 4.3 % among treated patients.[101]

Treatments targeting the nitric oxide pathway

In PAH, decreased expression of the endothelium-derived nitric oxide is present. Nitric oxide exerts its vasodilatory and antiproliferative effect on smooth muscle cells via activation of soluble guanylate cyclase, which in turn increases the production of cyclic guanosine monophosphate (cGMP). This pathway is regulated by a negative feedback loop where cGMP is degraded by phosphodiesterases, among which PDE5 is substantially expressed in the pulmonary vasculature.[78, 102]

Sildenafil, a PDE5i available for oral and intravenous administration, was approved by the FDA in 2005 and 2009, respectively.[6, 103, 104] Oral sildenafil was evaluated in several RCTs on patients with PAH, demonstrating improved symptoms, exercise capacity, and/or hemodynamics.[105-109] Tadalafil, approved by the FDA in 2009,[6] is a once daily oral PDE5i, and was evaluated in PHIRST-1 trial (NCT00125918) with favorable results on symptoms, exercise capacity, hemodynamics, and time to clinical worsening at the highest dose.[110]

Riociguat, approved by the FDA in 2013,[6] is an oral soluble guanylate cyclase stimulator independent of nitric oxide availability, leading to increased cGMP. Riociguat also acts in synergy with endogenous nitric oxide. The PATENT-1 trial (NCT00810693) demonstrated improved exercise capacity, hemodynamics, WHO-FC, and time to clinical worsening.[111]

Rebalancing transforming growth factor-β signaling: the bone morphogenetic protein–growth differentiation factor, and the transforming growth factor-β–activin–nodal branches

Under physiological conditions, two signaling branches that belong to the TGF-β superfamily (the BMP–growth differentiation factor (GDF) and the TGF-β–activin–nodal) are in balance, achieving vascular homeostasis. In PAH, however, signaling through the BMP–GDF branch is downregulated, whereas the TGF-β–activin–nodal branch is upregulated, leading to a proliferative and antiapoptotic milieu, driving vascular remodeling (figure 7).[9, 112, 113]

Sotatercept, approved by the FDA in 2024,[114] is a subcutaneously administrated first-in-class fusion protein consisting of the human activin receptor type IIA (ActRIIA) fused to the Fc domain of human immunoglobulin G, acting as a ligand trap for members of the TGF-β superfamily. Sotatercept rebalances vascular homeostasis through growth-inhibition, proapoptotic signaling, and alleviated inflammation (figure 6, figure 7).[9, 112, 113, 115] Sotatercept was evaluated in the RCTs (PULSAR, NCT03496207; and STELLAR, NCT04576988), with favorable effect on exercise capacity, hemodynamics,[112, 115], WHO-FC, and time to death or clinical worsening.[112] Additionally, the recent ZENITH trial (NCT04896008), performed on adults with WHO-FC III – IV, and classified as "high-risk" according to REVEAL Lite 2, showed a 76 % lower risk of a composite of death from any cause, lung transplantation, or hospitalization ≥ 24 hours with sotatercept versus placebo.[116]

Initial combination therapy

RCTs evaluating initial combination therapies include the AMBITION (NCT01178073),[117] and TRITON (NCT02558231) trials.[118] The AMBITION trial investigated initial combination therapy of oral ambrisentan and tadalafil versus monotherapy with either agent in treatment-naïve patients with predominantly IPAH, HPAH, or CTD-APAH. Improved time to clinical failure, functional capacity and NT-proBNP was demonstrated compared to initial monotherapy with either agent.[117] The TRITON trial investigated initial oral dual-combination therapy with macitentan and tadalafil versus initial oral triple-combination therapy with macitentan, tadalafil and selexipag in treatment-naïve patients with predominantly IPAH, HPAH, DPAH, or CTD-APAH. Initial oral triple-combination therapy was not superior to oral double-combination therapy, where both treatment groups demonstrated improvement in exercise capacity, hemodynamics, and NT-proBNP.[118] A retrospective analysis of the French registry in newly diagnosed patients with IPAH, HPAH, and anoreixgen-induced PAH, found survival benefit with initial triple-combination therapy including parental prostacyclin versus initial dual-combination therapy in patients classified as intermediate- or high risk according to the SPAHR/COMPERA strategy.[119]

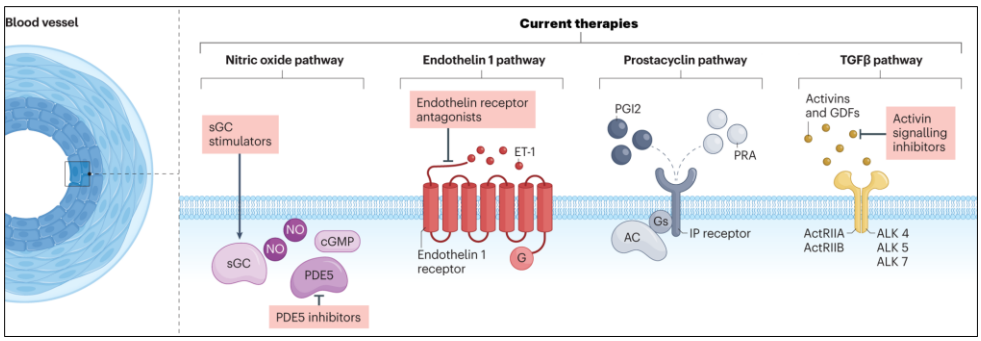

Figure 6. Main targets in pulmonary arterial hypertension specific therapies
AC, adenylyl cyclase; ActR, activin receptor type; ALK, activin receptor-like kinase; cGMP, cyclic guanosine monophosphate; ET-1, endothelin-1, GDFs, growth differentiation factors; Gs, stimulatory G protein alpha subunit; IP, I prostanoid; NO, nitric oxide; PDE5, phosphodiesterase type 5; PGI2, prostacyclin; PRA, prostacyclin receptor agonist; sGC, soluble guanylate cyclase; TGF- β, transforming growth factor-β.[120] Minimally adapted with permission (license: 6011240087062).

Calcium channel blockers

In addition to PAH specific therapy, patients with HPAH, IPAH and DPAH with a positive response at vasoreactivity testing (a reduction in mPAP ≥ 10 mmHg at an absolute value ≤ 40 mmHg, with an increased or unchanged cardiac output (CO)) may respond favorably to treatment with high-dose CCBs.[11, 12] Long-term responders to CCBs have 5-year survival rates of 98 % and 100 % on CCB monotherapy, and CCB with initial PAH specific therapy, respectively.[42] Historically, nifedipine and diltiazem have been evaluated in patients with PAH,[121-123] and more recent registry data also include amlodipine.[42]

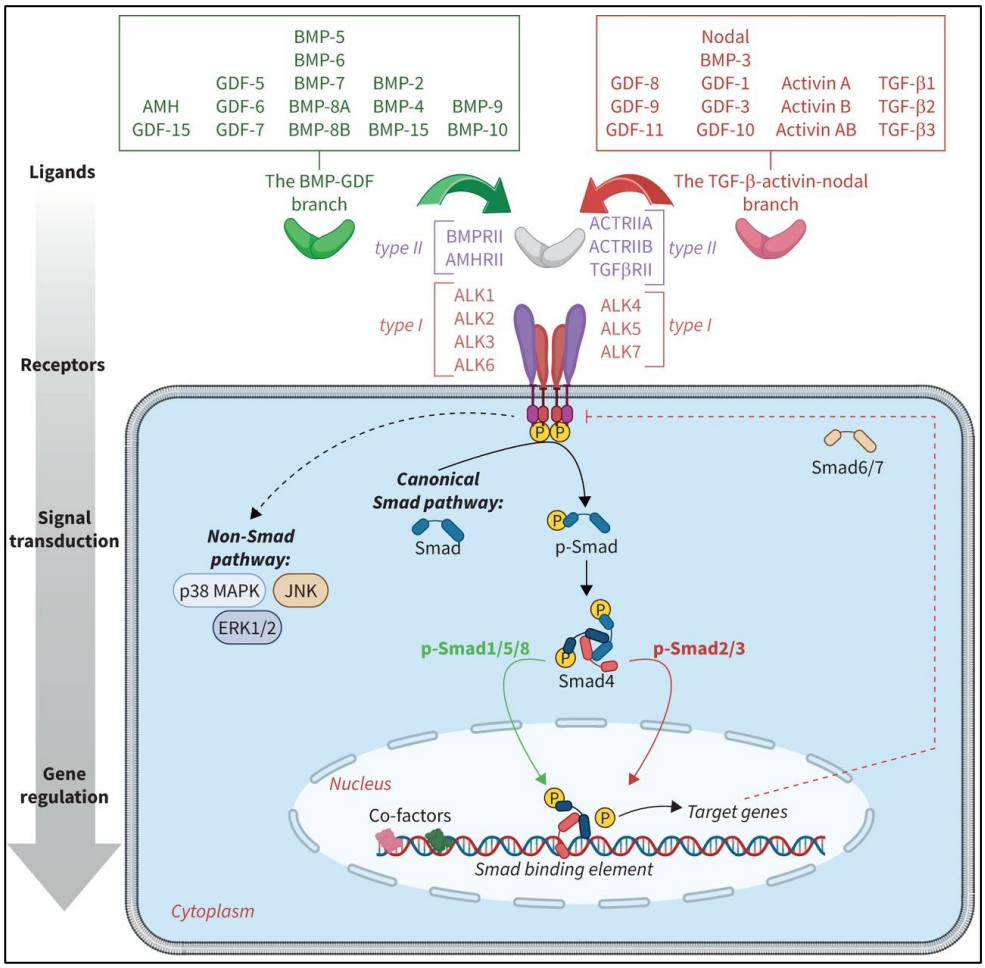

Figure 7. Transforming growth factor-β (TGF-β) signaling in pulmonary arterial hypertension (PAH): the bone morphogenetic protein (BMP)–growth differentiation factor (GDF), and the TGF-β–activin–nodal branches

A variety of ligands bind to type II receptors, which activate type I receptors through phosphorylation to induce signal transduction cascades in two main branches: the TGF-β–activin–nodal, and the BMP–GDF branch. The TGF-β–activin–nodal branch is activated through small mothers against decapentaplegic (Smad) 2/3 (red), whereas the BMP-GDF branch is activated through Smad 1/5/8 (green). Thereafter, phosphorylated (p)Smad 2/3, and pSmad 1/5/8 can oligomerize with Smad4, translocating into the nucleus followed by regulation of transcription of target genes. Inhibitory Smads (Smad6 and Smad7), accessory receptors, or other mediators may inhibit or facilitate signaling through pSmad 2/3–Smad4 complex (TGF-β-activin-nodal) or pSmad 1/5/8–Smad4 complex (BMP-GDF). Upon the activation of the ligand-receptor complex, several non-Smad or Smad-independent signaling (black dashed line) are activated. In PAH, signaling through the BMP–GDF branch is downregulated, whereas the TGF-β–activin–nodal branch is upregulated, leading to a proliferative and antiapoptotic milieu, driving vascular remodeling.[9, 113] AMH, anti-Müllerian hormone; BMPRII, BMP receptor type II; AMHRII, AMH receptor type II; ActRII, activin receptor type II; TGF-βRII, TGF-β receptor type II; ALK, activin receptor-like kinase; MAPK, mitogen-activated protein kinase; JNK, C-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase.[9] Reproduced with permission from the European Respiratory Society 2025.

Prognostic risk stratification

Among the first attempts of prognostic assessment in PAH date back to 1991. The study, based on 194 patients with PAH enrolled in the NIH registry, proposed a survival equation based on mPAP, mean right atrial pressure (mRAP), and cardiac index (CI).[66] Notably, the authors concluded that the equation "could be used as an adjunct in planning treatment strategies and allocating medical resources", before regulatory approval of the very first PAH-specific medication.[66, 74, 75]

In the proceedings of the 3rd WSPH, factors associated with prognosis were reported and it was concluded that evaluation of disease severity should be a function of end-organ consequences such as right heart failure, symptoms and functional limitation, and markers of decreased survival to optimize patient care.[124] In the following 2004 ESC guidelines on PAH, a single prognostic variable was considered to potentially provide less information compared to multiple variables.[125] The importance of multiparametric assessment was further emphasized in the 2009 ESC/ERS PH guidelines, receiving a recommendation class/level of evidence of 1C.[126] In the 2015 ESC/ERS PH guidelines, comprehensive multiparametric prognostic assessment was integrated into the evidence-based treatment algorithm, and a low-risk profile was proposed as a treatment goal.[21, 127] Due to the growing body of evidence including validation of the 2015 ESC/ERS PH guidelines' prognostic framework by several registries, where the SPAHR being the first,[128-133] the 2022 ESC/ERS PH guidelines further supported the risk-based, goal-oriented treatment approach, where both multiparametric risk assessment as well as achieving/maintaining a low-risk profile on optimized medical therapy received a recommendation class/level of evidence of 1B.[11, 12] Finally, in the proceedings of the 7th WSPH, the topic "risk stratification and treatment goals" was allocated a distinct task force, concluding that risk stratification is useful in prognostication and guiding treatment in PAH.[124, 134]

Prognostic survival equations

After the development of the first prognostic model, i.e., the NIH survival equation, several studies employed it to suggest improvement in survival by comparing observed survival rates in patients with IPAH treated with PAH-specific therapies, with predicted survival rates by the NIH equation.[135-138] In 2009, the NIH equation was reappraised using data from patients enrolled between 1991 and 2007 in the Pulmonary Hypertension Connection (PHC) registry, to account for improved management of PAH. The reappraised model, the PHC equation, was based on the same hemodynamic parameters as its predecessor.[139] In 2010, the French PAH survival equation was derived from a mixed cohort of incident patients diagnosed during the 2002 – 2003 enrolment period, and prevalent patients diagnosed within three years before enrolment. The French survival equation was based on sex, 6MWD, and CO.[140] In 2012, the newly proposed PHC and French equations at that time were validated for individual risk assessment by means of area under the receiver operating characteristics curve (AUC), which found AUCs of 0.56 and 0.53, respectively, deeming the equations non-optimal for use in individual patients. Additionally, the Scottish composite score,

introduced in 2012 and based on age, sex, etiology, mRAP, CO, and 6MWD, did not demonstrate a difference compared to the PHC equation in predicting survival.[141]

The Registry to Evaluate Early and Long-term PAH disease Management derived risk stratification strategies

In 2010, ten days after the French equation was made publicly available, the REVEAL survival equation was published online (according to metadata accessed via Crossref.org using Digital Object Identifiers (DOIs)).[140, 142] REVEAL – a multicenter, observational, prospective US-based registry – initially enrolled previously and newly diagnosed patients with PAH during 2006 – 2007, with follow-up data of at least five years after enrolment.[143] The enrolment period was subsequently further extended from 2007 – 2009 to include newly diagnosed patients with PAH with the same follow-up regimen.[68, 144] The REVEAL prognostic equation was derived from the population enrolled in 2006 – 2007, consisting of a mixed cohort of newly and previously diagnosed patients with PAH. The latter group comprised 86.5 % of the population, with mean and median times from diagnosis to enrolment of 3.3 and 2.2 years, respectively. The REVEAL equation identified 12 independent prognostic variables including PAH subgroup, male > 60 years of age (classified as one variable), renal insufficiency, WHO-FC, systolic blood pressure, heart rate (HR), 6MWD, natriuretic peptides, presence of pericardial effusion, diffusion capacity of lung for carbon monoxide (DLco) % predicted, mRAP, and PVR.[142] In 2011, the REVEAL equation was used to derive the risk score REVEAL 1.0, which was validated with newly diagnosed patients with PAH.[144] REVEAL 1.0 uses the same prognostic variables defined by the REVEAL equation.[142, 144] In 2019, a revised version of the risk score, REVEAL 2.0, was introduced, which in addition to the 12 variables used in its predecessor, included all-cause hospitalizations in the last six months (table 3).[133] REVEAL 2.0 predicts 1- and 5-year as well as clinical worsening defined as all-cause hospitalization or the introduction of a parenteral prostacyclin analog among patients surviving at least 1-year from enrolment.[133] Additionally, REVEAL 2.0 has been validated by several registries and RCT cohorts.[145-149]

An abridged version of REVEAL 2.0, REVEAL Lite 2, that includes only modifiable variables was developed and validated in 2020. REVEAL Lite 2 included WHO-FC, 6MWD, natriuretic peptides, renal insufficiency, systolic blood pressure, and HR (table 3).[150] Finally, the REVEAL-ECHO score, based on subjective assessment of the echocardiographic variables RV chamber enlargement, reduced RV systolic function, tricuspid regurgitation severity, and pericardial effusion, was recently proposed to potentially have a value in improving the classification capacity of REVEAL 2.0.[151]

The European Society of Cardiology / European Respiratory Society derived risk stratification strategies

In the 2015 ESC/ERS PH guidelines, a table including variables termed "determinants of prognosis" was proposed for risk stratification.[20, 21] Each variable had predefined cut-off values categorizing "low-risk", "intermediate-risk", and "high-risk",

corresponding to arbitrary 1-year mortality rates of < 5 %, 5 – 10 % and > 10 %, respectively (figure 8).[20, 21] Initially based on expert opinion and available literature, the strategy was first validated by the SPAHR registry which employed a simple yet novel calculation methodology to assess the overall risk score. Each parameter was assigned 1, 2, or 3 points depending on its classification as low-, intermediate-, or high-risk. The overall risk category was determined by the sum of the points divided by the number of available variables. The quotient was then rounded to the nearest integer. Quotients < 1.5, 1.5 – 2.5, and > 2.5 defined low-, intermediate-, and high-risk, respectively (figure 9, panel 1). The SPAHR strategy initially validated up to eight modifiable variables including WHO-FC, 6MWD, NT-proBNP, mRAP, CI, mixed venous oxygen saturation (SvO_2), pericardial effusion, and right atrial area.[128] Subsequently, the COMPERA registry used the SPAHR calculation methodology to validate all variables in the preceding SPAHR-study (subsequently referred to as the COMPERA strategy), except pericardial effusion and right atrial area (figure 9, panel 1, and figure 10).[129] The SPAHR and COMPERA strategy have been validated for predicting 1-, 3-, and 5-year mortality, transplant-free survival, as well as for use up to 5 years after diagnosis and at multiple follow-up assessments.[152-155] Additionally, the strategy was tested in multiple external cohorts,[133, 155-157] patients treated with add-on intravenous treprostinil,[158] and in RCT cohorts.[159, 160]

A French strategy was also introduced at the same time as the COMPERA study that defines the number of variables within the low-risk threshold among WHO-FC, 6MWD, and natriuretic peptides (French non-invasive), or WHO-FC, 6MWD, mRAP, and CI (French invasive). The French strategy is designed to identify whether a patient reaches treatment goals (i.e. achieve a low-risk category). [130]

Following the validation using the SAPHR strategy, an exploratory analysis in 2021 dividing the intermediate-risk group according to the overall risk score demonstrated worse survival rates in the intermediate-high (overall score 2.0 – 2.4) versus intermediate-low (overall score 1.5 – 1.99) risk scores.[152] Later in the same year, the COMPERA 2.0 was introduced,[131] and validated.[132] COMPERA 2.0 is a simplified four-strata risk stratification strategy that uses WHO-FC, 6MWD, and natriuretic peptides. Each variable is graded from 1 – 4 according to cut-off values derived from the 2015 ESC/ERS PH guidelines and a subsequent COMPERA registry analysis, as well as from REVEAL Lite 2.0.[20, 21, 131, 133, 134, 150] The overall score is calculated using the SPAHR strategy (figure 9, panel 2).[128]

In 2023, the Updated SPAHR was evaluated for baseline and several follow-up assessments.[153] The Updated SPAHR is a four-strata strategy based on the three-strata risk table defined by the 2022 ESC/ERS PH guidelines (figure 9, panel 1B, and figure 10).[11, 12, 153] It provides the flexibility of integrating more variables than the COMPERA 2.0, allowing a comprehensive assessment beyond that provided by COMPERA 2.0 to potentially fulfill the 2022 ESC/ERS PH guidelines' recommendation regarding comprehensive risk stratification during follow-ups when clinically needed.[11, 12, 161]

Finally, a recently proposed six-strata strategy was made by the COMPERA group. It involves a two-step calculation, where COMPERA 2.0 is initially applied. If COMPERA 2.0 returns an overall calculated risk within the intermediate risk group (i.e. intermediate-low or intermediate-high risk), the second step is employed based on SVI and/or SvO_2 where a patient can be classified as having a favorable or unfavorable hemodynamic profiles, defining two subsets of each intermediate-low and intermediate-high risk (figure 9, panel 3).

Table 3. The registry to evaluate early and long-term pulmonary arterial hypertension disease management (REVEAL) 2.0 and REVEAL Lite 2 risk stratification strategies

BNP, brain natriuretic peptide; CTD, connective tissue disease; DLco, diffusion capacity of lung for carbon monoxide; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-BNP; NYHA/WHO, New York Heart Association/World Health Organization; PE, pericardial effusion; PoPH, portopulmonary hypertension; PVR, pulmonary vascular resistance; RAP, right atrial pressure. Adopted from Benza R. et al.[133, 150]. Both publications are under the CC-BY-NC-ND license.

Determinants of prognosis[133, 150]	Scoring scale for respective variable[133, 150]					
	-2	-1	0	1	2	3
WHO group I subgroup			Other	CTD	Heritable	PoPH
Male > 60 years			No		Yes	
All-cause hospitalizations within 6 months			No	Yes		
eGFR < 60 ml/min/1.73m² or renal insufficiency¤			No	Yes		
Systolic blood pressure (mmHg)¤			≥ 110	< 110		
Heart rate (beats/ min)¤			≤ 96	> 96		
NYHA/WHO functional class¤#		I	II	III	IV	
Six-minute walk distance (m) ¤#	≥ 440	439 – 320	319 – 165	< 165		
NT-proBNP (ng/L) ¤# or BNP (ng/L) ¤#	< 300		300 – 1099		≥ 1100	
	< 50		50 – 199	200 – 799	≥ 800	
PE on echocardiogram			No	Yes		
DLco (% predicted) < 40 %			No	Yes		
RAP > 20 mmHg within 1 year			No	Yes		
PVR < 5 Wood units		Yes	No			
Overall risk calculation: REVEAL 2.0	≥ 7 out of 13 variables available (each row correspond to 1 variable). The sum of the points + 6 (correction factor) = low-risk (0 – 6 points), intermediate-risk (7 – 8 points), and high-risk (≥ 9 points).[133]					
Overall risk calculation: REVEAL Lite 2	≥ 3 out of 6 variables (denoted ¤), with at least 2 of the most predictive (denoted #). The sum of the points + 6 (correction factor) = low-risk (0 – 5 points), intermediate-risk (6 – 7 points), and high-risk (≥ 8 points).[150]					

Determinants of prognosis[a] (estimated 1-year mortality)	Low risk <5%	Intermediate risk 5–10%	High risk >10%
Clinical signs of right heart failure	Absent	Absent	Present
Progression of symptoms	No	Slow	Rapid
Syncope	No	Occasional syncope[b]	Repeated syncope[c]
WHO functional class	I, II	III	IV
6MWD	>440 m	165–440 m	<165 m
Cardiopulmonary exercise testing	Peak VO_2 >15 ml/min/kg (>65% pred.) VE/VCO_2 slope <36	Peak VO_2 11–15 ml/min/kg (35–65% pred.) VE/VCO_2 slope 36–44.9	Peak VO_2 <11 ml/min/kg (<35% pred.) VE/VCO_2 slope ≥45
NT-proBNP plasma levels	BNP <50 ng/l NT-proBNP <300 ng/l	BNP 50–300 ng/l NT-proBNP 300–1400 ng/l	BNP >300 ng/l NT-proBNP >1400 ng/l
Imaging (echocardiography, CMR imaging)	RA area <18 cm^2 No pericardial effusion	RA area 18–26 cm^2 No or minimal, pericardial effusion	RA area >26 cm^2 Pericardial effusion
Haemodynamics	RAP <8 mmHg CI ≥2.5 l/min/m^2 SvO_2 >65%	RAP 8–14 mmHg CI 2.0–2.4 l/min/m^2 SvO_2 60–65%	RAP >14 mmHg CI <2.0 l/min/m^2 SvO_2 <60%

Figure 8. The 2015 European Society of Cardiology/European Respiratory Society pulmonary hypertension guidelines' risk stratification framework

BNP, brain natriuretic peptide; CI, cardiac index; CMR, cardiac magnetic resonance; NT-proBNP, N-terminal pro-BNP; pred., predicted; RA, right atrium; RAP, right atrial pressure; SvO_2, mixed venous oxygen saturation; VE/VCO2, ventilatory equivalents for carbon dioxide; VO_2, oxygen consumption; WHO, World Health Organization; 6MWD, six-minute walk distance. Reproduced with permission,[20] (license: 6024890787484).

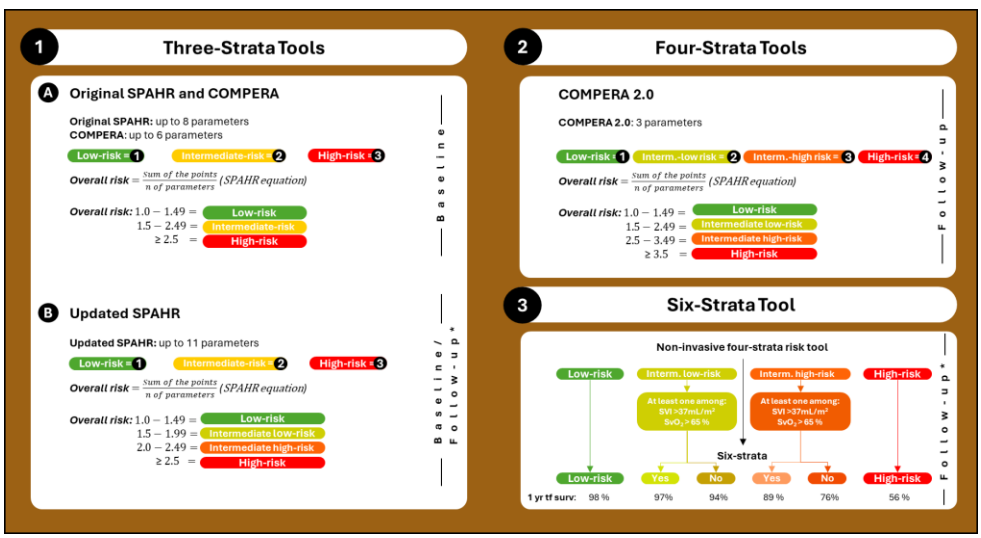

Figure 9. The European Society of Cardiology/European Respiratory Society derived risk stratification calculation methodology

Panel 1A shows the Swedish pulmonary arterial hypertension registry (SPAHR) methodology (SPAHR equation) that is also used by the comparative, prospective registry of newly initiated therapies for pulmonary hypertension (COMPERA) and COMPERA 2.0. Panel 1B shows the Updated SPAHR methodology. Panel 2 displays the calculation methodology of COMPERA 2.0 and panel 3 demonstrates the recently proposed 6-strata tool. SVI, stroke volume index; SvO_2, mixed venous oxygen saturation; 1 yr tf surv, 1-year transplantation free survival. A * denotes strategies awaiting further validation. Illustration by Ahmed A. Adapted from and modified by Ahmed A. et al.[153] under the CC-BY-NC license.

Determinants of prognosis		Risk Group Corresponding to 1-year Mortality			
		Low risk (<5%)	Intermediate risk (5 - 20%)		High risk (> 20%)
			Intermediate-low	Intermediate-high	
Clinical Observations	Clinical signs of right heart failure	Absent	Absent		Present
	Progression of symptoms	No	Slow		Rapid
	Syncope	No	Occasionally		Repeated syncope
Modifiable Parameters	WHO functional class	I or II	III		IV
	Six-minute walking distance	> 440 m	165 - 440 m		< 165 m
	Cardiopulmonary excercise testing	Peak VO_2 > 15 ml/min/kg (> 65 % predicted) VE/VO_2 slope < 36	Peak VO_2 11 - 15 ml/min/kg (35 - 65 % predicted) VE/VO_2 slope 36 - 44		Peak VO_2 < 11 ml/min/kg (< 35 % predicted) VE/VO_2 slope > 44
	Biochemical markers	NT-proBNP < 300 ng/l BNP < 50 ng/l	NT-proBNP 300 - 1100 ng/l BNP 50 - 800 ng/l		NT-proBNP > 1100 ng/l BNP > 800 ng/l
	Echocardiography	RA area < 18cm^2 TAPSE/sPAP > 0.32 mm/mmHg No pericardial effusion	RA area 18 - 26 cm^2 TAPSE/sPAP 0.19 - 0.32 mm/mmHg Minimal pericardial effusion		RA area > 26 cm^2 TAPSE/sPAP < 0.19 mm/mmHg ≥Moderate pericaridal effusion
	Cardiac magnetic resonance imaging	RVEF > 54 % SVI > 40 mL/m^2 RVESVI < 42mL/m^2	RVEF 37-54 % SVI 26 - 40 mL/m^2 RVESVI 42 - 54 mL/m^2		RVEF < 37 % SVI < 26 mL/m^2 RVESVI > 54 mL/m^2
	Hemodynamics	RAP < 8 mmHg CI ≥ 2.5 L/min/m^2 SVI > 38 mL/m^2 SvO_2 > 65 %	RAP 8 - 14 mmHg CI 2.0 - 2.4 L/min/m^2 SVI 31 - 38 mL/m^2 SvO_2 60 - 65 %		RAP > 14 mmHg CI < 2.0 L/min/m^2 SVI < 31 mL/m^2 SvO_2 < 60 %
Original SPAHR/COMPERA equation score		1 - 1.49	1.5 - 2.49		2.5 - 3.0
Updated SPAHR equation score with divided intermedaite risk		1 - 1.49	1.5 - 1.99	2.0 - 2.49	2.5 - 3.0

Figure 10. The 2022 European Society of Cardiology/European Respiratory Society pulmonary hypertension guidelines' risk stratification framework with emphasis on the Swedish pulmonary arterial hypertension registry (SPAHR) calculation methodology
BNP, brain natriuretic peptide; CI, cardiac index; CMR, cardiac magnetic resonance; NT-proBNP, N-terminal pro-BNP; RA, right atrium; RAP, right atrial pressure; RVEF, right ventricular ejection fraction; RVESVI, right ventricular end systolic volume index; SVI, stroke volume index; SvO_2, mixed venous oxygen saturation; TAPSE/sPAP, tricuspid annular plane systolic excursion/systolic pulmonary arterial pressure; VE/VCO_2, ventilatory equivalents for carbon dioxide; VO_2, oxygen consumption; WHO, World Health Organization. Illustration by Ahmed A. Adapted from Ahmed A. et al.[161] under the CC-BY-NC license.

Biomarkers

In 2015, the FDA and the NIH Joint Leadership Council identified a need to harmonize terminology used across various disciplines including regulators, clinical trialists, and therapeutic developers among others, with emphasis on terms related to endpoints and biomarkers.[162, 163] Thus, the FDA-NIH working group established the Biomarkers, EndpointS, and other Tools (BEST) resource, to improve communication and scientific understanding with the ultimate goal of accelerating development and refinement of medical products to improve health outcomes.[163]

A biomarker, i.e., a portmanteau of "biological marker"[164], is according to the BEST resource "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions. Biomarkers may include molecular, histologic, radiographic, or physiological characteristics. A biomarker is not a measure of how an individual feels, functions, or survives",[163] as such characteristics are a part of the definition of a clinical endpoint that reflects the effect of a therapeutic

intervention.[162, 163] The BEST resource provides classification of biomarkers along with their corresponding definitions, such as diagnostic-, prognostic-, and multicomponent biomarkers.[163]

A growing body of evidence supports that the pathogenesis of both heart failure and PH/PAH are ascribed to a complex and intricate interplay of several adverse pathways.[9, 165] In heart failure, several mechanisms have been described including hemodynamic overload, ischemia-related dysfunction, ventricular remodeling, excessive neurohumoral stimulation, inadequate or excessive proliferation of the extracellular matrix (ECM), and abnormal myocyte calcium cycling (figure 11).[165-167] On the same note, ECM remodeling, reduced anticoagulant properties as part of endothelial dysfunction, local unadapted release of inflammatory mediators, RV maladaptation, and metabolic dysregulation including a shift from oxidative phosphorylation to glycolysis (i.e. the Warburg effect),[168] have been described in PH/PAH.[9, 10, 120, 169] Blood-borne biomarkers can reflect some of these processes that are recognized in the pathobiology of these diseases (figure 3).[165, 166, 170, 171] Beyond the widely used natriuretic peptides, novel and particularly multi-component biomarkers, may provide deeper pathophysiological understanding, enable earlier diagnosis by e.g. screening in high risk populations such as the DETECT algorithm,[172] support clinical decision-making, and prompt the incorporation of precision medicine by enabling a more precise individual phenotyping.[173-176]

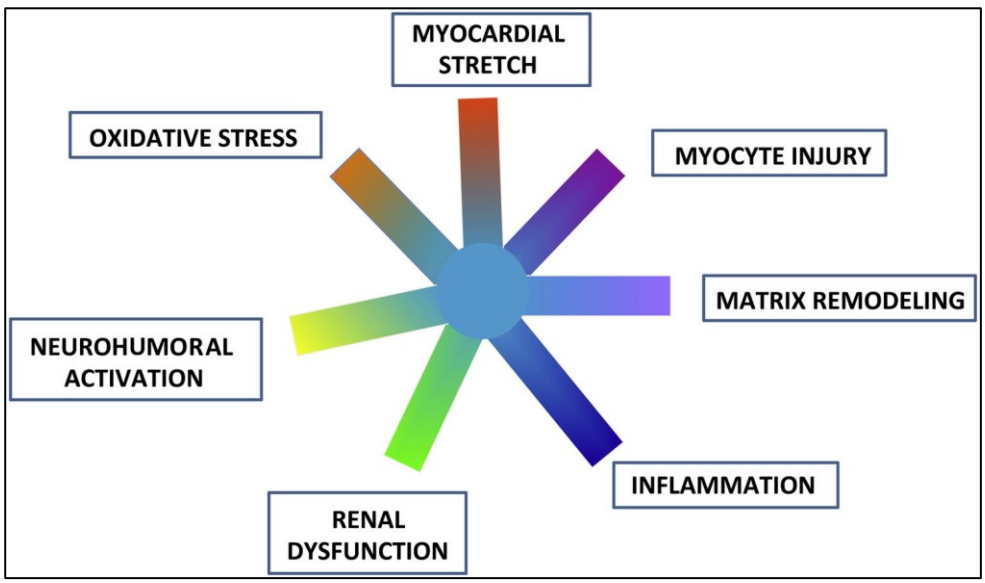

Figure 11. Major biomarker classes in heart failure[165]
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Aims

Paper I – II

As PH is a common condition associated with advanced heart failure, the aims of papers I – II were to investigate the plasma level dynamics of circulating ECM proteins (paper I), and metabolic proteins (paper II) in relation to hemodynamics prior to and 1-year after HT, to identify ECM and metabolic proteins that may be related to advanced heart failure and associated PH.

Paper III

The aims of paper III were to investigate the plasma levels of cardiovascular disease associated proteins in relation to hemodynamics in patients with advanced heart failure and PH, and in a subpopulation pre-HT and 1-year after HT. Additionally, the prognostic value of selected proteins was investigated in patients with advanced heart failure and PH.

Paper IV

As there are multiple causes of PH, the aims of paper IV were to investigate the diagnostic and prognostic potential of plasma proteins involved in coagulation, inflammation, and metabolism in patients with PAH, among a population with CTEPH, HFpEF with PH, HFrEF with PH, and HF without PH, and healthy participants.

Paper V

As PAH represent a severe subgroup of PH, the aims of paper V were to evaluate the ESC/ERS-derived three- and four-strata risk stratification strategies using the SUS-Lund PAH cohort and to establish a comprehensive internet-based calculator to facilitate risk assessment in PAH.

Materials and methods

Lund Cardio Pulmonary Registry and blood sampling

Papers I – IV were based on Lund Cardio Pulmonary registry (LCPR), an ongoing prospective cohort of Region Skåne Biobank, established in 2011 by Göran Rådegran. LCPR contains blood samples and clinical data collected from adult enrollees (≥ 18 years of age), referred to SUS-Lund for hemodynamic evaluation as part of specialized diagnostic workups, including pre- and post-HT assessment, evaluation of valvular disease, PH, or unexplained dyspnea. Additionally, healthy volunteers are enrolled in LCPR, who, unlike the former group, only undergo a routine clinical examination and peripheral blood sampling. The aim of LCPR is to advance biomarker research within the fields of PH and heart failure, particularly regarding diagnosis, treatment response and prognostic assessment.

The participants were not fasting, and venous blood was sampled from patients at the time of RHC, whereas venous blood from healthy volunteers was sampled at the routine clinical examinations. Mixed venous samples were drawn during the RHC from the introducer in vena jugularis interna, whereas venous samples were drawn peripherally from healthy controls. Blood samples were collected using 6 mL EDTA BD Vacutainer tubes, centrifuged at 2000 g for 10 min at 20°C after sampling. Plasma aliquots were subsequently stored in LCPR at –80°C until retrieved for analysis.

Ethics

All participants enrolled in LCPR provided written informed consent, and the participants in the SUS-Lund PAH cohort were informed about study participation. The papers conform with the standards outlined in the declaration of Helsinki and Istanbul and were approved by the regional ethics board in Lund, Sweden (papers I – IV, dnr: 2010/114, 2010/442, 2011/368, 2011/777, 2014/92, 2015/270; paper V, dnr: 2010/114, 2011/777).

Hemodynamic evaluation and diagnostic procedures

Hemodynamics were assessed by RHC, performed in the supine position at rest, predominantly via the right internal jugular vein, using a Swan Ganz catheter (Baxter Health Care Corp, Santa Ana, CA). Mean arterial pressure (mAP), sPAP, diastolic pulmonary arterial pressure (dPAP), PAWP, mRAP, SvO_2, arterial oxygen saturation (SaO_2), and CO (thermodilution) were measured during the RHCs. HR was measured by ECG. Diastolic pulmonary pressure gradient (DPG), CI, stroke volume (SV), SV index (SVI), TPG, pulmonary arterial compliance (PAC), PVR, and left and right ventricular stroke work indices (LVSWI and RVSWI) were calculated using the following formulae: DPG=dPAP–PAWP; CI=CO/body surface area (BSA); SV=CO/HR; SVI=CI/HR; TPG=mPAP–PAWP; PVR=TPG/CO; PAC=SV/(sPAP–dPAP); LVSWI=(mAP–PAWP)×SVI, and RVSWI=(mPAP–mRAP)×SVI. BSA was calculated using Du Bois and Du Bois formula $(\text{weight(kg)}^{0.425}\times\text{height(cm)}^{0.725}\times 0.007184)$.[177] In case of multiple assessments with RHC before HT, the hemodynamic data closest to HT or the data before implantation of left ventricular (LV) assist device were used.

The PH centre at SUS-Lund constitutes one of the PH university centers that report to the SPAHR registry.[41, 153] PH-LHD and PAH was diagnosed by experienced cardiologists and defined according to prevailing PH guidelines as that time.[20, 21, 126] For patients with PAH, the diagnostic RHC in treatment-naïve patients defined baseline. Classification of heart failure and identification of intracardiac shunts were performed with echocardiography, magnetic resonance imaging, and arterial gas analysis during RHC. HFpEF and HFrEF were defined as EF $\geq$ 50% and < 50%, respectively. Provocative testing was assessed if needed including fluid challenge to rule out occult diastolic dysfunction. CTEPH was identified with pulmonary scintigraphy. High-resolution computed tomography and spirometry with diffusion capacity were performed for assessment of lung disease including interstitial lung disease, pulmonary fibrosis, and emphysema, to exclude patients with PH due to hypoxia and/or lung disease (WHO group III PH).

Renal function

The creatinine-based estimates of the patients' glomerular filtration rate (eGFR) were calculated using the revised Lund-Malmö equation (LME). Included variables in the LME in addition to creatinine are age and sex. The LME was derived using a Swedish cohort, and had a more stable performance as well as higher accuracy across GFR, age and BMI-intervals compared with the Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations.[178]

Proteomic analyses

In papers I – IV, plasma aliquots were retrieved from LCPR and analyzed in March 2017 with proximity extension assay (PEA), using multiplex immunoassay reagent kits (Cardiovascular II, Cardiovascular III, and Oncology II panels). The proteins in the panels were divided into subgroups depending on the mechanisms they are involved in, i.e. proteins associated with ECM, and metabolism among other mechanisms. The proteins analyzed are listed in papers I – IV, respectively.[179-182] The PEA technique is based on DNA oligonucleotide labelled antibodies. Upon pairwise binding of the oligonucleotide-labelled antibodies to the target protein, the oligonucleotides come into proximity, hybridize, and are re-extended by a DNA-polymerase. Subsequently, the DNA template is quantified by microfluidic qPCR (Biomark HD, Fluidigm, San Francisco, CA, USA). Each panel corresponds to a plate on which the multiplex analysis was performed. In each plate, inter-plate controls were added in triplicates, and the median of the inter-plate triplicates was used to adjust for inter-plate variations (Olink proteomics, Uppsala, Sweden). By default, the readouts of the proteins' levels from the Olink assays are expressed on an arbitrary $\log_2$ scale (AU($\log_2$)), reflecting the inverted Ct (cycle threshold) values.[183] In papers I – IV, as the $\log_2$ scale did not provide normally distributed data and to simplify interpretation of results, the logarithmic values were transformed to a linear protein expression scale (AU), using the following formula: linear AU=$2^{AU(log_2)}$. Assay validation data and panel information are available at www.olink.com.

General statistical approaches and software use

Data distribution was assessed by histograms followed by visual evaluation. Nonparametric testing was generally used as appropriate, and values were mostly expressed as medians (25^{th} – 75^{th} percentiles) unless otherwise stated. A p-value (α) < 0.05 was considered statistically significant unless false discovery rate (FDR) was used,[184] to minimize type I errors (false positives). Analyses were usually performed according to certain steps that were determined *á priori* to limit the number of statistical tests performed. Cox regressions and the Kaplan-Meier method were used to assess prognostic associations and to plot survival curves, respectively. GraphPad Prism (GraphPad Software, La Jolla CA, USA), and R statistical software (R Foundation for Statistical Computing, Vienna, Austria) were used to perform statistical analyses.

Papers I – II

Study population

In papers I – II, the initial study population comprised 20 healthy volunteers and 29 patients with advanced heart failure evaluated pre-HT and at the 1-year follow-up post-HT. The healthy volunteers did not have a medical history of myocardial infarction, heart failure, diabetes mellitus, or atrial fibrillation. Patients with missing hemodynamic data or exhibiting PH (mPAP ≥ 25 mmHg) at the 1-year follow-up post-HT were excluded (n = 3). The final study population comprised 20 healthy volunteers and 26 HT recipients. The study included all eligible HT patients with a 1-year follow-up and healthy volunteers enrolled in LCPR between October 2011 and February 2017. The demographics of the study population in papers I and II are shown in table 4.

Study set-up and statistical analyses

Twenty and 21 plasma proteins associated with ECM (paper I) and metabolism (paper II), respectively, in addition to NT-proBNP were investigated. To address the aims of papers I – II, and to limit the number of statistical tests performed, two criteria were predefined: (i) a significant change in plasma levels pre-HT versus post-HT (difference between a deteriorated and restored hemodynamic state), and in pre-HT versus healthy volunteers (difference between "diseased" and "healthy"), and (ii) post-HT proteins' levels that develop towards the healthy controls' plasma levels, displaying a "pattern of normalization". Plasma proteins which fulfilled both criteria were correlated with hemodynamics. The correlations were based on changes (post-HT – pre-HT values; Δ) between the plasma proteins' levels and hemodynamic variables. Subsequently, the plasma proteins were ranked based on the strength and the number of correlations present. The Wilcoxon signed-rank test and Mann-Whitney U test were used to assess paired and unpaired data, respectively. Tukey's fence was used to define outliers. Correlations were expressed by Spearman's rank coefficient.

Table 4. Population characteristics in papers I – II

Variable	Controls (n=20)		Pre-HT (n=26)		Post-HT (n=26)	
	n (%)	median (IQR)	n (%)	median (IQR)	n (%)	median (IQR)
Female	10 (50)		5 (19.2)			
Age (y)	20 (100)	41 (27 – 51)	26 (100)	50 (45 – 61)[a]	26 (100)	52 (47 – 63)
BSA (m^2)	19 (95)	1.9 (1.8 – 2.0)	25 (96.2)	2 (1.8 – 2.1)	26 (100)	2 (1.8 – 2.1)
Creatinine (µmol/L)			25 (96.2)	108 (90 – 123)	26 (100)	114 (97 – 142)
eGFR (mL/min/1.73m^2)			25 (96.2)	63 (55 – 71)	26 (100)	53 (43 – 72)
NT-proBNP (AU)	20 (100)	1.1 (1.1 – 1.2)	26 (100)	24 (11 – 40)[a]	26 (100)	2 (1.4 – 5.8) [a, b]

Table 4 (continued). Population characteristics in papers I – II

Variable	Controls (n=20)		Pre-HT (n=26)		Post-HT (n=26)	
	n (%)	median (IQR)	n (%)	median (IQR)	n (%)	median (IQR)
EF < 50			24 (92.3)		–	
EF ≥ 50			2 (7.7)		–	
Cardiomyopathy						
DCM			17 (65.4)		–	
HCM			3 (11.5)		–	
ICM			3 (11.5)		–	
Other etiology			3 (11.5)		–	
PH-LHD			19 (73.1)		–	
Ipc-PH			10 (52.6)[c]		–	
Cpc-PH			9 (47.4)		–	
Atrial fibrillation			13 (50)		–	
Diabetes mellitus			3 (11.5)		9 (34.6)	
Hypertension			5 (19.2)		3 (11.5)	
β-blockers			25 (96.2)		9 (34.6)	
ACEI			11 (42.5)		–	
ARB			11 (42.5)		10 (38.5)	
MRA			22 (84.6)		3 (11.5)	
Furosemide			24 (92.3)		12 (46.2)	
Cordarone			4 (15.4)		–	
Prednisolone			1 (3.8)		25 (96.2)	
Cyclosporine			–		3 (11.5)	
Tacrolimus			–		23 (88.5)	
MMF			–		21 (80.8)	
Azathioprine			–		5 (19.2)	
Sildenafil			–		1 (3.8)	

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AU, arbitrary units; BSA, body surface area; Cpc-PH, combined post-capillary and pre-capillary pulmonary hypertension; DCM, dilated cardiomyopathy; EF; ejection fraction; eGFR, estimated glomerular filtration rate; HCM, hypertrophic cardiomyopathy; HT, heart transplantation; ICM, ischemic cardiomyopathy; Ipc-PH, isolated post-capillary pulmonary hypertension; IQR, inter-quartile range; MMF, mycophenolate mofetil; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-brain natriuretic peptide; PH-LHD, pulmonary hypertension associated with left heart disease.

$P < 0.0003$ was considered statistically significant; false discovery rate <0.01.

[a] $P < 0.0003$; false discovery rate <0.01, versus controls.

[b] $P < 0.0003$; false discovery rate <0.01, versus pre-HT.

[c] One patient exhibited severe orthopnea during evaluation with right heart catheterization, and pulmonary arterial wedge pressure could not be measured. The patient underwent a second right heart catheterization as a part of the pre-HT evaluation, which revealed Ipc-PH. Adapted from papers I – II,[179, 180] under the CC-BY-NC license.

Paper III

Study population

In paper III, the initial population comprised 20 healthy volunteers (used in papers I – II) and 70 patients with heart failure and associated WHO group II PH (LHF-PH). Patients missing hemodynamics or those with an mPAP ≥ 25 mmHg after HT were excluded (n=3). The final study population comprised 20 healthy volunteers and 67 patients with LHF-PH, of whom 19 underwent HT and had available hemodynamics at the 1-year follow-up post HT. Data were censored on August 21st, 2020. The demographics of the LHF-PH and the HT subgroup are presented in table 5.

Study set-up and statistical analyses

The use of nonparametric tests, correlation analyses, and FDR were described above under papers I – II . Eighteen plasma proteins associated with cardiovascular disease were analyzed in addition to NT-proBNP. The proteins are listed in paper III.[181] One of the proteins, adrenomedullin peptides and precursor levels (ADM), was measured with the Olink assay utilizing polyclonal antibodies that could detect pro-adrenomedullin N-20 terminal peptide, mid-regional pro-adrenomedullin (MR-proADM), adrenomedullin, as well as the unprocessed adrenomedullin protein. The same criteria described under paper I – II were applied on the 19 HT patients to identify candidate plasma proteins potentially associated with hemodynamics and prognosis. The proteins that displayed significant baseline- (n=67, LHF-PH), and Δ correlations (n=19, HT subpopulation) between plasma proteins and hemodynamics/NT-proBNP qualified for prognostic analyses with Cox regressions and the Kaplan-Meier method.

For the Kaplan-Meier analysis, the optimal thresholds of the proteins to differentiate patients with better or worse outcomes, defined as event-free survival (all-cause mortality or HT), or all-cause mortality, were determined using ROC curves and Youden's indices. Kaplan-Meier curves were compared using the log-rank test.

Table 5. Population characteristics in paper III

Variable	LHF-PH (n = 67)		Pre-HT (n = 19)		Post-HT (n = 19)	
	n (%)	median (IQR)	n (%)	median (IQR)	n (%)	median (IQR)
Female	28 (42)		3 (16)		3 (16)	
Age (years)	67 (100)	63 (51 – 75)	19	52 (49 – 64)	19	52 (49 – 64)
BSA (m^2)	67 (100)	1.9 (1.8 – 2.1)	19	2 (1.8 – 2)	19	2 (1.8 – 2)
Creatinine (µmol/L)	63 (94)	108 (86 – 136)[a]	18	110 (90 – 125)[b]	19	104 (97 – 121)
eGFR (mL/min/1.73m^2)	63 (94)	54 (40 – 66)[c]	18	63 (54 – 71)[d]	19	71 (46 – 73)
NT-proBNP (AU)	67 (100)	13 (7.6 – 32)	19	28 (17 – 45)[e]	19	2 (1.5 – 6.5)
Atrial fibrillation	37 (55)		7 (37)		–	

Table 5 (continued). Population characteristics in paper III

Variable	LHF-PH (n = 67)		Pre-HT (n = 19)		Post-HT (n = 19)	
	n (%)	median (IQR)	n (%)	median (IQR)	n (%)	median (IQR)
Hypertension	27 (40)		2 (11)		1 (5)	
Diabetes mellitus	14 (21)		1 (5)		6 (32)	
EF < 50%	36 (54)		18 (95)		–	
EF ≥ 50%	31 (46)		1 (5)		–	
Ipc-PH	30 (45)		10 (53)		–	
Cpc-PH	37 (55)		9 (47)		–	
β-blockers	59 (88)		18 (95)		3 (16)	
ACEI	30 (45)		9 (47)		–	
MRA	28 (42)		8 (42)		2 (11)	
Furosemide	40 (60)		18 (95)		7 (37)	
Prednisolone	–		–		18 (95)	
Cyclosporine	1 (2)		–		2 (11)	
Tacrolimus	–		–		17 (90)	
MMF	–		–		15 (79)	
Azathioprine	1 (2)		–		3 (16)	

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AU, arbitrary units; BSA, body surface area; Cpc-PH, combined post-capillary and pre-capillary pulmonary hypertension; EF, ejection fraction; eGFR, estimated glomerular filtration rate; HT, heart transplantation; Ipc-PH, isolated post-capillary pulmonary hypertension; IQR, inter-quartile range; LHF-PH, left heart failure associated with PH; MMF, mycophenolate mofetil; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-brain natriuretic peptide.

[a] P = 0.88 vs. post-HT.

[b] P = 0.89 vs. post-HT.

[c] P = 0.19 vs. post-HT.

[d] P = 0.58 vs. post-HT.

[e] P < 0.0001 vs. post-HT.

Adapted from paper III,[181] under the CC-BY-NC license.

Paper IV

Study population

The initial population consisted of 20 healthy volunteers (previously described), and 155 patients who were enrolled in the LCPR. Patients missing hemodynamics (n = 3) or had comorbidities that influenced the definitive diagnosis (n = 2) were excluded. The final study cohort consisted of 170 participants: 20 healthy volunteers, 48 with PAH, 20 with CTEPH, 31 with HFpEF and PH (HFpEF-PH), 36 with HFrEF and PH (HFrEF-PH), and 15 with heart failure without PH that constitute a control group for dyspnea (HF-non-PH). The patients with PAH constituted the primary focus, from whom blood samples were collected at the diagnostic RHC and who were treatment-

naïve at that time. Additionally, a subgroup of the PAH population (n = 33) was included as an early treatment follow-up. Data were censored on April 8th, 2020. Population characteristics are presented in table 6.

Study set-up and statistical analyses

The focus of paper IV was to identify diagnostic and prognostic biomarker candidates in PAH. Forty-two plasma proteins associated with inflammation, coagulation, and metabolism were included. Initially, the plasma proteins were stratified according to the presence of a difference in the levels between the incident PAH population and the healthy volunteers, qualifying the proteins for further analysis. The qualified proteins were analyzed both for their diagnostic/differentiation and prognostic ability in PAH.

Diagnostic approach

PAH was compared to CTEPH, HFpEF-PH, HFrEF-PH, and dyspnea/HF-non-PH, using the Kruskal-Wallis test, followed by multiple comparisons. ROC analysis was performed to evaluate each protein's unadjusted ability to differentiate PAH from the other disease groups. Multivariable logistic regression models were thereafter fitted to combine candidate proteins adjusted for age and sex, followed by a ROC analysis to evaluate the models' ability to differentiate PAH from the other disease groups. The odds-derived cut-offs of the multivariable logistic regression models were used to derive sensitivity and specificity. DeLong's test was used to compare the AUCs of two ROC-curves. For internal validation, the optimism adjusted AUCs via a bootstrap procedure (n = 10 000 with resampling) were calculated.[185, 186]

Prognostic approach

All-cause mortality was defined as events in the prognostic analyses. Using the maximum follow-up time of the patients with PAH, levels of the plasma proteins at diagnosis were compared in survivors versus non-survivors. Proteins displaying a difference were subsequently analysed with ROC curves to extract the optimal thresholds for the Kaplan-Meier analyses. Multivariable Cox-regression models were also fitted to adjust for age, sex, and the ESC/ERS (SPAHR) risk scores. Multivariable logistic regression models were also fitted, on which ROC curves were based to display the prognostic ability as a means of AUCs. The AUCs were internally validated using the optimism adjusted AUCs via a bootstrap procedure (n = 10, 000 with resampling).

Table 6. Population characteristics in paper IV

Variable	All PAH (n=48)	PAH (baseline) (n=33)	PAH (follow-up) (n=33)	CTEPH (n=20)	HFpEF-PH (n=31)	HFrEF-PH (n=36)	HF-non-PH (n=15)
Female, n (%)	40 (83.3)	29 (87.9)	29 (87.9)	13 (65)	20 (64.5)	7 (19.4)	8 (53.3)
Age (years)	71.5 (64 – 76)	71 (60.5 – 76.5)	72 (61 – 76.5)	75 (70.8 – 77.8)	76 (69 – 83)	54 (47.3 – 59.5)	60 (46 – 76)
BSA (m^2)	1.75 (1.59–1.96)	1.73 (1.58–1.79)	1.72 (1.5–1.79)	1.83 (1.75 – 1.99)	1.89 (1.72–2.13)	2.01 (1.89–2.13)	1.96 (1.65–2.09)
WHO-FC (n) 1/2/3/4	1/9/28/2[h]	1/6/22/2[b]	2/10/15/0[f]	0/6/13/0[a]	–	–	–
6MWD (m)	242 (173 – 349)[b]	242 (184 – 346)	270 (222 – 338)[c]	300 (220 – 338)[c]	–	–	–
NT-proBNP (ng/L)	2149(865–3631)[b]	2104(767–3139)[b]	695 (243–1797)[c]	1473 (199–4538)[b]	1574 (876–2355)[i]	4512(3613–7230)[f]	1273(475–2972)[d]
NT-proBNP (AU)	8.8 (4.2 – 14.3)	7.9 (3.6 – 11.4)	2.1 (1.3 – 3)	6.1 (2 – 18)	7.6 (5 – 9.9)	30 (17 – 43)	8.9 (2.4 – 21)
Creatinine (µmol/L)	90 (71– 114)[b]	90 (70 – 113)[b]	–	88 (73 – 123)[b]	98 (79–118)[b]	121 (90 – 145)[a]	93 (81 – 123)[b]
eGFR (mL/min/1.73m^2)	59(44 – 69)[b]	60 (42 – 68)[b]	–	56 (46 – 62)[b]	48 (38 – 63)[b]	57 (43 – 70)[a]	56 (44 – 77)[b]
Comorbidities and medications, n (%)							
Atrial fibrillation	4 (8.3)	2 (6.1)	–	3 (15)	23 (76.7)[a]	14 (38.9)	8 (61.5)[b]
Hypertension	17 (35.4)	11 (33.3)	–	11 (55)	20 (74.1)[d]	7 (19.4)	7 (53.8)[b]
Ischemic heart disease	7 (14.9)	5 (15.2)	–	1 (5)	6 (25)[g]	6 (16.7)	6 (42.9)[a]
Stroke	2 (4.2)	2 (6.1)	–	1 (5)	6 (24)[f]	4 (11.1)	2 (15.4)[b]
Thyroid disease	11 (22.9)	10 (30.3)	–	1 (5)	2 (8.3)[g]	3 (8.3)	3 (21.4)[a]
Diabetes mellitus	12 (25)	8 (24.2)	–	0 (0)	10 (35.7)[c]	4 (11.1)	3 (21.4)[a]
β-blockers	16 (33.3)	9 (27.3)	–	9 (45)	23 (74.2)	35 (97.2)	11 (73.3)
MRA	12 (25)	7 (21.2)	–	3 (15)	8 (25.8)	28 (77.8)	7 (46.7)
ACEI	10 (20.8)	6 (18.2)	–	2 (10)	11 (35.5)	19 (52.8)	3 (20)
ARB	4 (8.3)	0 (0)	–	2 (10)	10 (32.3)	14 (38.9)	0 (0)
Furosemide	11 (22.9)	11 (33.3)	–	7 (35)	6 (19.4)	34 (94.4)	12 (80)

Table 6 (*continued*). Population characteristics in paper IV

Variable	All PAH (n=48)	PAH (baseline) (n=33)	PAH (follow-up) (n=33)	CTEPH (n=20)	HFpEF-PH (n=31)	HFrEF-PH (n=36)	HF-non-PH (n=15)
Hemodynamics							
mAP (mmHg)	96 (89.4–104)	94.3 (89 – 103)	86 (80 – 92)	99 (94 – 110)	99 (91 – 106)	79.5 (75.3–88.8)	89 (80 – 96)
mPAP (mmHg)	43 (37 – 55)	43 (37 – 55)	36 (32 – 48)	42 (35 – 54)	34 (29 – 46)	34.5 (29 – 40.8)	20 (17 – 22)
mRAP (mmHg)	7 (4 – 11)	6 (3 – 9.5)	6 (3 – 9.5)	5.5 (3.25 – 8)	10 (7 – 14)	14.5 (9 – 17)	6 (2 – 16)
PAWP (mmHg)	8 (6 – 11)	6 (5 – 9.5)	8 (5 – 11)	9.5 (7 – 13)	18 (17 – 23)	25 (19 – 28)[a]	15 (9 – 18)
Cardiac output (L)	3.8 (3 – 5.1)	3.9 (3 – 5)	4.8 (3.7 – 6)	4 (3.5 – 4.7)	4.4 (3.6 – 5.6)	3.2 (2.8 – 4)	3.3 (3 – 4.4)
Cardiac index (L/m²)	2.2 (1.8–2.8)	2.2 (1.8 – 2.8)	2.7 (2.1 – 3.4)	2.3 (1.9 – 2.5)	2.3(2.1 – 2.6)	1.6 (1.4 – 1.9)	1.9 (1.6 – 2.3)
SVI (mL/beat/m²)	28.7(22.6–34.9)	29.2 (23.7–35.4)	37 (29.5–42.8)	30.5 (26.3–32.5)	33.7 (27.8–42.2)	22.5 (18.2–27.2)	29 (25.2–31.9)
LVSWI (mmHg×mL/m²)	2484(2045–3214)	2579(2054–3188)	2779(2448–3535)	2508(2330–3187)	2551(2150–3209)	1152(957–1636)[a]	2168(1650–2716)
RVSWI (mmHg×mL/m²)	991(807–1245)	981 (822–1250)	1258 (917–1396)	1111(845–1298)	832 (667 – 1136)	440 (306 – 649)	382 (195 – 495)
PVR (WU)	9.5 (6.2–11.8)	9.6 (7.0 – 12.1)	5.8 (4.3 – 8.7)	9.3 (5.9 – 10.8)	3.6 (2.4 – 5.1)	3 (2.3 – 3.7)[a]	1.5 (1 – 2)
PAC (mL/mmHg)	1.07 (0.85–1.49)	1.06 (0.87–1.44)	1.54 (1.19–2.12)	0.96 (0.76–1.34)	1.78 (1.27 – 2.7)	1.97 (1.68–3.02)	3.33 (2.34–5.34)
TPG (mmHg)	35 (26 – 47)	37 (29 – 47)	29 (25 – 38.5)	36 (27 – 40.8)	14 (12 – 23)	10 (7 – 13)[a]	5 (4 – 6)
SaO_2 (%)	91 (87 – 95)	91 (88 – 94)	91 (88 – 95)	89 (86 – 94)	95 (91 – 97)	95 (92 – 96)	96 (95 – 97)
SvO_2 (%)	59 (51 – 66)	62 (54 – 66)	63 (58 – 72)	63 (55 – 68)	63 (57 – 67)	50 (47 – 55)	61 (59 – 69)

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AU, arbitrary unit; BSA, body surface area; CTEPH, chronic thromboembolic pulmonary hypertension; eGFR, estimate of glomerular filtration rate; HF, heart failure; HFpEF-PH, pulmonary hypertension associated with HF with preserved ejection fraction; HFrEF-PH, pulmonary hypertension associated with HF with reduced ejection fraction; LVSWI, left ventricular stroke work index; mAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAC, pulmonary arterial compliance; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RVSWI, right ventricular stroke work index; SaO_2, arterial oxygen saturation; SVI, stroke volume index; SvO_2, mixed venous oxygen saturation; TPG, transpulmonary pressure gradient; WHO-FC, World Health Organization functional class; 6MWD, six-minute walk distance.
[a–i] Indicate n–1 to n–9, respectively.
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Paper V

Study population

Paper V was based on a patient cohort evaluated at the Hemodynamic Lab at SUS-Lund, Sweden, between January 2000 and July 2021. The initial cohort consisted of 2117 evaluations performed on 402 patients. The Hemodynamic Lab at SUS-Lund is one of the seven PH centres in Sweden where repetitive assessments with RHCs have been performed the most extensively in patients with PAH, i.e. in 100 % at baseline and ~80 % of all follow-ups.[36, 41, 153] When inclusion and exclusion criteria were applied (figure 13), 812 assessments from 169 incident patients (defined as those with a baseline assessment) with IPAH, FPAH, PAH associated with systemic sclerosis (SSc-APAH), and other CTD-APAH (other CTD-APAH) were identified. To ensure one event per patient, assessments until the first that predicted the 1-, 3-, or 5- year mortality were included. Hence the study cohort had three compositions of follow-up assessments (figure 13). Data were censored at the earliest of the predefined follow-up times (1, 3, or 5 years), or July 26th, 2021.

Risk stratification

The SPAHR/COMPERA three-strata, the Updated SPAHR, as well as the COMPERA 2.0 strategies were evaluated. The calculation method for each is described in detail in the introduction section under "Prognostic Risk Stratification", and further elaborated in figures 9 and 10. The initial SPAHR three-strata validated up to eight variables including WHO-FC, 6MWD, NT-proBNP, mRAP, CI, SvO_2, pericardial effusion, and right atrial area. In paper V, signs of right heart failure, progression of symptoms, and syncope were additionally evaluated in the SPAHR three-strata and Updated SPAHR strategies.

Study set-up and statistics

In the SPAHR/COMPERA and Updated SPAHR analyses, the population was divided into two subgroups based on the number of available prognostic variables: 3 – 6 and 7 – 11, to evaluate whether the abundance of variables influences the prognostic accuracy. The threshold used to define the two subgroups was selected to ensure an adequate number of events in each. For an assessment to qualify for analysis, the presence of ≥ 3 risk variables defined by the ESC/ERS PH guidelines was required. All-cause mortality was considered as an event. The prognostic accuracy of the risk stratification scores at specific time points (1, 3, and 5 years) was evaluated using ROC curves. To assess prognostic accuracy over time, time-dependent C-statistics were plotted using Uno's C-statistics.[187, 188] The C-statistic is the proportion of comparable participant pairs in a cohort for whom the predicted risk scores and observed event

times are concordant. It reflects the ability of a risk score/model to assign a higher risk to the participant who experiences the event earlier.[188]

Risk score calculator

An html-based webpage was developed for comprehensive calculation of risk scores, encompassing the SPAHR/COMEPRA, Updated SPAHR, COMPERA 2.0, as well as the FPHR invasive and non-invasive strategies. Coding was performed using JavaScript. The webpage was based on a macro-enabled Excel spreadsheet developed by the author, Salaheldin Ahmed, and Göran Rådegran, which has been clinically evaluated by Göran Rådegran on patients with PAH at the Hemodynamic Lab at SUS-Lund since 2019. The webpage also includes the REVEAL 2.0 and REVEAL Lite 2 strategies. The PAH risk stratification calculator can be reached using the following URL: (https://www.svefph.se/risk- stratification).

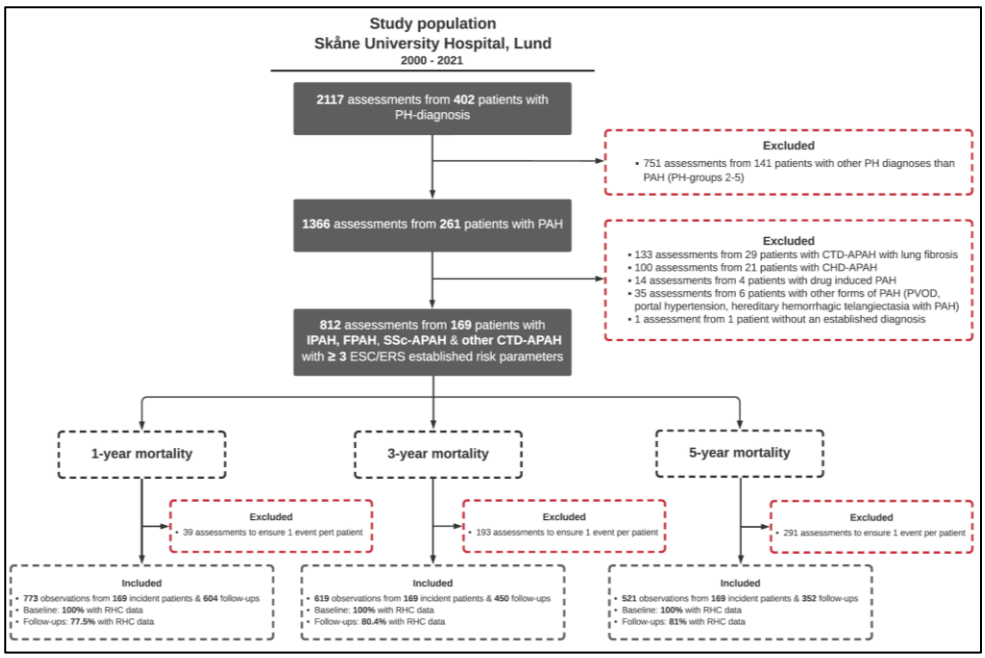

Figure 13. Flow-chart of the study population in paper V
APAH, associated pulmonary arterial hypertension; CHD-APAH, congenital heart disease-APAH; CTD-APAH, connective tissue disease-APAH; ESC/ERS, European Society of Cardiology/European Respiratory Society; FPAH, familial pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; PVOD, pulmonary veno-occlusive disease; SSc-APAH, systemic sclerosis-APAH. Reproduced from paper V,[153] under the CC-BY-NC license.

Table 7. Population characteristics in paper V

Variable	n (169)	Baseline	1-year		3-year		5-year	
			n (604)	Follow-up	n (450)	Follow-up	n (352)	Follow-up
Sex (female)	169	116 (68.6%)	604	467 (77.3%)	450	353 (78.4%)	352	279 (79.3%)
Age at diagnosis or at follow-ups (years)	169	70 (59 – 76)	604	69 (55 – 76)	450	65 (49 – 75)	352	60 (43 – 72)
Body mass index (kg/m²)	162	25 (23 – 28)	546	25 (22 – 28)	398	26 (22 – 29)	312	26 (22 – 29)
Event (death)	169	31 (18.3%)	604	45 (7.5%)	450	41 (9.1%)	352	16 (4.5%)
Time-to-event (days)	169	1068 (452 – 1893)	604	1167 (613 – 2129)	450	1583 (1001 – 2659)	352	1915 (995 – 3063)
Familial PAH	169	9 (5.3%)	604	64 (10.6%)	450	62 (13.8%)	352	62 (17.6%)
Idiopathic PAH	169	104 (61.5%)	604	366 (60.6%)	450	280 (62.2%)	352	216 (61.4%)
SSc-APAH	169	46 (27.2%)	604	148 (24.5%)	450	87 (19.3%)	352	60 (17%)
Other CTD-APAH	169	10 (5.9%)	604	26 (4.3%)	450	21 (4.7%)	352	14 (4%)
Diabetes mellitus	167	27 (16.2%)						
Atrial fibrillation	167	26 (15.6%)						
Stroke	167	9 (5.4%)						
Ischemic heart disease	167	34 (20.4%)						
Thyroid dysfunction	166	32 (19.3%)						
Anticoagulation	167	66 (39.5%)	603	255 (42.3%)	449	189 (42.1%)	352	136 (38.6%)
Diuretics	167	99 (59.3%)	602	384 (63.8%)	449	255 (56.8%)	351	180 (51.3%)
Calcium channel blocker	168	38 (22.6%)	601	134 (22.3%)	449	101 (22.5%)	351	101 (28.8%)
Endothelin receptor antagonist	145	125 (86.2%)	604	504 (83.4%)	450	383 (85.1%)	352	301 (85.5%)
Prostacyclin	145	6 (4.1%)	604	62 (10.3%)	450	51 (11.3%)	352	47 (13.4%)
PDE5i or sGC	145	75 (51.7%)	604	475 (78.6%)	450	346 (76.9%)	352	273 (77.6%)

Table 7 (*continued*). Population characteristics in paper V

Variable	n (169)	Baseline	1-year		3-year		5-year	
			n (604)	Follow-up	n (450)	Follow-up	n (352)	Follow-up
Total number of risk variables	169	7 (5.5 – 7)	604	6 (4 – 7)	450	6 (5 – 8)	352	6 (5 – 9)
Mean arterial pressure (mmHg)	143	96 (88 – 106)	399	91 (83 – 98)	310	92 (86 – 98)	242	91 (86 – 98)
Heart rate (beats/min)	138	78 (69 – 88)	506	72 (64 – 81)	368	72 (64 – 81)	284	71 (63 – 81)
Mean right atrial pressure (mmHg)	168	7 (3 – 11)	465	5 (3 – 8)	359	5 (2 – 7)	282	4 (2 – 7)
Mean pulmonary arterial pressure (mmHg)	169	46 (39 – 55)	467	40 (34 – 48)	362	40 (33 – 47)	285	39 (32 – 47)
Pulmonary arterial wedge pressure (mmHg)	169	8 (5 – 11)	465	8 (6 – 11)	359	8 (6 – 11)	282	8 (6 – 11)
Pulmonary vascular resistance (WU)	168	9.4 (6.8 – 13)	466	6.5 (4.4 – 8.8)	361	6.3 (4.1 – 8.3)	284	6.1 (3.8 – 8.2)
Cardiac index (L/min/m²)	167	2.2 (1.8 – 2.7)	459	2.8 (2.3 – 3.2)	355	2.9 (2.4 – 3.3)	280	2.9 (2.4 – 3.4)
SvO_2 (%)	110	58 (51 – 65)	395	66 (58 – 72)	316	68 (60 – 73)	252	70 (64 – 74)
NT-proBNP (ng/L)	125	2088 (684 – 4316)	492	519 (178 – 1559)	364	332 (141 – 869)	288	247 (111 – 586)
WHO-FC I	169	0 (0%)	555	88 (15.9%)	402	84 (20.9%)	315	84 (26.7%)
WHO-FC II	169	35 (20.7%)	555	252 (45.4%)	402	225 (56%)	315	169 (53.7%)
WHO-FC III	169	113 (66.9%)	555	190 (34.2%)	402	67 (16.7%)	315	60 (19%)
WHO-FC IV	169	21 (12.4%)	555	25 (4.5%)	402	6 (1.5%)	315	2 (0.6%)
Six-minute walk distance (m)	136	245 (171 – 350)	554	349 (240 – 481)	427	405 (285 – 508)	339	450 (347 – 520)
Pericardial effusion	129	20 (15.5%)	285	31 (10.9%)	228	27 (11.8%)	193	23 (11.9)
Right atrial area (cm²)	34	24 (18 – 28)	163	19 (15 – 24)	144	18 (14 – 23)	131	18 (14 – 22)
Signs of right ventricular failure	23	1 (4.3%)	94	6 (6.4%)	90	4 (4.4%)	88	3 (3.4%)
Progression of symptoms	23	19 (82.6%)	101	8 (7.9%)	97	6 (6.2%)	95	6 (6.3%)
Syncope	24	7 (29.2%)	109	3 (2.8%)	105	3 (2.9%)	103	3 (2.9%)

Continuous data are described as median (25^{th} – 75^{th} percentiles), and categorical data as n (%) of the available parameters. APAH, associated pulmonary arterial hypertension (PAH); CTD-APAH, connective tissue disease APAH; NT-proBNP, N-terminal pro-brain natriuretic peptide; PDE5i, phosphodiesterase-5 inhibitor; sGCs, soluble guanylate cyclase stimulator; SSc-APAH, systemic sclerosis-APAH; SvO_2, mixed venous oxygen saturation; WU, Wood units; WHO-FC, World Health Organization functional class. Adapted from paper V,[153] under the CC-BY-NC license.

Results

Papers I – II

Plasma levels of prolargin, matrix metalloproteinase-2 (MMP-2) [paper I]; soluble receptor for advanced glycation end products (sRAGE), and insulin-like growth factor protein 7 (IGFBP7) [paper II] were higher in patients with heart failure versus healthy volunteers. The levels of the four proteins decreased post-HT compared to pre-HT, approaching the levels of the healthy volunteers. The p-values are displayed in figure 14.

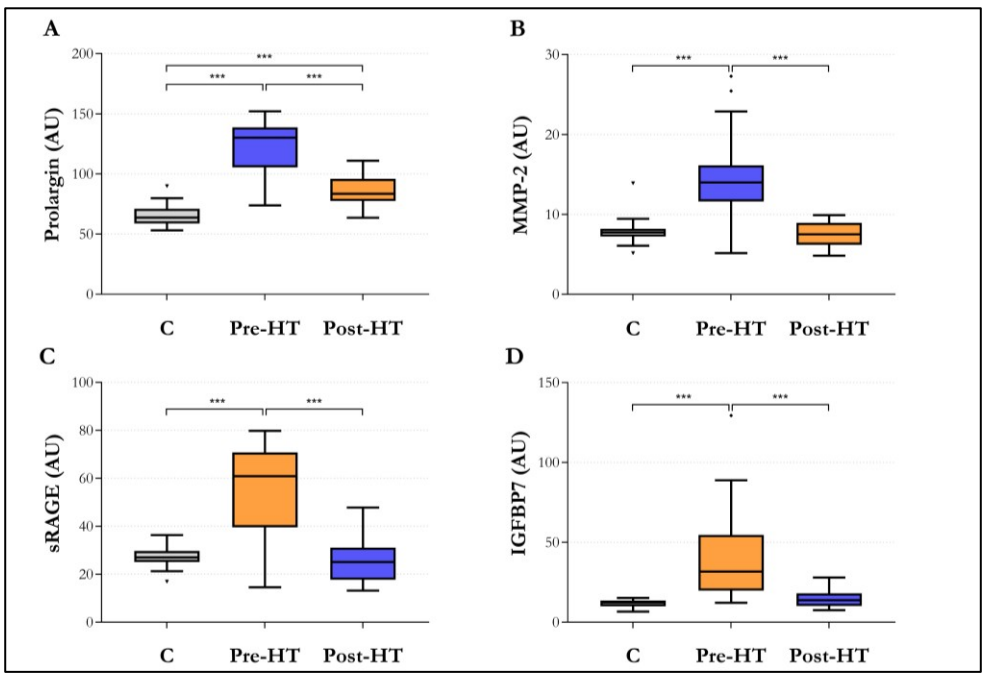

Figure 14
Plasma levels of A, prolargin; B, matrix metalloproteinase 2 (MMP-2); C, soluble receptor for advanced glycation end products (sRAGE); D, insulin-like growth factor binding protein 7 (IGFBP7) in healthy volunteers (controls, C), pre-heart transplantation (pre-HT), and post-HT. AU, arbitrary units. The p-values for post-HT versus C for MMP-2, sRAGE, and IGFBP7 were approximately 0.58, 0.14, and 0.12, respectively. *** $p < 0.0001$. Adapted from papers I – II,[179, 180] under the CC-BY NC license.

Hemodynamic improvement in papers I – II was characterized by decreased levels of mPAP, mRAP, PAWP, and PVR, as well as increased PAC, CI, and SVI post-HT compared to pre-HT. The complete list of hemodynamic variables including the Δ and p-values pre-HT versus post-HT are displayed in table 8. The Δ of prolargin, MMP-2, sRAGE, and IGFBP7 correlated with the Δ of several hemodynamic parameters. The complete list on the correlations and their p-values are displayed in table 9.

Table 8. Hemodynamic variables pre-heart transplantation and post-heart transplantation

Hemodynamic variable	Pre-HT (n=26)	Post-HT (n=26)	Δ (Post-HT−Pre-HT)	P-value
mAP (mmHg)	82 (77 – 93)[a]	102 (91 – 108)	15 (9 – 27)[a]	<0.0001*
mPAP (mmHg)	29 (24 – 38)[a]	14 (12 – 17)	-15 (-26 – -7.5)[a]	<0.0001*
PAWP (mmHg)	20 (18 – 25)[b]	7 (4 – 9.3)	-17 (-21 – -6.5)[b]	<0.0001*
mRAP (mmHg)	14 (7.5 – 18)[a]	3 (1 – 4)[a]	-12 (-15 – -3.3)[b]	<0.0001*
Heart rate (beats/min)	73 (69 – 82)[a]	82 (73 – 89)	7 (-4 – 15)[a]	0.063
CO (L/min)	3.3 (2.6 – 4.1)[a]	5.5 (5 – 6.5)	2.2 (1.2 – 2.9)[a]	<0.0001*
CI (L/min/m²)	1.8 (1.4 – 2.2)[a]	2.8 (2.6 – 3.2)	1.1 (0.65 – 1.6)[a]	<0.0001*
SV (mL/beat)	48 (35 – 58)[a]	72 (66 – 78)	23 (14 – 34)[a]	<0.0001*
SVI (mL/beat/m²)	25 (18 – 29)[a]	36 (33 – 40)	12 (6.5 – 18)[a]	<0.0001*
DPG (mmHg)	1 (0 – 3.8)[b]	2 (-0.25 – 4)	0 (-2 – 3.5)[b]	0.8
TPG (mmHg)	8.5 (6 – 12)[b]	8 (5 – 10)	-1.5 (-6 – 2)[b]	0.17
PAC (mL/mmHg)	2.2 (1.8 – 3.1)[a]	5.4 (4.1 – 6.6)	3.2 (1.3 – 4)[a]	0.00029*
PVR (WU)	2.4 (1.4 – 3.5)[b]	1.4 (0.89 – 1.9)	-1.3 (-1.9 – -0.036)[b]	<0.0001*
PVRI (WU/m²)	5.1 (2.9 – 6.9)[b]	2.8 (1.7 – 3.7)	-2.4 (-4 – -0.42)[b]	<0.0001*
LVSWI(mmHg×mL/m²)	1541(1052–2007)[b]	3344(3167–3810)	1675 (1224–2532)[b]	<0.0001*
RVSWI(mmHg×mL/m²)	362 (294 – 615)[a]	429 (317 – 516)[a]	62 (-119 – 245)[b]	0.64
a-VO_2 diff (mL O_2/L)	74 (63 – 81)[a]	42 (40 – 51)[c]	-32 (-40 – -19)[d]	<0.0001*
SaO_2 (%)	96 (94 – 97)[a]	97 (96 – 98)[c]	1.7 (-0.2 – 2.8)[d]	0.046
SvO_2 (%)	52 (47 – 60)[a]	69 (66 – 72)	17 (11 – 24)[a]	<0.0001*

Values are displayed as medians (25^{th} – 75^{th} percentiles). a-VO_2 diff, arteriovenous oxygen difference; CI, cardiac index; CO, cardiac output; DPG, diastolic pulmonary pressure gradient; LVSWI, left ventricular stroke work index; mAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; PVRI, PVR index; RVSWI, right ventricular stroke work index; SaO_2, arterial oxygen saturation; SV, stroke work; SVI, SV index; SvO_2, mixed venous oxygen saturation; TPG, transpulmonary pressure gradient; WU; Wood units. P-values < 0.0003 were considered statistically significant, denoted *, (false discovery rate < 0.01).

[a–d] Indicate n–1 to n–4, respectively.

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Table 9. Δ correlations between key proteins and hemodynamics/N-terminal pro-brain natriuretic peptide

Δ Variable	Prolargin (AU)	MMP-2 (AU)	IGFBP7 (AU)	sRAGE (AU)
mPAP (mmHg)	0.4 (0.049)[a]	0.58 (0.0025)[a]	0.041 (0.85)[a]	0.7 (<0.0001)*[a]
PVR (WU)	0.37 (0.071)[b]	0.46 (0.022)[b]	0.14 (0.52)[b]	0.65 (0.00062)*[b]
PAC (mL/mmHg)	-0.082 (0.7)[a]	0.041 (0.85)[a]	0.27 (0.19)[a]	-0.52 (0.0074)*[a]
mRAP (mmHg)	0.63 (0.00091)[b]	0.56 (0.0046)[b]	0.71 (0.00011)*[b]	0.18 (0.4)[b]
NT-proBNP (AU)	0.7 (0.000079)	0.56 (0.0029)	0.71 (<0.0001)*	0.3 (0.14)
PAWP (mmHg)	0.32 (0.13)[b]	0.48 (0.016)[b]	0.12 (0.57)[b]	0.73 (<0.0001)*[b]
CI (L/min/m²)	-0.64 (0.00057)[a]	-0.19 (0.36)[a]	-0.19 (0.37)[a]	-0.078 (0.71)[a]
SVI (mL/beat/m²)	-0.73 (0.000033)[a]	-0.25 (0.23)[a]	-0.15 (0.47)[a]	-0.16 (0.46)[a]
LVSWI (mmHg × mL/m²)	-0.49 (0.015)[b]	-0.31 (0.15)[b]	-0.33 (0.12)[b]	-0.22 (0.31)[b]

Values are displayed as [Spearman r coefficients (p-value)]. CI, cardiac index; LVSWI, left ventricular stroke work index; MMP-2, matrix metalloproteinase 2; mPAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance. SVI, stroke volume index; WU, Wood units. P-values < 0.02 for prolargin and MMP-2, and <0.021 for IGFBP7 and sRAGE were considered statistically significant, denoted *, (false discovery rate < 0.1).

[a] indicates n–1; [b], n–2. Adapted from papers I – II,[179, 180] under the CC-BY-NC license.

Paper III

The patients were followed up for a median of 4.6 (25th – 75th percentiles: 2.9 – 6.8) years. During the follow-up period, 36 out of 67 patients (53.7 %) underwent HT, and 25 (37.3 %) died. Of the 25 patients who died, 8 (32 %) had undergone HT.

Out of 18 plasma proteins, ADM displayed a difference pre-HT versus post-HT, a difference in LHF-PH versus healthy volunteers' levels, and the post-HT values displayed a normalization pattern towards the healthy volunteers' levels. Additionally, plasma ADM displayed significant correlations between baseline hemodynamics and NT-proBNP in the LHD-PH group, as well as significant Δ correlations with hemodynamics and NT-proBNP in the HT group, (table 10).

High baseline plasma ADM was associated with worse survival and event-free survival compared to low levels according to Kaplan-Meier analyses (figure 15). Baseline plasma ADM was also associated with survival in univariable Cox-regression analysis (Hazard ratio (HR): 95 % confidence interval (CI); 1.007: 1.00 – 1.015), p-value = 0.049. The association remained significant after adjusting for NTpro-BNP (HR: 95 % CI; 1.01: 1.000 – 1.021), p-value = 0.041.

Table 10. Baseline and Δ correlations in paper III

Variable	Baseline correlations with ADM LHF-PH cohort (n=67)	Δ correlations with ADM HT cohort (n=19)
Mean arterial pressure (mmHg)	-0.22 (0.071)	-0.16 (0.52)
Mean pulmonary arterial pressure (mmHg)	0.28 (0.020)	0.2 (0.41)
Pulmonary arterial wedge pressure (mmHg)	0.26 (0.034)	0.36 (0.13)
Mean right atrial pressure (mmHg)	0.67 (<0.0001)[a]	0.61 (0.0077)[a]
Cardiac output (L/min)	-0.0426 (0.73)	-0.33 (0.17)
Stroke volume index (mL/min/m²)	-0.23 (0.057)	-0.52 (0.022)
N-terminal pro-brain natriuretic peptide (AU)	0.55 (<0.0001)	0.75 (0.00025)

Values are displayed as Spearman r coefficients (p-value). ADM, adrenomedullin peptides and precursor levels; AU, arbitrary units, HT, heart transplantation; LHF-PH, heart failure and associated WHO group II pulmonary hypertension. Statistical significance for baseline and Δ correlations were defined as $p < 0.0026$ and $p < 0.033$, respectively, corresponding to false discovery rates of 0.01 and 0.25. [a] indicates n – 1. Adapted from paper III,[181] under the CC-BY-NC license.

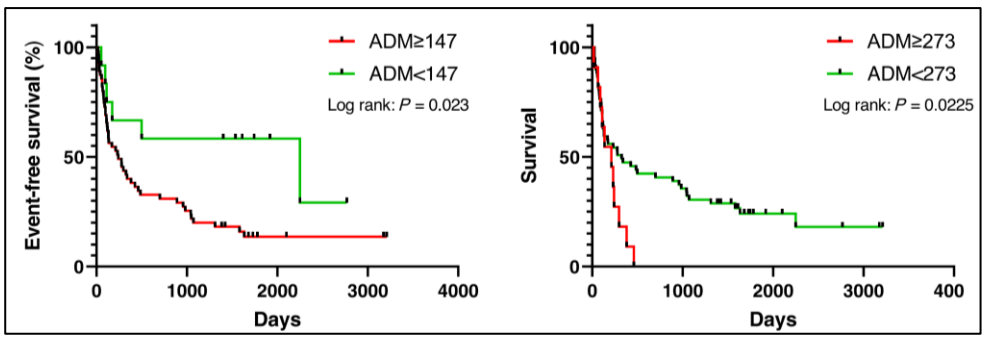

Figure 15. Main findings related to plasma adrenomedullin peptides and precursor levels (ADM)
Kaplan-Meier estimates of event-free survival (all-cause mortality or transplantation; left panel), and survival (all-cause mortality; right panel), according to optimal ADM cut-offs. Adapted from paper III,[181] under the CC-BY-NC license.

Paper IV

Population characteristics

The PAH population had a median follow-up of 3.6 (25th – 75th percentiles: 2.1 – 4.8) years. By the end of the follow-up period, 32 out of the 48 PAH patients had died. Three patients underwent lung transplantation, of whom two died after 0.83 and 5.33 years. The PAH population comprised IPAH (n = 21), SSc-APAH (n = 21), other CTD-APAH (n = 4), and FPAH (n = 2). The median time to the first early treatment follow-up was 3.8 (25th – 75th percentiles: 3.0 – 4.2) months. Among the 33 PAH patients with treatment follow-up, 23 were on monotherapy with ERAs (n = 17), or PDE5i (n = 6). Eight patients were on double combination therapy (ERA and PDE5i), and 1 patient was on triple combination therapy (ERA, PDE5i, and prostacyclin

analogue). One patient was treated with only CCB due to being acute responder at vasoreactivity testing. Additionally, nine patients were treated with CCBs for rheumatologic symptoms at baseline and at the early follow-up, and 1 patient only at follow-up. The characteristics of the patients are displayed in table 6, and the healthy volunteers in table 4.

Main results

Plasma ADAMTS13 levels were lower in the healthy volunteers compared to patients with PAH. Patients with PAH had the lowest levels of ADAMTS13 compared to the other disease groups (figure 16, A and B). A ROC analysis revealed that ADAMTS13, in univariable analysis, differentiated PAH from all other disease groups with an AUC of 0.90 (95% CI: 0.85 – 0.95). ADAMTS13 combined with age and sex displayed an AUC of 0.91 (95% CI: 0.86 – 0.96) (figure 16, C), and an optimism-adjusted AUC of 0.90.

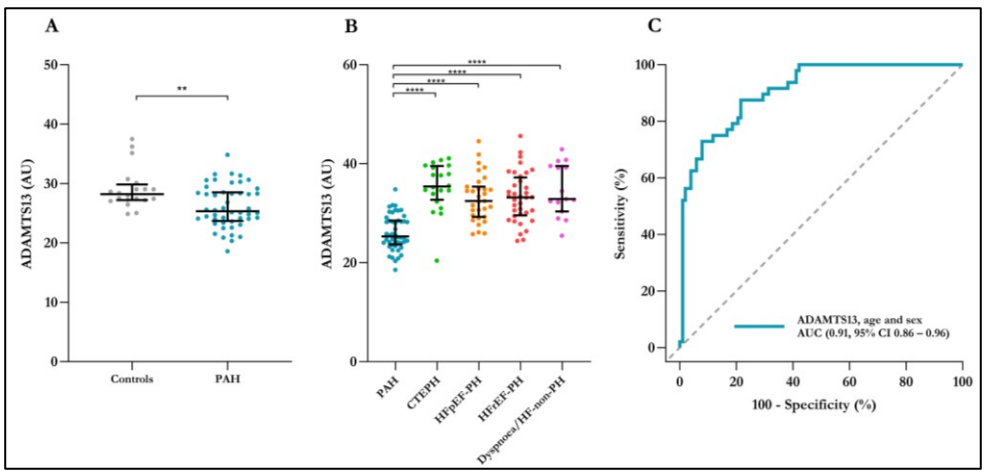

Figure 16. Main findings related to a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13)

AU, arbitrary units; AUC, area under the receiver operating characteristics curve; CTEPH, chronic thromboembolic pulmonary hypertension (PH); HFpEF-PH, PH associated with heart failure (HF) with preserved ejection fraction; HFrEF-PH, PH associated with HF with reduced ejection fraction; PAH, pulmonary arterial hypertension. ** indicates $p < 0.0055$; **** $p<0.0001$. Adapted from paper IV,[182] under the CC-BY license.

Baseline levels of plasma von Willebrand factor (vWF) were lower in survivors versus non-survivors (figure 17, A). Lower levels were also associated with better survival according to Kaplan-Meier estimates (figure 17, B). Cox-regression analysis showed that vWF was associated with survival (HR: 95 % CI; 1.002: 1 – 1.005), p-value = 0.045. The association remained significant after adjusting for age, sex, and the ESC/ERS SPAHR three-strata risk score (HR: 95 % CI; 1.002: 1 – 1.004), p-value = 0.041.

The prognostic ability, i.e. the AUC, of baseline vWF levels in patients with PAH combined with age, sex, and the ESC/ERS SPAHR three-strata risk score was 0.94 (95% CI: 0.88 – 1) (figure 17, C), and an optimism-adjusted AUC of 0.90.

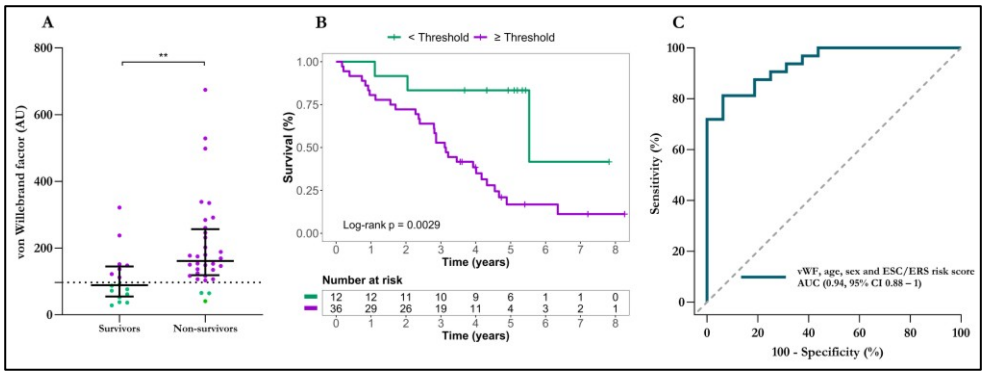

Figure 17. Main findings related to von Willebrand factor (vWF)
Survivors versus non-survivors, stratified by the maximum follow-up time (median 3.6 years) (A). Optimal threshold displayed by the dotted line. Plasma vWF levels according to the threshold are displayed using Kaplan-Meier survival analysis (B). Receiver operating characteristic curve and its area under the curve (AUC) for vWF, age, sex and the European society of cardiology / European Respiratory Society risk score (ESC/ERS) using the Swedish pulmonary arterial hypertension registry (SPAHR) three-strata strategy. AU, arbitrary units. ** indicates p = 0.0016. Adapted from paper IV,[182] under the CC-BY license.

Paper V

Population characteristics

The median follow-up time for the 169 incident patients diagnosed between 2001 and 2020 was 2.9 (25th – 75th percentiles: 1.3 – 5.1) years. By the end of the follow-up time, 115 patients had died. Within 1 year from diagnosis, 76 had died; within 3 years, 110 had died; and within 5 years 113 had died. Using the SPAHR three-strata strategy and all available variables at baseline, 28 patients were classified as low risk, 120 as intermediate-risk, and 21 as high-risk. The observed 1-year mortality rates according to risk group were 0 %, 18.3 %, and 42.9 %, respectively. Baseline characteristics are displayed in table 7.

Prognostic accuracy of risk stratification at baseline

For predicting the 1-, 3-, and 5-year mortality, the SPAHR three-strata and Updated SPAHR strategies, both based on 3 – 6 variables, demonstrated the highest AUCs (1-year: 0.73 for both; 3-year: 0.69 and 0.67, respectively; and 5-year: 0.73 and 0.68, respectively).

Prognostic accuracy of risk stratification at follow-up assessments

For predicting the 1- and 3-year mortality, the updated SPAHR (based on 7 – 11 variables) and COMPERA 2.0 strategies demonstrated the highest AUCs (1-year: 0.87 and 0.86, respectively; 3-year: 0.89 and 0.87, respectively). For predicting the 5-year mortality, the Updated SPAHR and the SPAHR three-strata strategies (both based on

7 – 11 variables) displayed the highest mean AUCs, followed by COMPERA 2.0 (0.92, 0.90, and 0.84 respectively). Notably, the Updated SPAHR strategy displayed higher mean AUCs than the SPAHR three-strata strategy (both based on 7 – 11 variables) (1-year: 0.87 and 0.78, respectively; 3-year: 0.89 and 0.86, respectively; and 5-year: 0.92 and 0.90, respectively).

Prognostic accuracy of risk stratification using the cumulative time-dependent area under the receiver operating characteristic curve (uAUC)

Based on baseline variables, the original SPAHR three-strata and the Updated SPAHR strategies (both based on 3 – 6 variables) displayed the highest uAUCs for predicting mortality within 5 years, followed by COMPERA 2.0 (figure 18, A). Based on follow-up variables, the Updated SPAHR strategy (7 – 11 variables) displayed the highest uAUC (0.90) for predicting mortality within 5 years, followed by the SPAHR three-strata (7 – 11 variables), and COMEPRA 2.0 (figure 18, B).

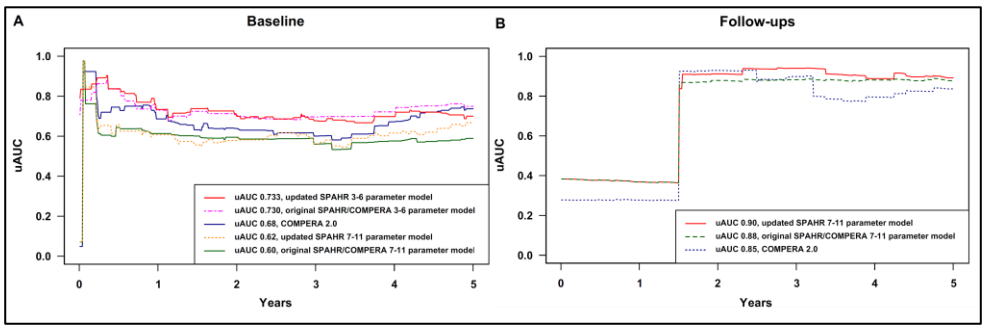

Figure 18. The cumulative time-dependent area under the receiver operating characteristic curves (uAUC) of several European Society of Cardiology/European Respiratory Society (ESC/ERS) derived risk stratification strategies

The uAUC of several risk score strategies at baseline (A), and at follow-up assessments (B). COMPERA, the Comparative, Prospective Registry of Newly Initiated Therapies for pulmonary hypertension; SPAHR, Swedish pulmonary Arterial Hypertension Registry. Adapted from paper V,[153] under the CC-BY-NC license.

Risk score calculator

To facilitate implementation of risk stratification, a web-based risk calculator was developed that included the SPAHR three-strata, Updated SPAHR, French invasive/non-invasive, and COMPERA 2.0 strategies (https://www.svefph.se/risk-stratification), (figure 19, A – C).

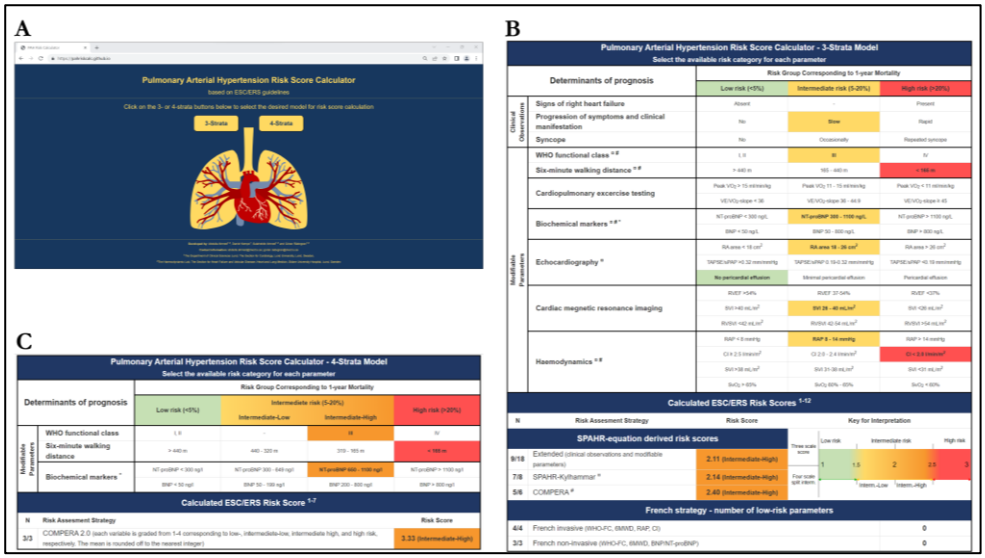

Figure 19. The web-based risk score calculator developed as a part of paper V
As of publication date of paper V (March 2023), the layout of the homepage of the risk score calculator is shown in A. Risk stratification strategies derived from the European Society of Cardiology / European Respiratory Society guidelines three-strata table (B), and the COMPERA 2.0 strategy (C). A – C constitute screen captures of the webpage. The webpage layout was designed by the author.

Discussion

Paper I

Plasma prolargin and MMP-2 were elevated in advanced heart failure and their decrease post-HT aligned with improved hemodynamics that were observed post-HT. The ECM is one of the major biomarker classes in heart failure, where it provides a scaffold for myocytes, determining their shape and size. Remodeling of the ventricles plays an important role in the progression of heart failure.[166]

Prolargin belongs to the family of small leucine-rich repeat proteoglycans, expressed in collagen-rich tissues including heart, lung, and vessels.[189, 190] Although there are relatively sparse data on this protein, it has been reported that fragmented prolargin functions by inhibiting all three complement pathways and thus hypothesized to limit the inflammatory milieu of e.g. rheumatoid arthritis.[191] Clinically, upregulation and activation of the complement pathways have been demonstrated in patients with congestive heart failure compared with healthy participants.[192] Treatment with complement inhibition using pexelizumab has been explored in patients undergoing coronary artery bypass surgery and urgent reperfusion therapy for ST elevation myocardial infarction. A systematic review and meta-analysis of > 15,000 patients demonstrated a beneficial effect in the former group on 30-day mortality, but no positive effect on the latter group.[193] We hypothesize that the increased plasma prolargin levels may reflect increased ECM turnover as a consequence of heart failure but this increase may also exert a protective mechanism to limit inflammation through complement inhibition. However, to establish a potential causal relationship and the role of this protein warrants further study.

MMP-2 is expressed by several tissues including cardiomyocytes, smooth muscle cells, alveolar cells, and endothelial cells.[190] MMP-2 participates in ECM degradation and remodelling.[194] It is involved in hypertension, remodeling following myocardial injury and healing, as well as pressure overload.[194-197] Our results confirm a previous report demonstrating increased levels of MMP-2 in congestive heart failure patients.[198] Paper I provides further evidence that the plasma levels decrease post-HT, approaching the plasma levels of healthy individuals. Additionally, MMP-2 is suggested to be involved in regulating vascular tone.[194, 199] For instance, MMP-2 cleaves big endothelin-1 to the biologically active endothelin-1 that mediates vasoconstriction.[199] Altogether, paper I further establishes the link between MMP-2 levels and advanced heart failure, pre-HT and post-HT, as well as the relation with invasive hemodynamics.

Paper II

In advanced heart failure, derangement of energy metabolism occurs, including insulin resistance in the myocardium, a decline in glucose and fatty acid utilization, as well as impaired oxidative phosphorylation.[200] In paper II, we found that plasma sRAGE and IGFBP7 out of 21 metabolism associated proteins were elevated in advanced heart failure, normalized towards healthy volunteers' levels post-HT, and correlated with the hemodynamic improvement post-HT.

The receptor for advanced glycation end-products, RAGE, is a multiligand receptor that can interact with advanced glycation end-products (AGEs).[201] Soluble RAGE (sRAGE) likely acts as a decoy receptor and may reflect the extent of RAGE activation.[202] Key processes that contribute to the increased expression of AGEs and are involved in heart failure and vascular dysfunction are hyperglycemia and oxidative stress.[203] In cardiomyocytes from transgenic mice that overexpressed RAGE, a reduced intracellular calcium concentration was found compared to normal cardiomyocytes.[204] Additionally, the AGE–RAGE interaction may promote fibrosis through stimulating collagen I and connective tissue growth factor expression.[205] Conversely, inhibition of RAGE in pressure-overloaded mice attenuated cardiac remodeling including fibrosis and hypertrophy.[206] Furthermore, RAGE activation reduces nitric oxide availability in human coronary artery endothelial cells,[207] and upregulates mRNA expression of endothelin-1 in human aortic endothelial cells.[208] Altogether, our results of high plasma sRAGE (that may reflect RAGE activation) in pre-HT align with the described pathogenetic mechanisms in heart failure including hemodynamic overload, cardiac remodeling, and abnormal myocyte calcium cycling.[165]

IGFPB7 is a secreted glycoprotein of the IGFBP superfamily.[209] IGFBP7 is associated with cardiac hypertrophy and failure,[210] vascular homeostasis,[211] and potentially fibrosis. Previous studies have reported elevated circulating levels of IGFBP7 in patients with heart failure,[210] and shown that treatment with sildenafil versus placebo,[212] or sacubitril/valsartan versus valsartan in patients with HFpEF decreased the levels of IGFBP7.[213] Our study aligns with these findings and provides further evidence on the dynamics of IGFBP7 in relation to advanced heart failure and post-HT. A recent study published after paper II, validated our hypothesis (figure 4, B1, paper II), that IGFBP7 is secreted by cardiomyocytes in heart failure, and mediates the elevated plasma levels. IGFBP7 was proposed as a driver of chronic inflammation.[214]

Paper III

In paper III, we found that plasma ADM levels were high in advanced heart failure with PH. The levels decreased post-HT approaching the healthy volunteers levels. Plasma ADM correlated with hemodynamics at baseline, and Δ ADM was associated

with improved hemodynamics. Additionally, lower baseline levels of ADM were associated with better prognosis in patients with LHF-PH.

Adrenomedullin is a potent vasodilator with inotropic and natriuretic properties.[166, 215] ADM is present in a multitude of tissues including kidneys, heart, and lungs.[166] Production of adrenomedullin is stimulated by cardiac pressure and volume overload.[215] Circulating adrenomedullin has been shown to be elevated in heart failure, where concentrations were higher in NYHA class III – IV compared to I – II.[216] A recent study found that bioactive plasma adrenomedullin in patients with heart failure had the strongest correlation with mRAP measured by RHC. In a population seeking the emergency department with acute dyspnea, bioactive adrenomedullin was associated with a need for hospitalization, and with 30- and 90-day mortality among those diagnosed with heart failure, independently of NT-proBNP. Additionally, in this population, bioactive adrenomedullin was independently associated with pleural effusion and the use of in-hospital diuretics.[217] Our results support these findings where we report the presence of baseline and Δ correlations between ADM and NT-proBNP as well as mRAP. Altogether this suggests that ADM is a plausible biomarker for systemic congestion. However, further studies are needed to better define and validate its role in clinical practice. This includes comparing it with ultrasound-based imaging, determining whether it provides additional value beyond existing systemic congestion markers such as liver injury and renal function biomarkers,[217] evaluating its dynamics following diuretic administration, and identifying conditions and medications that may influence its expression or clearance.

Paper IV

Early diagnosis of PAH remains challenging, due to non-specific early symptoms such as dyspnea on minimal exertion, fatigue, and palpitations.[11, 12] Screening of certain at-risk individuals may shorten the diagnostic delay that has not markedly improved in the last decades despite major treatment advances.[11, 12, 55] Beyond the objective of diminishing the diagnostic delay, there is a need to identify and validate biomarkers that enhance risk assessment of patients with PAH for more precise prognostication and treatment guidance.[134] In paper IV, ADAMTS13 was found as a discriminator of incident (newly diagnosed) patients with PAH from other dyspnea-associated conditions. Additionally, plasma vWF at diagnosis was prognostic in PAH.

ADAMTS13 is a circulating metalloproteinase, and vWF is a glycoprotein that mediates platelet adhesion to the site of vascular injury.[218] ADAMTS13 cleaves ultra large vWF multimers to small vWF fragments that have less procoagulant activity.[219] A deficiency in ADAMTS13 leads to thrombotic thrombocytopenic purpura, a disorder characterized by occlusion of arterioles and capillaries due to accumulation of ultra large vWF and excessive platelet aggregation. High plasma vWF and low levels of ADAMTS13 are associated with development of myocardial infarction, preeclampsia,

and ischemic stroke.[218] ADAMTS13 is produced by hepatic stellate and vascular endothelial cells. Considering the number of endothelial cells and the massive area they cover, it is possible that they constitute a significant source of the plasma levels of ADAMTS13.[220] Thus, we hypothesize that the reduced levels of ADAMTS13 in PAH may be related to the dysfunctional endothelium (figure 3, paper IV).

A recent study proposed a dysregulated ADAMTS13–vWF axis in patients with CTEPH and assessed plasma ADAMTS13 levels in IPAH. The authors found no difference in ADAMTS13 levels between patients with IPAH and healthy controls,[221] inconsistent with our results. As the PAH population in paper IV comprised different PAH etiologies, we performed a subgroup analysis between the largest subgroups (IPAH and SSc-APAH) and found no difference in ADAMTS13 levels. ($p = 0.29$). However, when the SSc-APAH and IPAH subgroups were each compared to the healthy volunteers levels, ADAMTS13 appeared lower in SSc-APAH versus controls ($p = 0.0035$), but there was weak evidence of a difference between IPAH and controls ($p = 0.06$). Although these analyses may be underpowered, they collectively suggest that the lower levels of ADAMTS13 compared to the healthy volunteers are, to a certain extent, driven by the SSc-APAH rather than the IPAH subgroup. We found no difference in ADAMTS13 levels among treatment-naïve versus patients at first follow-up, which may be attributed to the short follow-up period. Alternatively, under the speculative assumption that ADAMTS13 is related to PAH pathogenesis, the absence of disease modifying agents in the PAH population may explain the lack of a difference at the early treatment follow-up versus at diagnosis.

As PAH is a rare disease with non-specific symptoms, screening of large populations may neither be feasible nor appropriate.[55] The performance of a diagnostic test can be assessed by metrics such as positive predictive value (the proportion of those who tested positive who truly have the condition, i.e., true positives, PPV), and negative predictive value (the proportion of those who tested negative who truly do not have the condition, i.e., true negatives, NPV). Both PPV and NPV are influenced by the test's sensitivity, specificity, and the prevalence of the condition in the screened population.[163] As PAH is highly severe and early treatment may improve outcomes,[11, 12, 56] it can be argued that a high NPV and sensitivity are desired.[163] This approach may, however, come at the expense of a potentially lower specificity and PPV, potentially leading to unnecessary medical interventions. However, such drawbacks can be limited by identifying a high-risk population for screening. On another note, to utilize a single biomarker such as ADAMTS13 for screening of PAH which is a heterogenous disease with a complex pathogenesis is likely suboptimal. A multi-marker panel is, in this case, more plausible to be used for screening in a selected high-risk population.

High levels of vWF has previously been associated with increased risk of death in PAH, independent of demographics, baseline hemodynamics and PAH treatment.[222] In paper IV, we further validated these findings and demonstrated that vWF provided prognostic information independent of the ESC/ERS risk stratification score (SPAHR strategy). However, this analysis was based on baseline plasma vWF being used as a

covariate rather than being integrated into the ESC/ERS risk score. To more comprehensively evaluate the prognostic value of vWF, establishing cutoff values for low-, intermediate-, and high-risk, and to assess whether a revised score incorporating vWF outperforms the standard ESC/ERS risk stratification score. Plasma vWF was, however, measured in AU and assigning cutoffs for low-risk, intermediate-risk and high-risk will not provide generalizable information for validation in future studies. As such, future studies measuring absolute concentrations would provide more insight.

High levels of plasma vWF have been associated with histological evidence of endothelial cell injury in PAH,[223] and dysfunctional endothelial cells release large amounts of structurally deranged vWF with decreased biological activity. In PH, the deranged properties of vWF includes diminished levels of large multimeric vWF and elevations of low molecular weight polymers.[224] Thus, further investigations with focus on the biological activity and molecular weight of vWF may further advance the understanding of the ADAMTS13–vWF axis.

Finally, ADAMTS13 and vWF are two examples of biomarkers with established analytical methods, currently used to diagnose thrombotic thrombocytopenic purpura and von Willebrand disease, respectively.[225, 226] Their potential repurposing in PAH could enable faster clinical implementation, provided that sufficient evidence and validation data are available.[173]

Paper V

The 2022 ESC/ERS PH guidelines and the proceedings of the 7th WSPH recommend regular prognostic risk assessment in PAH at baseline and follow-ups.[11, 12, 70, 134] One of the new recommendations of the 2022 ESC/ERS PH guidelines was the use of a simplified three-parametric, four-strata risk assessment strategy during follow-up assessments, with incorporation of additional parameters when needed for a more comprehensive overview.[11, 12] The guidelines did, however, not elaborate on how to integrate additional variables at follow-up. On another note, several studies from the US and Europe reported underutilization of risk stratification, where only approximately 6 out of 10 clinicians employ risk assessment tools in patients with PAH.[227, 228] Time constraints represent the most frequently reported barrier to the use of risk assessment. Therefore, technology-based, time-saving solutions have been recommended.[227, 229] In paper V, we evaluated the Updated SPAHR strategy, that enables calculation of a comprehensive four-strata score at follow-up based on the three-strata risk assessment table. Additionally, we establish a web-based risk stratification calculator, incorporating multiple strategies to facilitate risk assessment.

We analyzed unique clinical data with high percentages of hemodynamic variables, i.e. 100% at baseline and ~80% at repetitive follow-ups. At baseline, the SPAHR three-strata and the Updated SPAHR strategies , both based on 3 – 6 variables, provided the highest accuracies (AUCs) in predicting the 1-, 3-, and 5-year mortality in PAH.

Including more than 6 variables at baseline did not markedly improve prognostication. This may reflect the lack of weighting, where variables with less prognostic value diminish the impact of highly prognostic variables. Furthermore, the prognostic value of some variables may vary over time, with certain variables being more prognostic at baseline and others more relevant at follow-up. Notably, the relatively low number of patients and events limited the ability to further analyze certain variables, which could have provided a better understanding on the impact of both the number and type of variable included. Nonetheless, our findings support the recommendation in the 2022 ESC/ERS PH guidelines of using a three-strata strategy at baseline.[11, 12]

We furthermore found that follow-up assessments had a better prognostic accuracy than baseline assessments, consistent with previous studies.[128, 130, 230] Specifically, at follow-ups, we demonstrate that the Updated SPAHR based on 7 – 11 variables, and COMPERA 2.0 strategies provided the highest prognostic accuracies, displaying higher values than the SPAHR three-strata strategy. These results support the use of a four-strata strategy at follow-up assessments, in line with the 2022 ESC/ERS PH guidelines' recommendations.[11, 12] Additionally, our results suggests the Updated SPAHR as a plausible strategy for utilization at follow-ups, when a more comprehensive multi-variable overview is required. Future studies are encouraged to further validate the Updated SPAHR strategy and the newly introduced variables of cardiac imaging, which have been suggested to enhance risk stratification.

The rationale for including multiple follow-up assessments per patient was to mimic the clinical setting, where risk scores are calculated independently at each time using newly obtained values for the risk variables. Although this provides information on the prognostic accuracy of serial follow-ups, the method may introduce survival bias, as patients who live longer tend to have more follow-up assessments. For instance, in the 5-year mortality cohort, the uAUC during the first ~1.5 years displayed a low plateau (figure 18, B). Further analysis revealed that it was associated to the scarcity of events within that time, after which the number of events increased. Altogether, this implies that larger multicenter studies are needed to further validate our findings.

Although reaching treatment goals is reassuring, pathological changes within the pulmonary vasculature and right ventricle can still occur even if no signs of clinical deterioration are apparent.[231, 232] Specifically, it is not unusual for young patients to present with misleadingly reassuring risk profiles. This underscores the importance of using a more comprehensive strategy for risk stratification than the simplified three-parametric four-strata tool, while simultaneously remaining vigilant to deteriorations in individual variables. Importantly, apart from risk stratification, a holistic view considering all available prognostic information including clinical gestalt, etiology, demographics, and comorbidities are essential.[231]

Strengths and limitations

The use of invasive hemodynamics as part of the thorough diagnostic workup and for follow-up assessments, the inclusion of treatment-naïve patients with PAH, and employing proximity extension assay as an analytical multiplex method constitutes major strengths. Although proximity extension assay provides high sensitivity and specificity,[183] the method does not provide absolute concentrations. However, the present studies are regarded as initial hypothesis generating where further clinical and analytical validation of biomarker candidates are required. Furthermore, we acknowledge the retrospective design, the younger healthy control population compared to the older patient groups, and the relatively low number of patients with PAH as limitations of the present work. However, the number of patients with PAH with respect to the inclusion criteria, study objectives, and the single centered setting are in line with other similar studies.[173] The strengths and limitations specific to each study are further discussed in papers I – V, respectively.[153, 179-182]

Future perspective

Despite major advances in recent years in the identifying the underlying mechanisms, as well as treatment options and strategies in PAH, survival remains unsatisfactory.[9] The identification of several biomarker candidates that predict response to therapy to guide treatment decisions and outcomes, or differentiate PAH, outline a field that is still in its infancy. Collaborative efforts are needed to establish tools that enable precision medicine for PAH. One promising direction involves integrating multi-marker panels by means of artificial intelligence and machine learning to enhance prognostication and phenotyping,[9, 10] to better describe the heterogeneous etiologies present in PAH.[9, 10, 173]

In the field of prognostic risk stratification, several challenges remain. One issue is aligning treatment goals to the underlying prognosis which is affected by modifiable and non-modifiable variables. An important part among the latter is the incorporation of comorbidities. Both the timing variable assessment, along with its relative influence in relation to other variables (weighting) should be some of the objectives to further optimize risk stratification tools. Additionally, the development of risk stratification tools that are sensitive to changes – whether improvements or deteriorations – in variables associated with treatment response are essential for such tools to genuinely guide treatment decisions. In this context, the emerging disease-modifying treatment has highlighted the relevance of hemodynamic variables, some of which have yet to be thoroughly evaluated due to the limitations of retrospective registry data. Additionally, treatment algorithms are affected by the range of available therapies, which in turn depends on the number of risk stratae a tool can define.

Ultimately, while the future of risk stratification holds some uncertainties, one certainty remains: the importance of never ceasing to be a clinician when evaluating patients.

Conclusions

Paper I – II

Plasma prolargin and MMP-2, as well as sRAGE and IGFBP7, may reflect abnormal changes in the ECM and metabolism, respectively, i.e, two pathways recognized the pathophysiology of heart failure. These four proteins may offer additional insight into the underlying pathophysiology of heart failure and associated PH. Their potential clinical relevance warrants further investigation.

Paper III

Plasma ADM appears to be a marker of cardiac overload in heart failure. In our population of advanced heart failure and associated PH, we confirm this finding and its prognostic potential. Further studies are encouraged to establish a deeper understanding of the pathophysiological effects of ADM in heart failure and PH. Additional, further analytical and clinical validation of ADM are warranted.

Paper IV

ADAMTS13 may be a promising biomarker for early PAH detection, potentially as part of a multi-marker panel. We confirm the prognostic value of vWF in PAH, though its value to the European risk stratification strategy remains to be elucidated. Repurposing ADAMTS13 and vWF as clinical biomarkers in PAH warrants further validation.

Paper V

Risk stratification can be facilitated by the introduced web-based risk score calculator, enabling broader implementation of the 2022 ESC/ERS PH guidelines. The updated SPAHR may be a plausible tool for comprehensive risk stratification at follow-up assessments when more variables are needed, providing a means to implement the recommendations of the 2022 ESC/ERS PH guidelines.

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Mattias Arvidsson, former PhD student and colleague. We have shared so many moments of deep dives into methodological and technical research challenges, some of which kept me sleepless at night. I am grateful for your support and for the fruitful collaborations throughout our projects.

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Ahmad Mahmoud, fellow PhD student, friend, and colleague. Thank you for your wonderful company. May there be many more evenings with intellectual and non-intellectual discussions about life, science, and politics, enriched by the taste of thyme and olives, and the soothing scent of warm sage tea. And hopefully someday, shared to the gentle rustling of leaves while beholding the beauty of olive trees.

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References

1 Karamanou M, Stefanadis C, Tsoucalas G, Laios K, Androutsos G. Galen's (130-201 AD) Conceptions of the Heart. *Hellenic J Cardiol* 2015; **56:**197-200.

2 West JB. Ibn al-Nafis, the pulmonary circulation, and the Islamic Golden Age. *J Appl Physiol (1985)* 2008; **105:**1877-1880.

3 Sadeghi S, Ghaffari F, Heydarirad G, Alizadeh M. Galen's place in Avicenna's The Canon of Medicine: Respect, confirmation and criticism. *Journal of Integrative Medicine* 2020; **18:**21-25.

4 Haddad SI, Khairallah AA. A Forgotten Chapter In The History Of The Circulation Of The Blood. *Ann Surg* 1936; **104:**1-8.

5 Fughelli P, Stella A, Sterpetti AV. Marcello Malpighi (1628–1694). *Circulation Research* 2019; **124:**1430-1432.

6 Anderson RJ, Malhotra A, Kim NH. Pulmonary hypertension: evolution of pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. *J Thorac Dis* 2016; **8:**S562-565.

7 Newman JH. Pulmonary hypertension. *Am J Respir Crit Care Med* 2005; **172:**1072-1077.

8 von Romberg E. Über sklerose der lungenarterie. *Dtsch Arch Klin Med* 1891; **48:**197-206.

9 Guignabert C, Aman J, Bonnet S, Dorfmüller P, Olschewski AJ, Pullamsetti S, Rabinovitch M, Schermuly RT, Humbert M, Stenmark KR. Pathology and pathobiology of pulmonary hypertension: current insights and future directions. *European Respiratory Journal* 2024**:**2401095.

10 Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, Rabinovitch M. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J* 2019; **53**

11 Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, Carlsen J, Coats AJS, Escribano-Subias P, Ferrari P, Ferreira DS, Ghofrani HA, Giannakoulas G, Kiely DG, Mayer E, Meszaros G, Nagavci B, Olsson KM, Pepke-Zaba J, Quint JK, Rådegran G, Simonneau G, Sitbon O, Tonia T, Toshner M, Vachiery JL, Vonk Noordegraaf A, Delcroix M, Rosenkranz S, Group EESD. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: Developed by 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 for Heart and Lung Transplantation (ISHLT) and the European Reference Network on rare respiratory diseases (ERN-LUNG). *European Heart Journal* 2022; **43:**3618-3731.

12 Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, Carlsen J, Coats AJS, Escribano-Subias P, Ferrari P, Ferreira DS, Ghofrani HA, Giannakoulas G, Kiely DG, Mayer E, Meszaros G, Nagavci B, Olsson KM, Pepke-Zaba J, Quint JK, Rådegran G, Simonneau G, Sitbon O, Tonia T, Toshner M, Vachiery J-L, Vonk Noordegraaf A, Delcroix M, Rosenkranz S. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *European Respiratory Journal* 2022**:**2200879.

13 ERS. Continuing Professional Development – Pulmonary Vascular Diseases. 2024

14 Tanner FC, Brooks N, Fox KF, Gonçalves L, Kearney P, Michalis L, Pasquet A, Price S, Bonnefoy E, Westwood M, Plummer C, Kirchhof P, Group ESCSD. ESC Core Curriculum for the Cardiologist. *European Heart Journal* 2020; **41:**3605-3692.

15 Kovacs G, Bartolome S, Denton CP, Gatzoulis MA, Gu S, Khanna D, Badesch D, Montani D. Definition, classification and diagnosis of pulmonary hypertension. *European Respiratory Journal* 2024**:**2401324.

16 Hoeper MM, Humbert M, Souza R, Idrees M, Kawut SM, Sliwa-Hahnle K, Jing Z-C, Gibbs JSR. A global view of pulmonary hypertension. *The Lancet Respiratory Medicine* 2016; **4:**306-322.

17 Hoeper MM, McLaughlin VV, Dalaan AMA, Satoh T, Galiè N. Treatment of pulmonary hypertension. *The Lancet Respiratory Medicine* 2016; **4:**323-336.

18 Weatherald J, Humbert M. The 'great wait' for diagnosis in pulmonary arterial hypertension. *Respirology* 2020; **25:**790-792.

19 Ford HJ, Brunetti C, Ferrari P, Meszaros G, Moles VM, Skaara H, Torbicki A, Gibbs JSR. Exploring the patient perspective in pulmonary hypertension. *European Respiratory Journal* 2024**:**2401129.

20 Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M, Group ESD. 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). *European Heart Journal* 2015; **37:**67-119.

21 Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *European Respiratory Journal* 2015; **46:**903-975.

22 Maron BA, Bortman G, De Marco T, Huston JH, Lang IM, Rosenkranz SH, Vachiéry J-L, Tedford RJ. Pulmonary hypertension associated with left heart disease. *European Respiratory Journal* 2024**:**2401344.

23 Rosenkranz S, Gibbs JS, Wachter R, De Marco T, Vonk-Noordegraaf A, Vachiéry JL. Left ventricular heart failure and pulmonary hypertension. *Eur Heart J* 2016; **37:**942-954.

24 Chandrashekhar Y, Westaby S, Narula J. Mitral stenosis. *The Lancet* 2009; **374:**1271-1283.

25 Amsallem M, Mercier O, Kobayashi Y, Moneghetti K, Haddad F. Forgotten No More: A Focused Update on the Right Ventricle in Cardiovascular Disease. *JACC: Heart Failure* 2018; **6:**891-903.

26 Houston BA, Brittain EL, Tedford RJ. Right Ventricular Failure. *New England Journal of Medicine* 2023; **388:**1111-1125.

27 McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, Burri H, Butler J, Čelutkienė J, Chioncel O, Cleland JGF, Coats AJS, Crespo-Leiro MG, Farmakis D, Gilard M, Heymans S, Hoes AW, Jaarsma T, Jankowska EA, Lainscak M, Lam CSP, Lyon AR, McMurray JJV, Mebazaa A, Mindham R, Muneretto C, Francesco Piepoli M, Price S, Rosano GMC, Ruschitzka F, Kathrine Skibelund A, Group ESD. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) With the special contribution of the Heart Failure Association (HFA) of the ESC. *European Heart Journal* 2021; **42:**3599-3726.

28 Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, Rocca H-PBL, Choi D-J, Chopra V, Chuquiure-Valenzuela E, Giannetti N, Gomez-Mesa JE, Janssens S, Januzzi JL, Gonzalez-Juanatey JR, Merkely B, Nicholls SJ, Perrone SV, Piña IL, Ponikowski P, Senni M, Sim D, Spinar J, Squire I, Taddei S, Tsutsui H, Verma S, Vinereanu D, Zhang J, Carson P, Lam CSP, Marx N, Zeller C, Sattar N, Jamal W, Schnaidt S, Schnee JM, Brueckmann M, Pocock SJ, Zannad F, Packer M. Empagliflozin in Heart Failure with a Preserved Ejection Fraction. *New England Journal of Medicine* 2021; **385:**1451-1461.

29 Crespo-Leiro MG, Metra M, Lund LH, Milicic D, Costanzo MR, Filippatos G, Gustafsson F, Tsui S, Barge-Caballero E, De Jonge N, Frigerio M, Hamdan R, Hasin T, Hülsmann M, Nalbantgil S, Potena L, Bauersachs J, Gkouziouta A, Ruhparwar A, Ristic AD, Straburzynska-Migaj E, McDonagh T, Seferovic P, Ruschitzka F. Advanced heart failure: a position statement of the Heart Failure Association of the European Society of Cardiology. *European Journal of Heart Failure* 2018; **20:**1505-1535.

30 Peled Y, Ducharme A, Kittleson M, Bansal N, Stehlik J, Amdani S, Saeed D, Cheng R, Clarke B, Dobbels F, Farr M, Lindenfeld J, Nikolaidis L, Patel J, Acharya D, Albert D, Aslam S, Bertolotti A, Chan M, Chih S, Colvin M, Crespo-Leiro M, D'Alessandro D, Daly K, Diez-Lopez C, Dipchand A, Ensminger S, Everitt M, Fardman A, Farrero M, Feldman D, Gjelaj C, Goodwin M, Harrison K, Hsich E, Joyce E, Kato T, Kim D, Luong M-L, Lyster H, Masetti M, Matos LN, Nilsson J, Noly P-E, Rao V, Rolid K, Schlendorf K, Schweiger M, Spinner J, Townsend M, Tremblay-Gravel M, Urschel S, Vachiery J-L, Velleca A, Waldman G, Walsh J. International Society for Heart and Lung Transplantation Guidelines for the Evaluation and Care of Cardiac Transplant Candidates-2024. *The Journal of Heart and Lung Transplantation* 2024; **43:**1529-1628.e1554.

31 Stevenson LW, Pagani FD, Young JB, Jessup M, Miller L, Kormos RL, Naftel DC, Ulisney K, Desvigne-Nickens P, Kirklin JK. INTERMACS Profiles of Advanced Heart Failure: The Current Picture. *The Journal of Heart and Lung Transplantation* 2009; **28:**535-541.

32 Costard-Jäckle A, Fowler MB. Influence of preoperative pulmonary artery pressure on mortality after heart transplantation: Testing of potential reversibility of pulmonary hypertension with nitroprusside is useful in defining a high risk group. *Journal of the American College of Cardiology* 1992; **19:**48-54.

33 Gjesdal G, Rylance RT, Bergh N, Dellgren G, Braun OÖ, Nilsson J. Waiting list and post-transplant outcome in Sweden after national centralization of heart transplant surgery. *The Journal of Heart and Lung Transplantation* 2024; **43:**1318-1325.

34 Khush KK, Hsich E, Potena L, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Jr., Perch M, Sadavarte A, Toll A, Singh TP, Zuckermann A, Stehlik J. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-eighth adult heart transplantation report — 2021; Focus on recipient characteristics. *The Journal of Heart and Lung Transplantation* 2021; **40:**1035-1049.

35 Scandiatransplant Organ Exchange Organization (Aarhus University Hospital, Skejby, Denmark).2025

36 Rådegran G, Barbro K, Björn E, Flemming L, Bengt R, Berg BS, Carola G, Roger H, Monica K, Björn K, Magnus N, Liselotte P, Henrik R, Maria S, Bengt U, Kent W, Gerhard W, Maria W, Kjell J, and. Characteristics and survival of adult Swedish PAH and CTEPH patients 2000–2014. *Scandinavian Cardiovascular Journal* 2016; **50:**243-250.

37 Frost AE, Badesch DB, Barst RJ, Benza RL, Elliott CG, Farber HW, Krichman A, Liou TG, Raskob GE, Wason P, Feldkircher K, Turner M, McGoon MD. The Changing Picture of Patients With Pulmonary Arterial Hypertension in the United States: How REVEAL Differs From Historic and Non-US Contemporary Registries. *Chest* 2011; **139:**128-137.

38 Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G. Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med* 2006; **173:**1023-1030.

39 Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK, Levy PC, Reid LM, Vreim CE, Williams GW. Primary Pulmonary Hypertension. *Annals of Internal Medicine* 1987; **107:**216-223.

40 Hoeper MM, Huscher D, Pittrow D. Incidence and prevalence of pulmonary arterial hypertension in Germany. *International Journal of Cardiology* 2016; **203:**612-613.

41 The Swedish Pulmonary Arterial Hypertension Registry (SPAHR) annual report 2023. 2024

42 Gerhardt F, Fiessler E, Olsson KM, Kayser MZ, Kovacs G, Gall H, Ghofrani HA, Badr Eslam R, Lang IM, Benjamin N, Grünig E, Halank M, Lange TJ, Ulrich S, Leuchte H, Held M, Klose H, Ewert R, Wilkens H, Pizarro C, Skowasch D, Wissmüller M, Hellmich M, Olschewski H, Hoeper MM, Rosenkranz S. Positive Vasoreactivity Testing in Pulmonary Arterial Hypertension: Therapeutic

Consequences, Treatment Patterns, and Outcomes in the Modern Management Era. *Circulation* 2024; **149:**1549-1564.

43 Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA, 3rd, Loyd JE, Nichols WC, Trembath RC. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 2000; **26:**81-84.

44 Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, Hodge SE, Knowles JA. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000; **67:**737-744.

45 Soubrier F, Chung WK, Machado R, Grünig E, Aldred M, Geraci M, Loyd JE, Elliott CG, Trembath RC, Newman JH, Humbert M. Genetics and Genomics of Pulmonary Arterial Hypertension. *Journal of the American College of Cardiology* 2013; **62:**D13-D21.

46 Clinical Genome Resource.

47 Austin ED, Aldred MA, Alotaibi M, Gräf S, Nichols WC, Trembath RC, Chung WK. Genetics and precision genomics approaches to pulmonary hypertension. *European Respiratory Journal* 2024**:**2401370.

48 Humbert M, Sitbon O, Guignabert C, Savale L, Boucly A, Gallant-Dewavrin M, McLaughlin V, Hoeper MM, Weatherald J. Treatment of pulmonary arterial hypertension: recent progress and a look to the future. *Lancet Respir Med* 2023; **11:**804-819.

49 Huertas A, Tu L, Humbert M, Guignabert C. Chronic inflammation within the vascular wall in pulmonary arterial hypertension: more than a spectator. *Cardiovascular Research* 2019; **116:**885-893.

50 Hautefort A, Girerd B, Montani D, Cohen-Kaminsky S, Price L, Lambrecht BN, Humbert M, Perros F. T-Helper 17 Cell Polarization in Pulmonary Arterial Hypertension. *CHEST* 2015; **147:**1610-1620.

51 McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, Rubin LJ, Tapson VF, Varga J. 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. *Journal of the American College of Cardiology* 2009; **53:**1573-1619.

52 Jonigk D, Golpon H, Bockmeyer CL, Maegel L, Hoeper MM, Gottlieb J, Nickel N, Hussein K, Maus U, Lehmann U, Janciauskiene S, Welte T, Haverich A, Rische J, Kreipe H, Laenger F. Plexiform lesions in pulmonary arterial hypertension composition, architecture, and microenvironment. *Am J Pathol* 2011; **179:**167-179.

53 Cool CD, Stewart JS, Werahera P, Miller GJ, Williams RL, Voelkel NF, Tuder RM. Three-dimensional reconstruction of pulmonary arteries in plexiform pulmonary hypertension using cell-specific markers. Evidence for a dynamic and heterogeneous process of pulmonary endothelial cell growth. *Am J Pathol* 1999; **155:**411-419.

54 Westöö C, Norvik C, Peruzzi N, van der Have O, Lovric G, Jeremiasen I, Tran P-K, Mokso R, de Jesus Perez V, Brunnström H, Bech M, Galambos C, Tran-Lundmark

K. Distinct types of plexiform lesions identified by synchrotron-based phase-contrast micro-CT. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 2021; **321:**L17-L28.

55 Weatherald J, Humbert M. The 'great wait' for diagnosis in pulmonary arterial hypertension. *Respirology* 2020; **25:**790-792.

56 Khou V, Anderson JJ, Strange G, Corrigan C, Collins N, Celermajer DS, Dwyer N, Feenstra J, Horrigan M, Keating D, Kotlyar E, Lavender M, McWilliams TJ, Steele P, Weintraub R, Whitford H, Whyte K, Williams TJ, Wrobel JP, Keogh A, Lau EM. Diagnostic delay in pulmonary arterial hypertension: Insights from the Australian and New Zealand pulmonary hypertension registry. *Respirology* 2020; **25:**863-871.

57 Brown LM, Chen H, Halpern S, Taichman D, McGoon MD, Farber HW, Frost AE, Liou TG, Turner M, Feldkircher K, Miller DP, Elliott CG. Delay in recognition of pulmonary arterial hypertension: factors identified from the REVEAL Registry. *Chest* 2011; **140:**19-26.

58 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:**448-456.

59 Escribano-Subias P, Blanco I, López-Meseguer M, Lopez-Guarch CJ, Roman A, Morales P, Castillo-Palma MJ, Segovia J, Gómez-Sanchez MA, Barberà JA. Survival in pulmonary hypertension in Spain: insights from the Spanish registry. *European Respiratory Journal* 2012; **40:**596-603.

60 Jing Z-C, Xu X-Q, Han Z-Y, Wu Y, Deng K-W, Wang H, Wang Z-W, Cheng X-S, Xu B, Hu S-S, Hui R-T, Yang Y-J. Registry and Survival Study in Chinese Patients With Idiopathic and Familial Pulmonary Arterial Hypertension. *CHEST* 2007; **132:**373-379.

61 The Swedish Pulmonary Arterial Hypertension Registry (SPAHR) annual report 2020. 2021

62 Ivarsson B, Johansson A, Kjellström B. The Odyssey from Symptom to Diagnosis of Pulmonary Hypertension from the Patients and Spouses Perspective. *Journal of Primary Care & Community Health* 2021; **12:**21501327211029241.

63 Runheim H, Kjellström B, Beaudet A, Ivarsson B, Husberg M, Pillai N, Levin L-Å, Bernfort L. Societal costs associated with pulmonary arterial hypertension: A study utilizing linked national registries. *Pulmonary Circulation* 2023; **13:**e12190.

64 Ahmed S, Ahmed A, Rådegran G. Structured evaluation of unclear dyspnea–An attempt to shorten the diagnostic delay in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. *Pulmonary Circulation* 2024; **14:**e12340.

65 Ahmed S, Ahmed A, Rådegran G. Structured evaluation of unclear dyspnea - of great importance to early identify patients with PAH and CTEPH and improve prognosis. *Lakartidningen* 2022; **119**

66 D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Kernis JT, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med* 1991; **115:**343-349.

67 Stupi AM, Steen VD, Owens GR, Barnes EL, Rodnan GP, Medsger TA, Jr. Pulmonary hypertension in the CREST syndrome variant of systemic sclerosis. *Arthritis Rheum* 1986; **29:**515-524.

68 Farber HW, Miller DP, Poms AD, Badesch DB, Frost AE, Rouzic EM-L, Romero AJ, Benton WW, Elliott CG, McGoon MD, Benza RL. Five-Year Outcomes of Patients Enrolled in the REVEAL Registry. *Chest* 2015; **148:**1043-1054.

69 Hoeper MM, Pausch C, Grünig E, Staehler G, Huscher D, Pittrow D, Olsson KM, Vizza CD, Gall H, Distler O, Opitz C, Gibbs JSR, Delcroix M, Ghofrani HA, Rosenkranz S, Park D-H, Ewert R, Kaemmerer H, Lange TJ, Kabitz H-J, Skowasch D, Skride A, Claussen M, Behr J, Milger K, Halank M, Wilkens H, Seyfarth H-J, Held M, Dumitrescu D, Tsangaris I, Vonk-Noordegraaf A, Ulrich S, Klose H. Temporal trends in pulmonary arterial hypertension: results from the COMPERA registry. *European Respiratory Journal* 2022; **59:**2102024.

70 Chin KM, Gaine SP, Gerges C, Jing Z-C, Mathai SC, Tamura Y, McLaughlin VV, Sitbon O. Treatment algorithm for pulmonary arterial hypertension. *European Respiratory Journal* 2024**:**2401325.

71 Grünig E, MacKenzie A, Peacock AJ, Eichstaedt CA, Benjamin N, Nechwatal R, Ulrich S, Saxer S, Bussotti M, Sommaruga M, Ghio S, Gumbiene L, Palevičiūtė E, Jurevičienė E, Cittadini A, Stanziola AA, Marra AM, Kovacs G, Olschewski H, Barberà J-A, Blanco I, Spruit MA, Franssen FME, Vonk Noordegraaf A, Reis A, Santos M, Viamonte SG, Demeyer H, Delcroix M, Bossone E, Johnson M. Standardized exercise training is feasible, safe, and effective in pulmonary arterial and chronic thromboembolic pulmonary hypertension: results from a large European multicentre randomized controlled trial. *European Heart Journal* 2021; **42:**2284-2295.

72 Weitzenblum E, Sautegeau A, Ehrhart M, Mammosser M, Pelletier A. Long-term oxygen therapy can reverse the progression of pulmonary hypertension in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1985; **131:**493-498.

73 Sonnweber T, Nairz M, Theurl I, Petzer V, Tymoszuk P, Haschka D, Rieger E, Kaessmann B, Deri M, Watzinger K, Steringer-Mascherbauer R, Tancevski I, Weiss G, Löffler-Ragg J. The crucial impact of iron deficiency definition for the course of precapillary pulmonary hypertension. *PLoS One* 2018; **13:**e0203396.

74 Epoprostenol wins FDA approval for primary pulmonary hypertension. *American Journal of Health-System Pharmacy* 1995; **52:**2645-2651.

75 Barst RJ, Rubin LJ, Long WA, McGoon MD, Rich S, Badesch DB, Groves BM, Tapson VF, Bourge RC, Brundage BH, Koerner SK, Langleben D, Keller CA, Murali S, Uretsky BF, Clayton LM, Jöbsis MM, Blackburn SD, Shortino D, Crow JW. A Comparison of Continuous Intravenous Epoprostenol (Prostacyclin) with Conventional Therapy for Primary Pulmonary Hypertension. *New England Journal of Medicine* 1996; **334:**296-301.

76 Galiè N, Channick RN, Frantz RP, Grünig E, Jing ZC, Moiseeva O, Preston IR, Pulido T, Safdar Z, Tamura Y, McLaughlin VV. Risk stratification and medical therapy of pulmonary arterial hypertension. *European Respiratory Journal* 2019; **53:**1801889.

77 Humbert M. Viewpoint: activin signalling inhibitors for the treatment of pulmonary arterial hypertension. *European Respiratory Journal* 2023; **62:**2301726.

78 Galiè N, Corris PA, Frost A, Girgis RE, Granton J, Jing ZC, Klepetko W, McGoon MD, McLaughlin VV, Preston IR, Rubin LJ, Sandoval J, Seeger W, Keogh A. Updated Treatment Algorithm of Pulmonary Arterial Hypertension. *Journal of the American College of Cardiology* 2013; **62:**D60-D72.

79 Rubin LJ, Mendoza J, Hood M, McGoon M, Barst R, Williams WB, Diehl JH, Crow J, Long W. Treatment of primary pulmonary hypertension with continuous intravenous prostacyclin (epoprostenol). Results of a randomized trial. *Ann Intern Med* 1990; **112:**485-491.

80 Badesch DB, Tapson VF, McGoon MD, Brundage BH, Rubin LJ, Wigley FM, Rich S, Barst RJ, Barrett PS, Kral KM, Jöbsis MM, Loyd JE, Murali S, Frost A, Girgis R, Bourge RC, Ralph DD, Elliott CG, Hill NS, Langleben D, Schilz RJ, McLaughlin VV, Robbins IM, Groves BM, Shapiro S, Medsger TA, Jr. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. *Ann Intern Med* 2000; **132:**425-434.

81 Krowka MJ, Frantz RP, McGoon MD, Severson C, Plevak DJ, Wiesner RH. Improvement in pulmonary hemodynamics during intravenous epoprostenol (prostacyclin): A study of 15 patients with moderate to severe portopulmonary hypertension. *Hepatology* 1999; **30:**641-648.

82 Rosenzweig EB, Kerstein D, Barst RJ. Long-term prostacyclin for pulmonary hypertension with associated congenital heart defects. *Circulation* 1999; **99:**1858-1865.

83 Nunes H, Humbert M, Sitbon O, Morse JH, Deng Z, Knowles JA, Le Gall C, Parent F, Garcia G, Hervé P, Barst RJ, Simonneau G. Prognostic factors for survival in human immunodeficiency virus-associated pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2003; **167:**1433-1439.

84 Simonneau G, Barst RJ, Galie N, Naeije R, Rich S, Bourge RC, Keogh A, Oudiz R, Frost A, Blackburn SD, Crow JW, Rubin LJ. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: a double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 2002; **165:**800-804.

85 Hiremath J, Thanikachalam S, Parikh K, Shanmugasundaram S, Bangera S, Shapiro L, Pott GB, Vnencak-Jones CL, Arneson C, Wade M, White RJ. Exercise improvement and plasma biomarker changes with intravenous treprostinil therapy for pulmonary arterial hypertension: A placebo-controlled trial. *The Journal of Heart and Lung Transplantation* 2010; **29:**137-149.

86 McLaughlin VV, Benza RL, Rubin LJ, Channick RN, Voswinckel R, Tapson VF, Robbins IM, Olschewski H, Rubenfire M, Seeger W. Addition of inhaled treprostinil to oral therapy for pulmonary arterial hypertension: a randomized controlled clinical trial. *J Am Coll Cardiol* 2010; **55:**1915-1922.

87 Jing ZC, Parikh K, Pulido T, Jerjes-Sanchez C, White RJ, Allen R, Torbicki A, Xu KF, Yehle D, Laliberte K, Arneson C, Rubin LJ. Efficacy and safety of oral treprostinil monotherapy for the treatment of pulmonary arterial hypertension: a randomized, controlled trial. *Circulation* 2013; **127:**624-633.

88 Tapson VF, Torres F, Kermeen F, Keogh AM, Allen RP, Frantz RP, Badesch DB, Frost AE, Shapiro SM, Laliberte K, Sigman J, Arneson C, Galiè N. Oral Treprostinil for the Treatment of Pulmonary Arterial Hypertension in Patients on Background

Endothelin Receptor Antagonist and/or Phosphodiesterase Type 5 Inhibitor Therapy (The FREEDOM-C Study): A Randomized Controlled Trial. *CHEST* 2012; **142:**1383-1390.

89 Tapson VF, Jing Z-C, Xu K-F, Pan L, Feldman J, Kiely DG, Kotlyar E, McSwain CS, Laliberte K, Arneson C, Rubin LJ. Oral Treprostinil for the Treatment of Pulmonary Arterial Hypertension in Patients Receiving Background Endothelin Receptor Antagonist and Phosphodiesterase Type 5 Inhibitor Therapy (The FREEDOM-C2 Study): A Randomized Controlled Trial. *CHEST* 2013; **144:**952-958.

90 Simonneau G, Torbicki A, Hoeper MM, Delcroix M, Karlócai K, Galiè N, Degano B, Bonderman D, Kurzyna M, Efficace M, Giorgino R, Lang IM. Selexipag: an oral, selective prostacyclin receptor agonist for the treatment of pulmonary arterial hypertension. *Eur Respir J* 2012; **40:**874-880.

91 Sitbon O, Channick R, Chin KM, Frey A, Gaine S, Galiè N, Ghofrani HA, Hoeper MM, Lang IM, Preiss R, Rubin LJ, Di Scala L, Tapson V, Adzerikho I, Liu J, Moiseeva O, Zeng X, Simonneau G, McLaughlin VV. Selexipag for the Treatment of Pulmonary Arterial Hypertension. *N Engl J Med* 2015; **373:**2522-2533.

92 Galiè N, Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, Badesch DB, McGoon MD, McLaughlin VV, Roecker EB, Gerber MJ, Dufton C, Wiens BL, Rubin LJ. Ambrisentan for the Treatment of Pulmonary Arterial Hypertension. *Circulation* 2008; **117:**3010-3019.

93 Rubin LJ, Badesch DB, Barst RJ, Galiè N, Black CM, Keogh A, Pulido T, Frost A, Roux S, Leconte I, Landzberg M, Simonneau G. Bosentan Therapy for Pulmonary Arterial Hypertension. *New England Journal of Medicine* 2002; **346:**896-903.

94 Channick RN, Simonneau G, Sitbon O, Robbins IM, Frost A, Tapson VF, Badesch DB, Roux S, Rainisio M, Bodin F, Rubin LJ. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet* 2001; **358:**1119-1123.

95 Humbert M, Barst RJ, Robbins IM, Channick RN, Galiè N, Boonstra A, Rubin LJ, Horn EM, Manes A, Simonneau G. Combination of bosentan with epoprostenol in pulmonary arterial hypertension: BREATHE-2. *European Respiratory Journal* 2004; **24:**353-359.

96 Galiè N, Beghetti M, Gatzoulis MA, Granton J, Berger RMF, Lauer A, Chiossi E, Landzberg M. Bosentan Therapy in Patients With Eisenmenger Syndrome. *Circulation* 2006; **114:**48-54.

97 Sitbon O, Gressin V, Speich R, Macdonald PS, Opravil M, Cooper DA, Fourme T, Humbert M, Delfraissy JF, Simonneau G. Bosentan for the treatment of human immunodeficiency virus-associated pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2004; **170:**1212-1217.

98 Galiè N, Rubin LJ, Hoeper MM, Jansa P, Al-Hiti H, Meyer GMB, Chiossi E, Kusic-Pajic A, Simonneau G. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. *The Lancet* 2008; **371:**2093-2100.

99 Galié N, Badesch D, Oudiz R, Simonneau G, McGoon MD, Keogh AM, Frost AE, Zwicke D, Naeije R, Shapiro S, Olschewski H, Rubin LJ. Ambrisentan therapy for pulmonary arterial hypertension. *J Am Coll Cardiol* 2005; **46:**529-535.

100 McGoon MD, Frost AE, Oudiz RJ, Badesch DB, Galie N, Olschewski H, McLaughlin VV, Gerber MJ, Dufton C, Despain DJ, Rubin LJ. Ambrisentan Therapy in Patients With Pulmonary Arterial Hypertension Who Discontinued Bosentan or Sitaxsentan Due to Liver Function Test Abnormalities. *Chest* 2009; **135:**122-129.

101 Pulido T, Adzerikho I, Channick RN, Delcroix M, Galiè N, Ghofrani H-A, Jansa P, Jing Z-C, Brun F-OL, Mehta S, Mittelholzer CM, Perchenet L, Sastry BKS, Sitbon O, Souza R, Torbicki A, Zeng X, Rubin LJ, Simonneau G. Macitentan and Morbidity and Mortality in Pulmonary Arterial Hypertension. *New England Journal of Medicine* 2013; **369:**809-818.

102 Sitbon O, Morrell N. Pathways in pulmonary arterial hypertension: the future is here. *Eur Respir Rev* 2012; **21:**321-327.

103 Vachiery J-L, Huez S, Gillies H, Layton G, Hayashi N, Gao X, Naeije R. Safety, tolerability and pharmacokinetics of an intravenous bolus of sildenafil in patients with pulmonary arterial hypertension. *British Journal of Clinical Pharmacology* 2011; **71:**289-292.

104 The US Food and Drug Administration. Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations. 2009

105 Rubin LJ, Badesch DB, Fleming TR, Galiè N, Simonneau G, Ghofrani HA, Oakes M, Layton G, Serdarevic-Pehar M, McLaughlin VV, Barst RJ. Long-term Treatment With Sildenafil Citrate in Pulmonary Arterial Hypertension: The SUPER-2 Study. *Chest* 2011; **140:**1274-1283.

106 Sastry BKS, Narasimhan C, Reddy NK, Raju BS. Clinical efficacy of sildenafil in primary pulmonary hypertension: A randomized, placebo-controlled, double-blind, crossover study. *Journal of the American College of Cardiology* 2004; **43:**1149-1153.

107 Galiè N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, Fleming T, Parpia T, Burgess G, Branzi A, Grimminger F, Kurzyna M, Simonneau G. Sildenafil Citrate Therapy for Pulmonary Arterial Hypertension. *New England Journal of Medicine* 2005; **353:**2148-2157.

108 Singh TP, Rohit M, Grover A, Malhotra S, Vijayvergiya R. A randomized, placebo-controlled, double-blind, crossover study to evaluate the efficacy of oral sildenafil therapy in severe pulmonary artery hypertension. *American Heart Journal* 2006; **151:**851.e851-851.e855.

109 Iversen K, Jensen AS, Jensen TV, Vejlstrup NG, Søndergaard L. Combination therapy with bosentan and sildenafil in Eisenmenger syndrome: a randomized, placebo-controlled, double-blinded trial†. *European Heart Journal* 2010; **31:**1124-1131.

110 Galiè N, Brundage BH, Ghofrani HA, Oudiz RJ, Simonneau G, Safdar Z, Shapiro S, White RJ, Chan M, Beardsworth A, Frumkin L, Barst RJ. Tadalafil Therapy for Pulmonary Arterial Hypertension. *Circulation* 2009; **119:**2894-2903.

111 Ghofrani HA, Galiè N, Grimminger F, Grünig E, Humbert M, Jing ZC, Keogh AM, Langleben D, Kilama MO, Fritsch A, Neuser D, Rubin LJ. Riociguat for the treatment of pulmonary arterial hypertension. *N Engl J Med* 2013; **369:**330-340.

112 Hoeper Marius M, Badesch David B, Ghofrani HA, Gibbs JSR, Gomberg-Maitland M, McLaughlin Vallerie V, Preston Ioana R, Souza R, Waxman Aaron B, Grünig E, Kopeć G, Meyer G, Olsson Karen M, Rosenkranz S, Xu Y, Miller B, Fowler M, Butler J, Koglin J, de Oliveira Pena J, Humbert M. Phase 3 Trial of Sotatercept for Treatment

of Pulmonary Arterial Hypertension. *New England Journal of Medicine* 2023; **388:**1478-1490.

113 Guignabert C, Humbert M. Targeting transforming growth factor-β receptors in pulmonary hypertension. *European Respiratory Journal* 2021; **57:**2002341.

114 Villanueva J, Wade J, Torres A, Hale G, Pham H. Sotatercept: The First FDA-Approved Activin A Receptor IIA Inhibitor Used in the Management of Pulmonary Arterial Hypertension. *American Journal of Cardiovascular Drugs* 2025; **25:**17-24.

115 Humbert M, McLaughlin V, Gibbs JSR, Gomberg-Maitland M, Hoeper Marius M, Preston Ioana R, Souza R, Waxman A, Escribano Subias P, Feldman J, Meyer G, Montani D, Olsson Karen M, Manimaran S, Barnes J, Linde Peter G, de Oliveira Pena J, Badesch David B. Sotatercept for the Treatment of Pulmonary Arterial Hypertension. *New England Journal of Medicine* 2021; **384:**1204-1215.

116 Humbert M, McLaughlin VV, Badesch DB, Ghofrani HA, Gibbs JSR, Gomberg-Maitland M, Preston IR, Souza R, Waxman AB, Moles VM, Savale L, Vizza CD, Rosenkranz S, Shi Y, Miller B, Mackenzie HS, Kim SS, Loureiro MJ, Patel MJ, Koglin J, Cornell AG, Hoeper MM. Sotatercept in Patients with Pulmonary Arterial Hypertension at High Risk for Death. *New England Journal of Medicine*; **0**

117 Galiè N, Barberà JA, Frost AE, Ghofrani HA, Hoeper MM, McLaughlin VV, Peacock AJ, Simonneau G, Vachiery JL, Grünig E, Oudiz RJ, Vonk-Noordegraaf A, White RJ, Blair C, Gillies H, Miller KL, Harris JH, Langley J, Rubin LJ. Initial Use of Ambrisentan plus Tadalafil in Pulmonary Arterial Hypertension. *N Engl J Med* 2015; **373:**834-844.

118 Chin KM, Sitbon O, Doelberg M, Feldman J, Gibbs JSR, Grünig E, Hoeper MM, Martin N, Mathai SC, McLaughlin VV, Perchenet L, Poch D, Saggar R, Simonneau G, Galiè N. Three- Versus Two-Drug Therapy for Patients With Newly Diagnosed Pulmonary Arterial Hypertension. *Journal of the American College of Cardiology* 2021; **78:**1393-1403.

119 Boucly A, Savale L, Jaïs X, Bauer F, Bergot E, Bertoletti L, Beurnier A, Bourdin A, Bouvaist H, Bulifon S, Chabanne C, Chaouat A, Cottin V, Dauphin C, Degano B, De Groote P, Favrolt N, Feng Y, Horeau-Langlard D, Jevnikar M, Jutant EM, Liang Z, Magro P, Mauran P, Moceri P, Mornex JF, Palat S, Parent F, Picard F, Pichon J, Poubeau P, Prévot G, Renard S, Reynaud-Gaubert M, Riou M, Roblot P, Sanchez O, Seferian A, Tromeur C, Weatherald J, Simonneau G, Montani D, Humbert M, Sitbon O. Association between Initial Treatment Strategy and Long-Term Survival in Pulmonary Arterial Hypertension. *Am J Respir Crit Care Med* 2021; **204:**842-854.

120 Mocumbi A, Humbert M, Saxena A, Jing Z-C, Sliwa K, Thienemann F, Archer SL, Stewart S. Pulmonary hypertension. *Nature Reviews Disease Primers* 2024; **10:**1.

121 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:**135-141.

122 Rich S, Kaufmann E, Levy PS. The Effect of High Doses of Calcium-Channel Blockers on Survival in Primary Pulmonary Hypertension. *New England Journal of Medicine* 1992; **327:**76-81.

123 Sitbon O, Humbert M, Jaïs X, Ioos V, Hamid AM, Provencher S, Garcia G, Parent F, Hervé P, Simonneau G. Long-Term Response to Calcium Channel Blockers in Idiopathic Pulmonary Arterial Hypertension. *Circulation* 2005; **111:**3105-3111.

124 Barst Robyn J, McGoon M, Torbicki A, Sitbon O, Krowka Michael J, Olschewski H, Gaine S. Diagnosis and differential assessment of pulmonary arterial hypertension. *JACC* 2004; **43:**S40-S47.

125 Galiè N, Torbicki A, Barst R, Dartevelle P, Haworth S, Higenbottam T, Olschewski H, Peacock A, Pietra G, Rubin LJ, Simonneau G, members TF. Guidelines on diagnosis and treatment of pulmonary arterial hypertension: The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. *European Heart Journal* 2004; **25:**2243-2278.

126 Galiè N, Hoeper MM, Humbert M, Torbicki A, Vachiery J-L, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G, Vahanian A, Auricchio A, Bax J, Ceconi C, Dean V, Filippatos G, Funck-Brentano C, Hobbs R, Kearney P, McDonagh T, McGregor K, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Vardas P, Widimsky P, Sechtem U, Al Attar N, Andreotti F, Aschermann M, Asteggiano R, Benza R, Berger R, Bonnet D, Delcroix M, Howard L, Kitsiou AN, Lang I, Maggioni A, Nielsen-Kudsk JE, Park M, Perrone-Filardi P, Price S, Domenech MTS, Vonk-Noordegraaf A, Zamorano JL, Authors/Task Force Members ECfPG, Document Reviewers. 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). *European Heart Journal* 2009; **30:**2493-2537.

127 Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *European Respiratory Journal*; **46:**903-975.

128 Kylhammar D, Kjellström B, Hjalmarsson C, Jansson K, Nisell M, Söderberg S, Wikström G, Rådegran G. A comprehensive risk stratification at early follow-up determines prognosis in pulmonary arterial hypertension. *Eur Heart J* 2018; **39:**4175-4181.

129 Hoeper MM, Kramer T, Pan Z, Eichstaedt CA, Spiesshoefer J, Benjamin N, Olsson KM, Meyer K, Vizza CD, Vonk-Noordegraaf A, Distler O, Opitz C, Gibbs JSR, Delcroix M, Ghofrani HA, Huscher D, Pittrow D, Rosenkranz S, Grünig E. Mortality in pulmonary arterial hypertension: prediction by the 2015 European pulmonary hypertension guidelines risk stratification model. *Eur Respir J* 2017; **50**

130 Boucly A, Weatherald J, Savale L, Jaïs X, Cottin V, Prevot G, Picard F, de Groote P, Jevnikar M, Bergot E, Chaouat A, Chabanne C, Bourdin A, Parent F, Montani D, Simonneau G, Humbert M, Sitbon O. Risk assessment, prognosis and guideline implementation in pulmonary arterial hypertension. *Eur Respir J* 2017; **50**

131 Hoeper MM, Pausch C, Olsson KM, Huscher D, Pittrow D, Grünig E, Staehler G, Vizza CD, Gall H, Distler O, Opitz C, Gibbs JSR, Delcroix M, Ghofrani HA, Park

DH, Ewert R, Kaemmerer H, Kabitz HJ, Skowasch D, Behr J, Milger K, Halank M, Wilkens H, Seyfarth HJ, Held M, Dumitrescu D, Tsangaris I, Vonk-Noordegraaf A, Ulrich S, Klose H, Claussen M, Lange TJ, Rosenkranz S. COMPERA 2.0: a refined four-stratum risk assessment model for pulmonary arterial hypertension. *Eur Respir J* 2022; **60**

132 Boucly A, Weatherald J, Savale L, de Groote P, Cottin V, Prévot G, Chaouat A, Picard F, Horeau-Langlard D, Bourdin A, Jutant E-M, Beurnier A, Jevnikar M, Jaïs X, Simonneau G, Montani D, Sitbon O, Humbert M. External validation of a refined four-stratum risk assessment score from the French pulmonary hypertension registry. *European Respiratory Journal* 2022; **59:**2102419.

133 Benza RL, Gomberg-Maitland M, Elliott CG, Farber HW, Foreman AJ, Frost AE, McGoon MD, Pasta DJ, Selej M, Burger CD, Frantz RP. Predicting Survival in Patients With Pulmonary Arterial Hypertension: The REVEAL Risk Score Calculator 2.0 and Comparison With ESC/ERS-Based Risk Assessment Strategies. *Chest* 2019; **156:**323-337.

134 Dardi F, Boucly A, Benza R, Frantz R, Mercurio V, Olschewski H, Rådegran G, Rubin LJ, Hoeper MM. Risk stratification and treatment goals in pulmonary arterial hypertension. *European Respiratory Journal* 2024**:**2401323.

135 Barst RJ, Galie N, Naeije R, Simonneau G, Jeffs R, Arneson C, Rubin LJ. Long-term outcome in pulmonary arterial hypertension patients treated with subcutaneous treprostinil. *Eur Respir J* 2006; **28:**1195-1203.

136 Kuhn KP, Byrne DW, Arbogast PG, Doyle TP, Loyd JE, Robbins IM. Outcome in 91 consecutive patients with pulmonary arterial hypertension receiving epoprostenol. *Am J Respir Crit Care Med* 2003; **167:**580-586.

137 McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension: the impact of epoprostenol therapy. *Circulation* 2002; **106:**1477-1482.

138 Provencher S, Sitbon O, Humbert M, Cabrol Sgn, Jaïs X, Simonneau Gr. Long-term outcome with first-line bosentan therapy in idiopathic pulmonary arterial hypertension. *European Heart Journal* 2006; **27:**589-595.

139 Thenappan T, Shah SJ, Rich S, Tian L, Archer SL, Gomberg-Maitland M. Survival in pulmonary arterial hypertension: a reappraisal of the NIH risk stratification equation. *Eur Respir J* 2010; **35:**1079-1087.

140 Humbert M, Sitbon O, Yaïci A, Montani D, O'Callaghan DS, Jaïs X, Parent F, Savale L, Natali D, Günther S, Chaouat A, Chabot F, Cordier J-F, Habib G, Gressin V, Jing Z-C, Souza R, Simonneau G. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. *European Respiratory Journal* 2010; **36:**549-555.

141 Lee WT, Ling Y, Sheares KK, Pepke-Zaba J, Peacock AJ, Johnson MK. Predicting survival in pulmonary arterial hypertension in the UK. *Eur Respir J* 2012; **40:**604-611.

142 Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS, Frost A, Barst RJ, Badesch DB, Elliott CG, Liou TG, McGoon MD. Predicting Survival in Pulmonary Arterial Hypertension. *Circulation* 2010; **122:**164-172.

143 McGoon MD, Krichman A, Farber HW, Barst RJ, Raskob GE, Liou TG, Miller DP, Feldkircher K, Giles S. Design of the REVEAL registry for US patients with pulmonary arterial hypertension. *Mayo Clin Proc* 2008; **83:**923-931.

144 Benza RL, Gomberg-Maitland M, Miller DP, Frost A, Frantz RP, Foreman AJ, Badesch DB, McGoon MD. The REVEAL Registry risk score calculator in patients newly diagnosed with pulmonary arterial hypertension. *Chest* 2012; **141:**354-362.

145 Benza RL, Miller DP, Foreman AJ, Frost AE, Badesch DB, Benton WW, McGoon MD. Prognostic implications of serial risk score assessments in patients with pulmonary arterial hypertension: a Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL) analysis. *J Heart Lung Transplant* 2015; **34:**356-361.

146 Anderson JJ, Lau EM, Lavender M, Benza R, Celermajer DS, Collins N, Corrigan C, Dwyer N, Feenstra J, Horrigan M, Keating D, Kermeen F, Kotlyar E, McWilliams T, Rhodes B, Steele P, Thakkar V, Williams T, Whitford H, Whyte K, Weintraub R, Wrobel JP, Keogh A, Strange G. Retrospective Validation of the REVEAL 2.0 Risk Score With the Australian and New Zealand Pulmonary Hypertension Registry Cohort. *CHEST* 2020; **157:**162-172.

147 Sitbon O, Chin KM, Channick RN, Benza RL, Di Scala L, Gaine S, Ghofrani H-A, Lang IM, McLaughlin VV, Preiss R, Rubin LJ, Simonneau G, Tapson VF, Galiè N, Hoeper MM. Risk assessment in pulmonary arterial hypertension: Insights from the GRIPHON study. *The Journal of Heart and Lung Transplantation* 2020; **39:**300-309.

148 Benza RL, Farber HW, Frost AE, Ghofrani H-A, Corris PA, Lambelet M, Nikkho S, Meier C, Hoeper MM. Application of the REVEAL risk score calculator 2.0 in the PATENT study. *International Journal of Cardiology* 2021; **332:**189-192.

149 Benza RL, Gomberg-Maitland M, Farber HW, Vizza CD, Broderick M, Holdstock L, Nelsen AC, Deng C, Rao Y, White RJ. Contemporary risk scores predict clinical worsening in pulmonary arterial hypertension - An analysis of FREEDOM-EV. *The Journal of Heart and Lung Transplantation* 2022; **41:**1572-1580.

150 Benza RL, Kanwar MK, Raina A, Scott JV, Zhao CL, Selej M, Elliott CG, Farber HW. Development and Validation of an Abridged Version of the REVEAL 2.0 Risk Score Calculator, REVEAL Lite 2, for Use in Patients With Pulmonary Arterial Hypertension. *Chest* 2021; **159:**337-346.

151 El-Kersh K, Zhao C, Elliott G, Farber HW, Gomberg-Maitland M, Selej M, Garcia-Ferrer J, Benza R. Derivation of a Risk Score (REVEAL-ECHO) Based on Echocardiographic Parameters of Patients With Pulmonary Arterial Hypertension. *Chest* 2023; **163:**1232-1244.

152 Kylhammar D, Hjalmarsson C, Hesselstrand R, Jansson K, Kavianipour M, Kjellström B, Nisell M, Söderberg S, Rådegran G. Predicting mortality during long-term follow-up in pulmonary arterial hypertension. *ERJ Open Research* 2021; **7:**00837-02020.

153 Ahmed A, Ahmed S, Kempe D, Rådegran G. Evaluation of the European Society of Cardiology/European Respiratory Society derived three- and four-strata risk stratification models in pulmonary arterial hypertension: introducing an internet-based risk stratification calculator. *European Heart Journal Open* 2023; **3:**1-14.

154 Quan R, Zhang G, Yu Z, Zhang C, Yang Z, Tian H, Yang Y, Wu W, Chen Y, Liu Y, Zhu X, Li S, Shen J, Zheng Z, Zhu X, Wang G, Wang Q, Zhou D, Ji Y, Yang T, Li W, Chen X, Qian Y, Lin Y, Gu Q, Xiong C, Shan G, He J. Characteristics, goal-oriented treatments and survival of pulmonary arterial hypertension in China: Insights from a national multicentre prospective registry. *Respirology* 2022; **27:**517-528.

155 Zelt JGE, Hossain A, Sun LY, Mehta S, Chandy G, Davies RA, Contreras-Dominguez V, Dunne R, Doyle-Cox C, Wells G, Stewart DJ, Mielniczuk LM. Incorporation of renal function in mortality risk assessment for pulmonary arterial hypertension. *J Heart Lung Transplant* 2020; **39:**675-685.

156 Mercurio V, Diab N, Peloquin G, Housten-Harris T, Damico R, Kolb TM, Mathai SC, Hassoun PM. Risk assessment in scleroderma patients with newly diagnosed pulmonary arterial hypertension: application of the ESC/ERS risk prediction model. *European Respiratory Journal* 2018; **52:**1800497.

157 Gong S-G, Wu W-H, Li C, Zhao Q-H, Jiang R, Luo C-J, Qiu H-L, Liu J-M, Wang L, Zhang R. Validity of the ESC Risk Assessment in Idiopathic Pulmonary Arterial Hypertension in China. *Frontiers in Cardiovascular Medicine* 2021; **Volume 8 - 2021**

158 Olsson KM, Richter MJ, Kamp JC, Gall H, Heine A, Ghofrani H-A, Fuge J, Ewert R, Hoeper MM. Intravenous treprostinil as an add-on therapy in patients with pulmonary arterial hypertension. *The Journal of Heart and Lung Transplantation* 2019; **38:**748-756.

159 Kuwana M, Blair C, Takahashi T, Langley J, Coghlan JG. Initial combination therapy of ambrisentan and tadalafil in connective tissue disease-associated pulmonary arterial hypertension (CTD-PAH) in the modified intention-to-treat population of the AMBITION study: post hoc analysis. *Annals of the Rheumatic Diseases* 2020; **79:**626-634.

160 Humbert M, Farber HW, Ghofrani H-A, Benza RL, Busse D, Meier C, Hoeper MM. Risk assessment in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. *European Respiratory Journal* 2019; **53:**1802004.

161 Ahmed A, Ahmed S, Rådegran G. Risk assessment in pulmonary arterial hypertension: A step towards clinical implementation based on the 2022 ESC/ERS pulmonary hypertension guidelines. *Pulmonary Circulation* 2023; **13:**e12253.

162 Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clinical Pharmacology & Therapeutics* 2001; **69:**89-95.

163 *Silver Spring (FDA), Bethesda (NIH). FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools)*. 2016.

164 Strimbu K, Tavel JA. What are biomarkers? *Curr Opin HIV AIDS* 2010; **5:**463-466.

165 Braunwald E. Heart Failure. *JACC: Heart Failure* 2013; **1:**1-20.

166 Braunwald E. Biomarkers in Heart Failure. *New England Journal of Medicine* 2008; **358:**2148-2159.

167 Ibrahim NE, Januzzi JL. Established and Emerging Roles of Biomarkers in Heart Failure. *Circulation Research* 2018; **123:**614-629.

168 Simon B, Rafael Sobrano F, Sue G, Andrea F, Tim L. Pathophysiology and new advances in pulmonary hypertension. *BMJ Medicine* 2023; **2:**e000137.

169 Hemnes AR, Celermajer DS, D'Alto M, Haddad F, Hassoun PM, Prins KW, Naeije R, Vonk Noordegraaf A. Pathophysiology of the right ventricle and its pulmonary vascular interaction. *European Respiratory Journal* 2024**;**2401321.

170 Rhodes CJ, Wharton J, Ghataorhe P, Watson G, Girerd B, Howard LS, Gibbs JSR, Condliffe R, Elliot CA, Kiely DG, Simonneau G, Montani D, Sitbon O, Gall H, Schermuly RT, Ghofrani HA, Lawrie A, Humbert M, Wilkins MR. Plasma proteome analysis in patients with pulmonary arterial hypertension: an observational cohort study. *The Lancet Respiratory Medicine* 2017; **5:**717-726.

171 Rhodes CJ, Wharton J, Swietlik EM, Harbaum L, Girerd B, Coghlan JG, Lordan J, Church C, Pepke-Zaba J, Toshner M, Wort SJ, Kiely DG, Condliffe R, Lawrie A, Gräf S, Montani D, Boucly A, Sitbon O, Humbert M, Howard LS, Morrell NW, Wilkins MR. Using the Plasma Proteome for Risk Stratifying Patients with Pulmonary Arterial Hypertension. *Am J Respir Crit Care Med* 2022; **205:**1102-1111.

172 Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D, Müller-Ladner U, Pope JE, Vonk MC, Doelberg M, Chadha-Boreham H, Heinzl H, Rosenberg DM, McLaughlin VV, Seibold JR, group obotDs. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Annals of the Rheumatic Diseases* 2014; **73:**1340-1349.

173 Ahmed S, Ahmed A, Rådegran G. Circulating biomarkers in pulmonary arterial hypertension: State-of-the-art review and future directions. *JHLT Open* 2024; **6:**1-16 100152.

174 Hemnes A, Rothman AMK, Swift AJ, Zisman LS. Role of biomarkers in evaluation, treatment and clinical studies of pulmonary arterial hypertension. *Pulmonary Circulation* 2020; **10:**2045894020957234.

175 Thenappan T, Ormiston ML, Ryan JJ, Archer SL. Pulmonary arterial hypertension: pathogenesis and clinical management. *Bmj* 2018; **360:**j5492.

176 Califf RM. Biomarker definitions and their applications. *Experimental Biology and Medicine* 2018; **243:**213-221.

177 Du Bois D, Du Bois EF. Clinical Calorimetry: Tenth Paper A Formula To Estimate The Approximate Surface Area If Height And Weight Be Known. *Archives of Internal Medicine* 1916; **XVII:**863-871.

178 Nyman U, Grubb A, Larsson A, Hansson LO, Flodin M, Nordin G, Lindström V, Björk J. The revised Lund-Malmö GFR estimating equation outperforms MDRD and CKD-EPI across GFR, age and BMI intervals in a large Swedish population. *Clin Chem Lab Med* 2014; **52:**815-824.

179 Ahmed A, Ahmed S, Arvidsson M, Bouzina H, Lundgren J, Rådegran G. Prolargin and matrix metalloproteinase-2 in heart failure after heart transplantation and their association with haemodynamics. *ESC Heart Failure* 2020; **7:**224-235.

180 Ahmed A, Ahmed S, Arvidsson M, Bouzina H, Lundgren J, Rådegran G. Elevated plasma sRAGE and IGFBP7 in heart failure decrease after heart transplantation in association with haemodynamics. *ESC Heart Failure* 2020; **7:**2340-2353.

181 Ahmed A, Kania K, Abdul Rahim H, Ahmed S, Rådegran G. Adrenomedullin peptides and precursor levels in relation to haemodynamics and prognosis after heart transplantation. *ESC Heart Failure* 2023; **10:**2427-2437.

182 Ahmed A, Ahmed S, Rådegran G. Plasma ADAMTS13 and von Willebrand factor in diagnosis and prediction of prognosis in pulmonary arterial hypertension. *Pulmonary Circulation* 2021; **11:**1-15 20458940211041500.

183 Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, Andersson AC, Lindstedt P, Stenvang J, Gullberg M, Fredriksson S. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* 2014; **9:**e95192.

184 Benjamini Y, Krieger AM, Yekutieli D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* 2006; **93:**491-507.
185 Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996; **15:**361-387.
186 Steyerberg EW, Harrell FE, Borsboom GJJM, Eijkemans MJC, Vergouwe Y, Habbema JDF. Internal validation of predictive models: Efficiency of some procedures for logistic regression analysis. *Journal of Clinical Epidemiology* 2001; **54:**774-781.
187 Uno H, Tianxi C, Lu T, and Wei LJ. Evaluating Prediction Rules for t-Year Survivors With Censored Regression Models. *Journal of the American Statistical Association* 2007; **102:**527-537.
188 Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. *Stat Med* 2011; **30:**1105-1117.
189 Hultgårdh-Nilsson A, Borén J, Chakravarti S. The small leucine-rich repeat proteoglycans in tissue repair and atherosclerosis. *J Intern Med* 2015; **278:**447-461.
190 Thul PJ, Åkesson L, Wiking M, Mahdessian D, Geladaki A, Ait Blal H, Alm T, Asplund A, Björk L, Breckels LM, Bäckström A, Danielsson F, Fagerberg L, Fall J, Gatto L, Gnann C, Hober S, Hjelmare M, Johansson F, Lee S, Lindskog C, Mulder J, Mulvey CM, Nilsson P, Oksvold P, Rockberg J, Schutten R, Schwenk JM, Sivertsson Å, Sjöstedt E, Skogs M, Stadler C, Sullivan DP, Tegel H, Winsnes C, Zhang C, Zwahlen M, Mardinoglu A, Pontén F, von Feilitzen K, Lilley KS, Uhlén M, Lundberg E. A subcellular map of the human proteome. *Science* 2017; **356**
191 Happonen KE, Fürst CM, Saxne T, Heinegård D, Blom AM. PRELP protein inhibits the formation of the complement membrane attack complex. *J Biol Chem* 2012; **287:**8092-8100.
192 Aukrust P, Gullestad L, Lappegård KT, Ueland T, Aass H, Wikeby L, Simonsen S, Frøland SS, Mollnes TE. Complement Activation in Patients With Congestive Heart Failure. *Circulation* 2001; **104:**1494-1500.
193 Testa L, Van Gaal WJ, Bhindi R, Biondi-Zoccai GGL, Abbate A, Agostoni P, Porto I, Andreotti F, Crea F, Banning AP. Pexelizumab in ischemic heart disease: A systematic review and meta-analysis on 15,196 patients. *The Journal of Thoracic and Cardiovascular Surgery* 2008; **136:**884-893.
194 Barhoumi T, Fraulob-Aquino JC, Mian MOR, Ouerd S, Idris-Khodja N, Huo K-G, Rehman A, Caillon A, Dancose-Giambattisto B, Ebrahimian T, Lehoux S, Paradis P, Schiffrin EL. Matrix metalloproteinase-2 knockout prevents angiotensin II-induced vascular injury. *Cardiovascular Research* 2017; **113:**1753-1762.
195 Hayashidani S, Tsutsui H, Ikeuchi M, Shiomi T, Matsusaka H, Kubota T, Imanaka-Yoshida K, Itoh T, Takeshita A. Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction. *Am J Physiol Heart Circ Physiol* 2003; **285:**H1229-1235.
196 Matsusaka H, Ide T, Matsushima S, Ikeuchi M, Kubota T, Sunagawa K, Kinugawa S, Tsutsui H. Targeted Deletion of Matrix Metalloproteinase 2 Ameliorates Myocardial Remodeling in Mice With Chronic Pressure Overload. *Hypertension* 2006; **47:**711-717.

197 Polyakova V, Hein S, Kostin S, Ziegelhoeffer T, Schaper J. Matrix metalloproteinases and their tissue inhibitors in pressure-overloaded human myocardium during heart failure progression. *J Am Coll Cardiol* 2004; **44:**1609-1618.

198 Yamazaki T, Lee JD, Shimizu H, Uzui H, Ueda T. Circulating matrix metalloproteinase-2 is elevated in patients with congestive heart failure. *Eur J Heart Fail* 2004; **6:**41-45.

199 Fernandez-Patron C, Radomski MW, Davidge ST. Vascular Matrix Metalloproteinase-2 Cleaves Big Endothelin-1 Yielding a Novel Vasoconstrictor. *Circulation Research* 1999; **85:**906-911.

200 Neubauer S. The Failing Heart — An Engine Out of Fuel. *New England Journal of Medicine* 2007; **356:**1140-1151.

201 Basta G. Receptor for advanced glycation endproducts and atherosclerosis: From basic mechanisms to clinical implications. *Atherosclerosis* 2008; **196:**9-21.

202 Creagh-Brown BC, Quinlan GJ, Hector LR, Evans TW, Burke-Gaffney A. Association between Preoperative Plasma sRAGE Levels and Recovery from Cardiac Surgery. *Mediators of Inflammation* 2013; **2013:**496031.

203 Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced Glycation End Products. *Circulation* 2006; **114:**597-605.

204 Petrova R, Yamamoto Y, Muraki K, Yonekura H, Sakurai S, Watanabe T, Li H, Takeuchi M, Makita Z, Kato I, Takasawa S, Okamoto H, Imaizumi Y, Yamamoto H. Advanced Glycation Endproduct-Induced Calcium Handling Impairment in Mouse Cardiac Myocytes. *Journal of Molecular and Cellular Cardiology* 2002; **34:**1425-1431.

205 Chung AC, Zhang H, Kong YZ, Tan JJ, Huang XR, Kopp JB, Lan HY. Advanced glycation end-products induce tubular CTGF via TGF-beta-independent Smad3 signaling. *J Am Soc Nephrol* 2010; **21:**249-260.

206 Liu Y, Yu M, Zhang Z, Yu Y, Chen Q, Zhang W, Zhao X. Blockade of receptor for advanced glycation end products protects against systolic overload-induced heart failure after transverse aortic constriction in mice. *European Journal of Pharmacology* 2016; **791:**535-543.

207 Ren X, Ren L, Wei Q, Shao H, Chen L, Liu N. Advanced glycation end-products decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. *Cardiovasc Diabetol* 2017; **16:**52.

208 Adamopoulos C, Piperi C, Gargalionis AN, Dalagiorgou G, Spilioti E, Korkolopoulou P, Diamanti-Kandarakis E, Papavassiliou AG. Advanced glycation end products upregulate lysyl oxidase and endothelin-1 in human aortic endothelial cells via parallel activation of ERK1/2-NF-κB and JNK-AP-1 signaling pathways. *Cell Mol Life Sci* 2016; **73:**1685-1698.

209 Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr Rev* 1999; **20:**761-787.

210 Chugh S, Ouzounian M, Lu Z, Mohamed S, Li W, Bousette N, Liu PP, Gramolini AO. Pilot study identifying myosin heavy chain 7, desmin, insulin-like growth factor 7, and annexin A2 as circulating biomarkers of human heart failure. *Proteomics* 2013; **13:**2324-2334.

211 Hooper AT, Shmelkov SV, Gupta S, Milde T, Bambino K, Gillen K, Goetz M, Chavala S, Baljevic M, Murphy AJ, Valenzuela DM, Gale NW, Thurston G,

Yancopoulos GD, Vahdat L, Evans T, Rafii S. Angiomodulin is a specific marker of vasculature and regulates vascular endothelial growth factor-A-dependent neoangiogenesis. *Circ Res* 2009; **105:**201-208.

212 Gandhi PU, Gaggin HK, Redfield MM, Chen HH, Stevens SR, Anstrom KJ, Semigran MJ, Liu P, Januzzi JL, Jr. Insulin-Like Growth Factor-Binding Protein-7 as a Biomarker of Diastolic Dysfunction and Functional Capacity in Heart Failure With Preserved Ejection Fraction: Results From the RELAX Trial. *JACC Heart Fail* 2016; **4:**860-869.

213 Januzzi JL, Packer M, Claggett B, Liu J, Shah AM, Zile MR, Pieske B, Voors A, Gandhi PU, Prescott MF, Shi V, Lefkowitz MP, McMurray JJV, Solomon SD. IGFBP7 (Insulin-Like Growth Factor–Binding Protein-7) and Neprilysin Inhibition in Patients With Heart Failure. *Circulation: Heart Failure* 2018; **11:**e005133.

214 Zhang L, Smyth D, Al-Khalaf M, Blet A, Du Q, Bernick J, Gong M, Chi X, Oh Y, Roba-Oshin M, Coletta E, Feletou M, Gramolini AO, Kim K-H, Coutinho T, Januzzi JL, Tyl B, Ziegler A, Liu PP. Insulin-like growth factor-binding protein-7 (IGFBP7) links senescence to heart failure. *Nature Cardiovascular Research* 2022; **1:**1195-1214.

215 Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, Oya H, Kyotani S, Nakanishi N, Goto Y, Masuda Y, Miyatake K, Kangawa K. Hemodynamic, renal, and hormonal effects of adrenomedullin infusion in patients with congestive heart failure. *Circulation* 2000; **101:**498-503.

216 Jougasaki M, Wei CM, McKinley LJ, Burnett JC, Jr. Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. *Circulation* 1995; **92:**286-289.

217 Egerstedt A, Czuba T, Bronton K, Lejonberg C, Ruge T, Wessman T, Rådegran G, Schulte J, Hartmann O, Melander O, Smith JG. Bioactive adrenomedullin for assessment of venous congestion in heart failure. *ESC Heart Fail* 2022; **9:**3543-3555.

218 Zander CB, Cao W, Zheng XL. ADAMTS13 and von Willebrand factor interactions. *Curr Opin Hematol* 2015; **22:**452-459.

219 Akyol O, Akyol S, Chen C-H. Update on ADAMTS13 and VWF in cardiovascular and hematological disorders. *Clinica Chimica Acta* 2016; **463:**109-118.

220 Turner N, Nolasco L, Tao Z, Dong JF, Moake J. Human endothelial cells synthesize and release ADAMTS-13. *J Thromb Haemost* 2006; **4:**1396-1404.

221 Newnham M, South K, Bleda M, Auger WR, Barberà JA, Bogaard H, Bunclark K, Cannon JE, Delcroix M, Hadinnapola C, Howard LS, Jenkins D, Mayer E, Ng C, Rhodes CJ, Screaton N, Sheares K, Simpson MA, Southwood M, Su L, Taboada D, Traylor M, Trembath RC, Villar SS, Wilkins MR, Wharton J, Gräf S, Pepke-Zaba J, Laffan M, Lane DA, Morrell NW, Toshner M. The ADAMTS13-VWF axis is dysregulated in chronic thromboembolic pulmonary hypertension. *Eur Respir J* 2019; **53**

222 Kawut SM, Horn EM, Berekashvili KK, Widlitz AC, Rosenzweig EB, Barst RJ. von Willebrand Factor Independently Predicts Long-term Survival in Patients With Pulmonary Arterial Hypertension. *CHEST* 2005; **128:**2355-2362.

223 Lip GYH, Blann A. von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? *Cardiovascular Research* 1997; **34:**255-265.

224 Lopes AA, Maeda NY, Bydlowski SP. Abnormalities in circulating von Willebrand factor and survival in pulmonary hypertension. *Am J Med* 1998; **105:**21-26.

225 James PD, Connell NT, Ameer B, Di Paola J, Eikenboom J, Giraud N, Haberichter S, Jacobs-Pratt V, Konkle B, McLintock C, McRae S, R. Montgomery R, O'Donnell JS, Scappe N, Sidonio R, Jr, Flood VH, Husainat N, Kalot MA, Mustafa RA. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. *Blood Advances* 2021; **5:**280-300.

226 Zheng XL, Vesely SK, Cataland SR, Coppo P, Geldziler B, Iorio A, Matsumoto M, Mustafa RA, Pai M, Rock G, Russell L, Tarawneh R, Valdes J, Peyvandi F. ISTH guidelines for the diagnosis of thrombotic thrombocytopenic purpura. *Journal of Thrombosis and Haemostasis* 2020; **18:**2486-2495.

227 Wilson M, Keeley J, Kingman M, Wang J, Rogers F. Current clinical utilization of risk assessment tools in pulmonary arterial hypertension: a descriptive survey of facilitation strategies, patterns, and barriers to use in the United States. *Pulmonary Circulation* 2020; **10:**2045894020950186.

228 Sahay S, Balasubramanian V, Memon H, Poms A, Bossone E, Highland K, Kay D, Levine DJ, Mullin CJ, Melendres-Groves L, Mathai SC, Soto FJ, Shlobin O, Elwing JM. Utilization of risk assessment tools in management of PAH: A PAH provider survey. *Pulm Circ* 2022; **12:**e12057.

229 Wilson M, Keeley J, Kingman M, McDevitt S, Brewer J, Rogers F, Hill W, Rideman Z, Broderick M. Clinical application of risk assessment in PAH: Expert center APRN recommendations. *Pulmonary Circulation* 2022; **12:**e12106.

230 Nickel N, Golpon H, Greer M, Knudsen L, Olsson K, Westerkamp V, Welte T, Hoeper MM. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. *European Respiratory Journal* 2011; **39:**589-596.

231 Gaine S, McLaughlin V. Pulmonary arterial hypertension: tailoring treatment to risk in the current era. *Eur Respir Rev* 2017; **26**

232 van de Veerdonk MC, Marcus JT, Westerhof N, de Man FS, Boonstra A, Heymans MW, Bogaard HJ, Vonk Noordegraaf A. Signs of right ventricular deterioration in clinically stable patients with pulmonary arterial hypertension. *Chest* 2015; **147:**1063-1071.

Papers I – V

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Abdulla Ahmed studied medicine at Lund University, Sweden, and graduated in 2021. His passion for cardiology began in 2017, during his medical studies, when he embarked on research in the field. Today, he is a clinician at Helsingborg Hospital, Sweden, while also aspiring to lead his own research group in the future. Beyond his professional pursuits, he enjoys practicing the art of calligraphy in his free time.

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