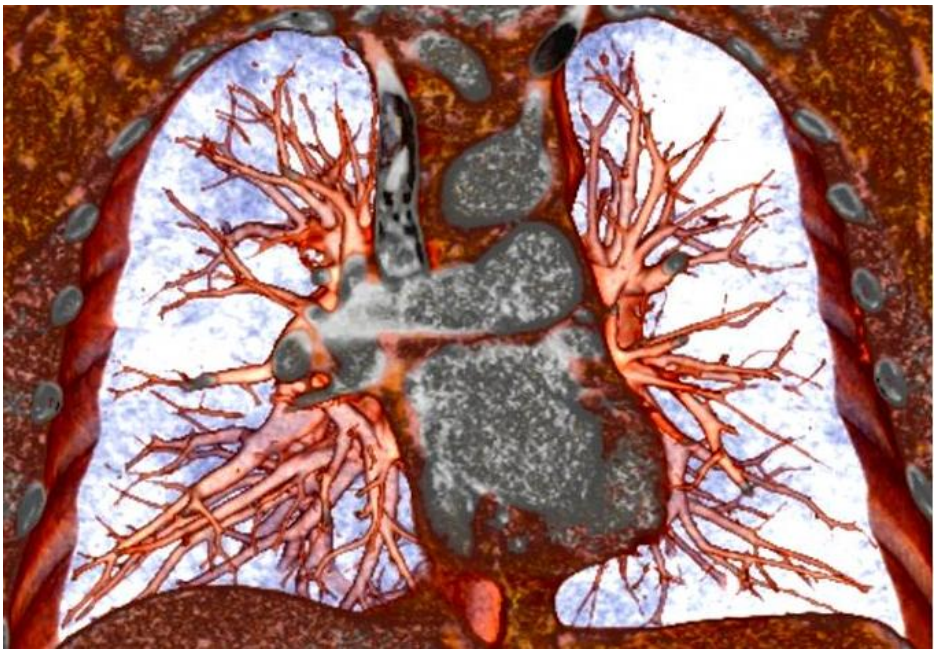
r10th MEETING ON PULMONARY HYPERTENSION RESEARCH



Friday, March 6th, 2026

Aula Pedro Lorenzo, segunda planta
Departamento de Farmacología y Toxicología
Facultad de Medicina, Universidad Complutense de Madrid

Organized by: Pulmonary Hypertension Program.
Biomedical Research Networking Center on Respiratory Diseases

ciber ES Instituto de Salud Carlos III GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES European Reference Networks ERN-LUNG RARE RESPIRATORY DISEASES

Welcome

Welcome to the 10th Pulmonary Hypertension Research Meeting, organized by the Pulmonary Hypertension Research Line of the Center for Biomedical Research in Respiratory Diseases (CIBERES).

The meeting aims to showcase the research currently being conducted in the field of pulmonary hypertension in Spain, promote the exchange of information among researchers, and encourage the development of collaborative actions.

As in previous editions, this meeting includes the participation of clinical researchers from various specialties, basic researchers from diverse disciplines, and translational researchers. The meeting has been organized by CIBERES, but it is not only aimed at researchers from this center; it is open to all researchers working in the field of pulmonary hypertension in Spain.

In this edition, 25 oral communications will be presented, and the invited lecture will be delivered by Prof. Andrea Olschewski, from the Department of Anaesthesiology and Intensive Care Medicine at the University of Graz.

It is our hope that the meeting will be fruitful and beneficial for all, and that it will facilitate future collaborations.

Prof. Joan Albert Barberà
Coordinator
Research Line on Pulmonary Hypertension
CIBERES

Meeting Program

Welcome 09:30-09:40

Prof. María Molina Molina
Scientific Director, CIBERES

Prof. Francisco Pérez-Vizcaíno
Director, Diffuse Respiratory Diseases Program, CIBERES

Prof. Joan Albert Barberà
Coordinator, Research Line on Pulmonary Hypertension, CIBERES

Invited Lecture 09:40-10:15

▪ Voltage, Vessels, and Vascular Remodeling: Ion Channels in Pulmonary Hypertension
Prof. Andrea Olschewski
Department of Anaesthesiology and Intensive Care Medicine
LBI for Lung Vascular Research, Medical University of Graz

Session: PH associated with Lung Disease 10:15-11:40
Chairs: Myriam Calle, Víctor Peinado

▪ Immune profile of the lung in animal models of COPD and pulmonary hypertension ▪ 10:15
Ana Hernández
Universidad Complutense de Madrid. CIBERES

▪ Wt1-Lineage HIF2 Protects Microvascular and Cardiac Function During Chronic Hypoxia: Implications for Pulmonary Hypertension ▪ 10:27
Silvia Martín
Instituto de Investigaciones Biomédicas Sols-Morreale (IIBM)

▪ Cell-type-specific divergence of cGMP-PKG signaling under cigarette smoke stress reveals PKG2-dependent necroptosis in pulmonary fibroblasts ▪ 10:39
Adelaida Bosacoma
IDIBAPS-Hospital Clínic de Barcelona. CIBERES

▪ TPD190, a nitric Oxide-Releasing PDE4 Inhibitor, reduces pulmonary artery remodelling and hypertension in *in vivo*, *in vitro* and *ex vivo* preclinical models relevant to Class III Pulmonary Hypertension ▪ 10:51

Paula Montero
Universidad de Valencia. Fundación de Investigación Hospital General Universitario de Valencia. CIBERES

▪ Role of nAChRs in oxidative stress-mediated vascular dysfunction in COPD ▪ 11:03

Rosa Andreu
IIS Princesa-Universidad Autónoma de Madrid

▪ Novel genetic variants linked to pulmonary hypertension in chronic lung diseases ▪ 11:15

Adelaida Bosacoma
IDIBAPS-Hospital Clínic de Barcelona. CIBERES

▪ Pulmonary Hypertension Associated with Chronic Lung Disease in Spain: results from the REHAR Registry ▪ 11:27

Álvaro Cantero
Hospital Universitari Vall d'Hebron, Barcelona

Coffee Break 11:40-12:00

SEPAR Session 12:00-12:55
Chairs: Isabel Blanco, Gregorio Pérez-Peñate

▪ Introduction ▪ 12:00

▪ Simplified risk stratification using cardiopulmonary exercise testing: a two-centre spanish experience ▪ 12:05

Amaya Martínez
Hospital Universitario Marqués de Valdecilla, Santander, SEPAR

▪ Development of a Nomogram for Genetic Risk of PAH ▪ 12:17

Fernando Vargas
Hospital Universitario Ramón y Cajal, Madrid, SEPAR

▪ Beyond BMI: Morphofunctional assessment, sarcopenia and prognosis in pulmonary hypertension. Morfhipul study ▪ 12:29

Esther Henríquez
Hospital Universitario Dr. Negrín. Universidad de Las Palmas de Gran Canaria, SEPAR

▪ Evaluation of microbial-derived metabolites in patients with acute pulmonary embolism: findings from the MICTEP study ▪ 12:41
Alberto García-Ortega
Hospital Universitario Doctor Peset, Valencia, SEPAR

Session: CTEPH and Pulmonary Hypertension, general 12:55-13:45
Chairs: Remedios Otero, Eduardo Oliver

▪ AI tool for automatic segmentation of left and right ventricle in preclinical MRI ▪ 12:55
Sebastián Acebal
CIC biomaGUNE, Donostia

▪ Non-Invasive Risk Stratification in Severe Pulmonary Hypertension: Derivation and Validation of the NIRV Score ▪ 13:07
Soha Esmaili
Instituto de Investigación Sanitaria San Carlos (IdISSC), Madrid

▪ Distinct translational dysregulation in endothelial cells reveals a unique molecular signature of chronic thromboembolic pulmonary hypertension ▪ 13:19
Olga Tura-Ceide
Girona Biomedical Research Institute (IDIBGI-CERCA), Parc Hospitalari Martí i Julià, CIBERES

▪ Impact of intermittent hypoxia on venous thrombosis: a combined murine model of deep vein thrombosis ▪ 13:31
María Melero
Hospital Virgen del Rocío, Sevilla

Fundación Contra la Hipertensión Pulmonar 13:45-13:55

Lunch Break 14:00-15:00

Session: Pulmonary Arterial Hypertension 15:00-16:50
Chairs: María Lázaro, Jesús Ruiz-Cabello

▪ Parathyroid hormone as an independent prognostic biomarker in pulmonary arterial hypertension ▪ 15:00
Rui Adão
Universidad Complutense de Madrid. CIBERES

▪ Transcriptomic Profiling of Pulmonary Endothelial Cells Harvested from Pulmonary Artery Catheter in Pulmonary Arterial Hypertension ▪ 15:12
Anna Sardiné
IDIBAPS-Hospital Clínic de Barcelona. CIBERES

▪ A Novel Partial *TBX4* Duplication Causing Small Patella Syndrome and Pulmonary Arterial Hypertension ▪ 15:24
Lucía Miranda
Instituto de Genética Médica y Molecular (INGEMM), CIBERER

▪ Metformin fails to reverse key features of pulmonary arterial hypertension: evidence for sex-dependent metabolic effects ▪ 15:36
Irene Fernández
CIC biomaGUNE, Donostia. CIBERES

▪ Effects of Physical Training in Patients with Pulmonary Arterial Hypertension ▪ 15:48
Kelly Casós
IDIBAPS-Hospital Clínic de Barcelona. CIBERES

▪ Genomic Architecture of Drug Response in Pulmonary Arterial Hypertension: Refining the Clinical Gold Standard via Precision Medicine ▪ 16:00
Manuel Rodríguez
Instituto de Genética Médica y Molecular (INGEMM), CIBERER

▪ β3-Adrenergic Receptor Agonists Attenuates Pulmonary Inflammation and Vascular Remodelling in Pulmonary Hypertension ▪ 16:12
Laura de la Bastida
CIB Margarita Salas

▪ Identification of Structural Variants in *BMPR2* by whole-genome sequencing in patients with pulmonary arterial hypertension ▪ 16:24
Mónica Mora
Instituto de Genética Médica y Molecular (INGEMM), CIBERER

▪ One Genome, Multiple Answers: Diagnostic and Predictive Value of Whole-Genome Sequencing in PAH and PVOD ▪ 16:36

Natalia Gallego
Instituto de Genética Médica y Molecular (INGEMM), CIBERER

Closing Remarks 16:50-17:00

Prof. Joan Albert Barberà
Coordinator, Research Line on Pulmonary Hypertension, CIBERES

Poster

▪ Update and management of the Spanish Pulmonary Hypertension Bank

Ada Soler
Biobanc Hospital Clínic-IDIBAPS, Barcelona

With the support of:

Session: PH associated with Lung Disease

Chairs:
Myriam Calle, Víctor Peinado

Immune profile of the lung in animal models of COPD and pulmonary hypertension

Ana Hernández
Universidad Complutense de Madrid. CIBERES

Hernández-García A[1,2], Barreira B[1,2], Andreu R[3], Morales-Cano D[1,2], Adão R[1,2], Peces-Barba G[4], Pérez-Vizcaino[1,2], Calzada MJ[2,3], Fernández-Malavé E[5], Cogolludo A[1,2]

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Pulmonary hypertension (PH) is a severe and progressive disease characterized by elevated pulmonary arterial pressure and increased vascular resistance, frequently associated with chronic lung diseases such as chronic obstructive pulmonary disease (COPD). In these patients, the development of PH is linked to worse clinical outcomes, impaired gas exchange, and increased mortality. Inflammation and pulmonary endothelial dysfunction are considered early and key pathogenic events in PH, with chronic hypoxia and cigarette smoke exposure playing major contributory roles.

The aim of this study was to characterize the immune profile of the lung in experimental animal models of COPD-associated PH induced by cigarette smoke or chronic hypoxia, and to explore the relationship between immune alterations and endothelial dysfunction. Mice or rats were exposed *in vivo* to cigarette smoke for two weeks or to the combination of S5416 plus hypoxia (SuHx) for three weeks. Wire myography was used to evaluate endothelium-dependent and -independent vasodilation in pulmonary arteries. Lung immune cell populations were characterized by flow cytometry.

Both cigarette smoke and SuHx exposure induced marked endothelial dysfunction, evidenced by impaired acetylcholine-mediated vasodilation in pulmonary arteries. Immunophenotyping revealed distinct alterations in lung immune populations depending on the model. Notably, a significant increase in γδ T cells was observed in mice exposed to cigarette smoke and in SuHx rats. Within this population, IL-17-producing $CD27^{-}$ $CCR6^{+}$ γδ T cells were consistently increased across models.

Functional studies showed that IL-17 induces endothelial dysfunction in pulmonary arteries, promoting endothelial apoptosis and impairing both endothelium-dependent and - independent vasodilation. Together, these findings suggest a pathogenic role for IL-17 and γδ T cells in pulmonary vascular injury.

In conclusion, chronic exposure to cigarette smoke and SuHx lead to pulmonary endothelial dysfunction, with IL-17-producing γδ T cells as potential contributors. Future studies will clarify the causal role of IL-17 in vascular dysfunction and its therapeutic potential in COPD-associated PH.

Research funded by Comunidad de Madrid (P2022/BMD-7224 to A.C. and M.C.), Ministerio de Ciencia e Innovación (PID2020-117939RB-I00 to A.C.) Fundación contra la Hipertensión Pulmonar (Empathy project)

Wt1-Lineage HIF2 Protects Microvascular and Cardiac Function During Chronic Hypoxia: Implications for Pulmonary Hyperten

Silvia Martín
Instituto de Investigaciones Biomédicas Sols-Morreale (IIBM)

Teresa Albendea-Gomez, Susana Mendoza-Tamajon, Rosana Castro-Mecinas, Beatriz Escobar, Susana Rocha Ferreira, Sonia Urra-Balduz, Jose Angel Nicolas-Avila, Eduardo Oliver, Maria Villalba-Orero and Silvia Martin-Puig

Background: Hypoxia is associated with the development of cardiovascular diseases, including cardiac hypertrophy and pulmonary hypertension. Endothelial hypoxia inducible factor 2 (HIF2) signaling mediates pulmonary arterial remodeling and the consequent elevation of right ventricular systolic pressure during chronic hypoxia. Accordingly, therapeutic strategies for pulmonary hypertension based on selective HIF2 inhibition have been proposed. However, the relevance of HIF2 beyond the pulmonary endothelium, as well as its role in cardiac adaptation to hypoxia, remains poorly understood. The Wt1 (Wilms tumor 1) lineage contributes to cardiac and pulmonary vascular compartments, including pericytes, endothelial cells and smooth muscle cells.

Methods: We generated a novel HIF2 mutant mouse model in the Wt1 lineage (*Hif2/Wt1* cKO) and characterized its response to chronic hypoxia. Structural and functional cardiac and pulmonary parameters were assessed using classical histology, immunohistochemistry, flow cytometry, echocardiography, and lung ultrasound analysis.

Results: The *Hif2/Wt1* cKO is protected against hypoxia-induced pulmonary arterial remodeling and elevation of right ventricular systolic pressure, in agreement with previous studies. However, *Hif2/Wt1* cKO mice exhibit alveolar congestion, inflammation, and hemorrhage associated with microvascular instability. Moreover, loss of HIF2 in the Wt1 lineage leads to cardiomegaly, capillary remodeling, right and left ventricular hypertrophy, systolic dysfunction, and left ventricular dilation, suggesting pulmonary-independent roles of HIF2 in the cardiac response to chronic hypoxia. These structural abnormalities are partially reversed upon reoxygenation, whereas cardiac functional parameters remain altered.

Conclusions: Our results confirm previous observations regarding the deleterious role of HIF2 signaling in arterial remodeling, but also reveal that cardiopulmonary HIF2 signaling prevents excessive endothelial proliferation during chronic hypoxia and exerts novel protective roles that ensure microvascular stability and organ function. Furthermore, our findings raise caution regarding the therapeutic use of HIF2-specific inhibitors for the treatment of pulmonary hypertension.

Cell-type-specific divergence of cGMP-PKG signaling under cigarette smoke stress reveals PKG2-dependent necroptosis in pulmonary fibroblasts

Adelaida Bosacoma
IDIBAPS-Hospital Clínic de Barcelona. CIBERES

Adelaida Bosacoma, Anna Sardiné, Kelly Casós, Rita Fernández-Hernández, Olga Tura-Ceide, Isabel Blanco, Joan A. Barberà and Victor I. Peinado

Cigarette smoke (CS) promotes pulmonary vascular remodeling in chronic obstructive pulmonary disease (COPD) by triggering oxidative and inflammatory signaling in the vessel wall. Soluble guanylate cyclase (sGC) stimulators improve pulmonary hemodynamics and prevent vascular remodeling in experimental COPD. However, downstream cGMP effector mechanisms may differ across vascular cell types.

In this study, we examined the effects of CS extract (CSE) and sGC stimulation (BAY 63-2521) on three types of cells: human pulmonary artery smooth muscle cells (PASMC), lung fibroblasts (NHLF), and pulmonary endothelial cells (HPAEC). CSE alone induced stress responses in all cell types. However, when combined with sGC stimulation, strikingly divergent outcomes were produced. PASMC preserved viability and exhibited normalization of stress markers.

Transcriptomic and protein analyses revealed a cell-type-specific distribution of cGMP effectors. PASMC predominantly expressed PKG1, while fibroblasts mainly expressed the membrane-anchored PKG2. Phosphoproteomic profiling showed that, after CSE incubation, sGC stimulation dampened MAPK/mTOR signaling in PASMC, which is consistent with an adaptive, cytoprotective repair state. In contrast, NHLF displayed dysregulated receptor tyrosine kinase signaling, including site-selective EGFR phosphorylation, altered integrin signaling, checkpoint activation, and impaired CREB activity.

Notably, CSE+BAY-exposed fibroblasts exhibited strong induction of TNFR1, FAS, and TRAIL receptors, loss of caspase-8 protein, FLIP-S accumulation, and RIPK1-RIPK3-MLKL necroptotic pathway activation. This death program was not observed in PASMCs, in which RIPK1 signaling remained nonlethal.

Together, these findings identify the context of PKG isoform expression as a critical determinant of cellular responses to sGC stimulation. PKG1 mediates stress buffering and survival in vascular smooth muscle, while PKG2 sensitizes pulmonary fibroblasts to receptor-driven necroptosis under oxidative stress. This duality provides a mechanistic framework linking the beneficial vascular effects of sGC stimulation to potential adverse stromal responses in smoke-exposed lungs.

Supported by SEPAR 1240/2022

TPD190, a nitric Oxide-Releasing PDE4 Inhibitor, reduces pulmonary artery remodelling and hypertension in *in vivo*, *in vitro* and *ex vivo* preclinical models relevant to Class III Pulmonary Hypertension

Paula Montero
Universidad de Valencia. Fundación de Investigación Hospital General Universitario de Valencia. CIBERES

Paula Montero, Inés Roger, Rubén Serrano, Oscar Villarroya, Javier Milara

Background: Pulmonary hypertension (PH) in idiopathic pulmonary fibrosis (IPF) portends a poor prognosis. Recent data from phase III clinical trials have shown positive results for nerandomilast, a PDE4B inhibitor, improving lung function and antifibrotic effects in IPF patients. However, its effects on pulmonary artery function are limited. A new class of nitric oxide (NO)-releasing PDE4 inhibitors, such as TPD190, has been developed improving the intracellular bioavailability of NO.

Objective: To evaluate the antifibrotic and anti-pulmonary artery remodeling and hypertension effects of TPD190, a new NO-releasing PDE4 inhibitor and its corresponding PDE4 inhibitor without nitrate esther, in a rat model of bleomycin-induced lung fibrosis and pulmonary hypertension, in precision cut lung slices (hPCLS) from patients with IPF and PH.

Methods: Wistar rats (n=12 per group) were undergoing intratracheal administration of 3.75 UI/kg7day bleomycin or vehicle at day 0 of the 28-day experimental procedure. At day 10, TPD190 (NO-PDE4i) or TPD191 (PDE4i) were administered at 5mg/kg/once a day until day 28. At the end of experimental procedure, lung fibrosis, heart hypertrophy, pulmonary artery tension and remodeling were evaluated by histochemistry, western blot and RT-PCR. Human PCLS generated from patients with IPF-PH were stimulated with a fibrotic and hypertensive cocktail (TGFβ1, LPA, IL-11, TNFα and PDGF) for 120 hours. TPD190 (NO-PDE4i), TPD191 (PDE4i) or nerandomilast 100nM were administered from 48h to 120h of the experimental procedure. Fibrosis and vascular remodeling markers were measured by RT-PCR.

Results: TPD190 and in a lesser extent TPD191 showed inhibitory effects on right heart hypertrophy, lung fibrosis (collagen I, hydoxiproline), hypertension (RVSP, mmHg) and pulmonary artery remodeling. TPD190 showed more efficacy than TPD191 and nerandomilast inhibiting profibrotic and pulmonary artery remodelling markers induced by fibrotic cocktail in hPCLS.

Conclusions: TPD190 showed improved antifibrotic and anti-pulmonary artery remodelling effects that its corresponding PDE4i and nerandomilast.

Role of nAChRs in oxidative stress-mediated vascular dysfunction in COPD

Rosa Andreu
IIS Princesa-Universidad Autónoma de Madrid

Rosa Andreu-Martínez, Onofre Munar-Rubert, Jorge Rodríguez-Pérez, Noelia López, Bianca Barreira, Laura Sánchez-Carretero, Adele Cardeñosa, Ana Marcos-Jimenez, Luis Gandia, Ramón Moreno-Balsalobre, Héctor Milian, Francisco Perez-Vizcaino, Edgar Fernández-Malavé, Germán Peces-Barba, Cecilia Muñoz-Calleja, Ángel Cogolludo and María J. Calzada

Tobacco smoke is the main risk factor for developing COPD. Although current therapies alleviate symptoms, their effectiveness in preventing cardiovascular complications, particularly vascular dysfunction and pulmonary hypertension, remains limited. Previous studies by our group show that cigarette smoke directly contributes to pulmonary artery dysfunction. However, further research into the molecular mechanisms involved is needed to design more effective targeted treatments.

In vitro studies on human pulmonary artery smooth muscle cells (hPASMC) exposed to tobacco smoke extract, together with murine models of tobacco exposure, identified molecular pathways associated with oxidative stress-induced cell damage and alterations in calcium regulation, which in turn contribute to the dysfunction of the contractile machinery of vascular smooth muscle.

These integrated approaches demonstrate that these effects are mediated by the activation of nicotinic acetylcholine receptors (nAChRs), particularly the α7 subtype. Furthermore, the use of nAChR antagonists, or the deletion of the α7 nAChR in a murine model, protects pulmonary artery function against tobacco-induced damage. Notably, the expression of α7 nAChR in the pulmonary arteries of COPD patients increases with disease severity and is inversely correlated with respiratory function.

Taken together, our findings indicate that aberrant activation of nAChRs, particularly α7, plays a key role in COPD-associated vascular dysfunction and suggest that selective antagonists of these receptors could constitute a promising therapeutic strategy to prevent or mitigate its vascular complications.

Novel genetic variants linked to pulmonary hypertension in chronic lung diseases

Adelaida Bosacoma
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Adelaida Bosacoma, Agustín R. Garcia, Irene Madrigal, Juan-José Lozano, Julia Sidorova, Daniel Aguilar, Anna Sardiné, Isabel Blanco, Olga Tura-Ceide, Rita Fernández-Hernández, Clara Martín-Ontiyuelo, Diego A. Rodríguez-Chiaradía, Manuel López-Meseguer, Fernanda Hernandez-Gonzalez, Jesús Ribas, Xavier Pomares, María Molina-Molina, Jacobo Sellares, Victor I. Peinado, Joan A. Barberà

The potential genetic determinants of pulmonary hypertension (PH) in chronic lung disease (CLD) are poorly understood. This study aimed to characterize the genetic landscape of PH associated with chronic obstructive pulmonary disease (COPD) and interstitial lung disease (ILD) and compare it with idiopathic pulmonary arterial hypertension (iPAH), using a targeted sequencing approach.

We analyzed 63 patients with COPD and 51 with ILD, stratified in: without PH (17 with COPD, 19 with ILD), with nonsevere PH (18 COPD, 15 ILD), and with severe PH (28 COPD, 17 ILD); and 38 patients with iPAH. A 150-genes custom panel was sequenced in blood-isolated DNA. We analyzed the number of variants per patient, as well as the prevalence of pathogenic and variants of uncertain significance (VUS) unique to patients with PH.

Patients with COPD-PH showed more variants per patient (42.2 in nonsevere and 48.3 in severe PH) than those without PH (34.2). Conversely, ILD-PH patients had significantly fewer variants (37.4 nonsevere, 36.1 severe) compared to those without PH (48.4). No pathogenic or likely pathogenic variants were observed in patients with CLD, whereas in IPAH, 5 patients (13.1%) showed pathogenic variants in *BMPR2* (n=3), *ACVRL1* (n=1), and *TOPBP1* (n=1). VUS in PAH-associated genes were observed in 3 patients with severe COPD-PH (10.7%) (*BMPR2*:1*; BMPR1B*:2), and in one with nonsevere ILD-PH (6.7%) (*SMAD9)*. VUS in other genes, present in >1 PH patient were observed in 5 patients with severe COPD-PH (17.8%) (*THBS1*:2; *C3*:2; *LOXL2*:2*; PTK2*:2), and 3 with ILD-PH (9.4%) (*C3*:1*; TNFAIP62*:2). Eight iPAH patients (21%) showed VUS in other genes (*CYP1B1*:3; *NCOR2*:2; *PTGS2*:2; *PTK2*:1). Variants exclusive to severe CLD-PH were found in genes involved in matrix remodeling and inflammation.

COPD-PH was associated with a trend toward increased genetic variability, whereas a significant opposite pattern was found in ILD-PH. Pathogenic variants in PAH-associated genes were detected only in iPAH, whereas some patients with CLD-PH harbored VUS in these genes, suggesting a potential role in PH development. New variants unique to severe COPD- and ILD-PH, not previously linked to PH, were identified. Functional validation is required to confirm their pathogenicity and assess their potential as biomarkers or therapeutic targets.

Supported by PI18/00383 (ISCiii), FCHP

Pulmonary Hypertension Associated with Chronic Lung Disease in Spain: results from the REHAR Registry

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Background: Pulmonary hypertension (PH) is frequent and impactful in respiratory disease, but its characteristics in the Spanish population are unknown.

Methods: We analysed the clinical characteristics and outcomes of patients with chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD) and combined pulmonary fibrosis and emphysema (CPFE) enrolled in the Spanish Registry of PH Associated with Respiratory Disease (REHAR) between January 2002 and December 2024.

Results: Of the 447 patients, 216 had COPD, 186 had ILD and 45 had CPFE. In each group, severe PH showed distinct features compared to non-severe PH, notably worse hypoxemia. Survival was worse in ILD and CPFE compared to COPD (p =0.028), and in severe PH compared to non-severe PH in COPD and CPFE, both according to the 6th World Symposium on PH (6WSPH) criteria (p =0.048 and p =0.021, respectively) and to the European Society of Cardiology/European Respiratory Society (ESC/ERS) 2022 guidelines (p =0.005 and p =0.027, respectively).

However, in ILD none of the two classifications was associated with worse survival (p =0.086 and p =0.270 for 6WSPH and ESC/ERS, respectively); no PVR threshold could discriminate survival. Comparing the 6WSPH and the ESC/ERS hemodynamic classifications, 31% of patients changed hemodynamic category, those switching category having specific profiles. Treatment with pulmonary vasodilators was infrequent and associated with reduced survival.

Conclusions: This study highlights clinical differences in a Spanish population with COPD, ILD and CPFE and in severe PH compared to non-severe PH, with hemodynamic compromise having a different impact on survival in COPD, ILD and CPFE. Our results underscore the significant heterogeneity within Group 3 PH.

SEPAR Session

Chairs:
Isabel Blanco, Gregorio Pérez-Peñate

Simplified risk stratification using cardiopulmonary exercise testing: a two-centre spanish experience

Amaya Martínez
Hospital Universitario Marqués de Valdecilla, Santander, SEPAR

Amaya Martínez Meñaca, Alejandro Cruz Utrilla, Víctor Manuel Mora Cuesta, Raquel Luna López, Teresa Segura De La Cal, Ángela Flox Camacho, Pilar Alonso Lecue, José Manuel Cifrián Martínez, Pilar Escribano Subías

Introduction: Risk stratification in pulmonary arterial hypertension (PAH) is a key tool for clinical decision-making both at diagnosis and during follow-up. The 2022 ESC/ERS guidelines propose a simplified four-strata risk assessment based on three variables. The aim of this study was to evaluate the impact on risk classification of replacing the 6-minute walk test (6MWT) with cardiopulmonary exercise testing (CPET) within this simplified risk scale.

Materials and methods: This was a cross-sectional study including prevalent patients with a diagnosis of PAH from two specialised centres. Patients were selected if functional class, NT-proBNP, 6MWT, and CPET were available within a period of less than three months, with no changes in PAH-specific therapy during that interval.

Results: A total of 180 patients were included, predominantly women (69.4%), with a mean age of 44.5 ± 0.9 years. The most frequent subtype was idiopathic PAH (53.3%). During a median follow-up of 131.5 months, 38 patients died and 22 underwent lung transplantation. Compared with survivors, these patients had worse functional class, higher levels of cardiac biomarkers, and more frequently received systemic prostacyclins and combination therapies. Application of the original four-strata model classified most patients as low or intermediate–low risk. However, when 6MWT distance was replaced by oxygen consumption, a relevant shift in risk stratification was observed, mainly from intermediate–low to intermediate–high risk.

Conclusions: The use of oxygen consumption instead of 6MWT distance improves the identification of higher-risk patients within the four-strata risk assessment model, with potential prognostic and therapeutic implications in PAH.

Development of a Nomogram for Genetic Risk of PAH

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Introduction: Heritable pulmonary arterial hypertension (PAH) is a subgroup within PAH in which individuals carry pathogenic genetic mutations such as in BMPR2, TBX4, or EIF2AK4. Heritable PAH is typically indistinguishable from idiopathic PAH (iPAH), which is why genetic testing is recommended for all iPAH cases, due to its prognostic value and for genetic counseling purposes.

The objective of this study is to determine the variables that characterize patients with heritable PAH and to construct a predictive model in the form of a nomogram to identify which subjects with PAH are more likely to carry pathogenic mutations.

Methods: Single-center retrospective study conducted at the Pulmonary Hypertension Unit of the 12 de Octubre University Hospital. Data from 823 patients, between 2006 and 2023, diagnosed with PAH were collected, and 302 patients classified as iPAH prior to genetic testing were selected, of whom 59 had a positive result. Univariate and multivariate analyses were performed, and a nomogram was developed following the analysis of the results.

Results: Hemoglobin and age were the statistically significant variables in the multivariate analysis. Considering these two variables and five variables that were statistically significant in the univariate analysis (DLCO, hypertension, syncope at presentation, mPAP, and smoking), a nomogram was developed with an area under the ROC curve of 0.829 and a Hosmer–Lemeshow goodness-of-fit test p-value of 0.833.

Conclusions: The developed nomogram demonstrates excellent predictive ability in internal calibration tests. Its application, pending external validation, could be important for establishing priority in requesting genetic tests to rule out heritable PAH, or even for modifying current clinical practice, as a threshold could be determined—based on an agreed-upon probability—to establish a diagnosis of iPAH without requiring genetic testing.

Beyond BMI: Morphofunctional assessment, sarcopenia and prognosis in pulmonary hypertension. Morfhipul study

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Background: Chronic disease–related malnutrition has a direct impact on muscle mass and function. There is a limited information regarding nutritional status and body composition in patients with pulmonary hypertension (PH). Moreover, data on the prevalence of malnutrition and sarcopenia, as well as their impact on the development of complications and mortality in these patients are scarce.

Objetive: To analyse the prevalence of nutritional and muscular deterioration in patients with PH (Groups I, III, and IV) and evaluates (secondary objective) the association between nutritional and muscular status, and prognosis and quality of life in PH patients.

Methods: This is a pilot, prospective, descriptive, comparative cohort study including patients with and without pulmonary hypertension (PH Groups I, III, and IV), in which nutritional status and body composition are being evaluated in patients from the Pulmonary Vascular Unit of HUGC Dr. Negrín. Demographic, anthropometric, and nutritional variables were assessed using validated tools (MUST, SARC-F, and GLIM criteria), laboratory parameters, and a quality-of-life questionnaire (EmPHasis-10).

Results: To date, 33 patients have been evaluated (12 in Group I, 13 in Group III, and 8 in Group IV). According to BMI, 6% (n = 2) had a BMI < 18.5 kg/m². Comprehensive morphofunctional assessment detected malnutrition in 42% of PH Group I (4 severe and 1 moderate), 54% of PH Group III (6 moderate and 1 severe), and 25% of PH Group IV patients (1 severe and 1 moderate).
In addition, 20% of the total cohort (n = 6) were identified as being at risk of sarcopenia (SARC-F questionnaire). However, probable sarcopenia, defined by the presence of dynapenia (functional impairment assessed by handgrip dynamometry and the chair stand test), was detected in 88% of patients (n = 29): 92% in PH Group I, 85% in PH Group III, and 88% in PH Group IV. Finally, sarcopenia was confirmed by muscle ultrasound in 32% (n = 9) of these patients.

Conclusión: based on these preliminary data, malnutrition and sarcopenia are present in a high proportion of this cohort of patients with pulmonary hypertension, which supports the need for a targeted and comprehensive therapeutic approach.

Evaluation of microbial-derived metabolites in patients with acute pulmonary embolism: findings from the MICTEP study

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Introduction: Functional analysis of microbiome with microbial-derived metabolites (MDM) has emerged with a crucial role for several inflammatory and cardiovascular diseases. However, the data on the relationships of pulmonary embolism (PE) and the microbiome are scarce.

Methods: We collected serum samples from a multicentric cohort including 96 patients with acute PE at hospital admission and 30 healthy controls to compare MDM levels. Additionally, we investigated differences in MDM levels among patients based on predisposing risk factors (i.e., unprovoked, provoked and cancer-associated thrombosis [CAT]). MDM levels and inflammation and coagulation-related markers were quantified by liquid chromatography-mass spectrometry and flow cytometry respectively.

Results: Compared to healthy controls, patients with acute PE showed significantly higher serum levels of trimethylamine N-oxide (TMAO) (11.5 μM vs 6.7 μM; p=0.02) and acetate (48.3 μM vs 33.0 μM; p=0.04); and lower levels of propionate (3.8 μM vs 5.3 μM; p=0.007), butyrate (4.03 μM vs. 7.68 μM; p=0.009), isobutyrate (5.0 μM vs 7.32 μM; p=0.002), and valerate (0.4 μM vs 0.63 μM; p<0.001). Valerate showed the best discrimination between PE and controls (area under the ROC curve 0.758 [95% CI 0.66–0.86]). In the multinomial analysis, higher values of TMAO and acetate were associated with a higher probability of unprovoked PE. MDM levels exhibited different correlation with inflammation-related markers highlighting TGF-β1, CCL2, CXCL10.

Conclusion: These findings reveal imbalances in the serological concentrations of MDMs in patients with acute PE and highlight the potential role of the microbiome and its functional metabolites as novel predisposing risk factors for PE.

Session: CTEPH and Pulmonary Hypertension, general

Chairs:
Remedios Otero, Eduardo Oliver

AI tool for automatic segmentation of left and right ventricle in preclinical MRI

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Pulmonary arterial hypertension (PAH) is a rare disease in which distal pulmonary arteries remodel, increasing pulmonary blood pressure and causing right ventricular (RV) enlargement and eventual heart failure (Thenappan et al., 2018). The Sugen–hypoxia mouse model best reproduces these vascular and cardiac changes. Cardiac Magnetic Resonance Imaging (CMR) enables visualization of these alterations and accurate quantification of ventricular volumes. Here, we propose replacing the current manual analysis with an artificial intelligence (AI)–based approach to shorten analysis time, reduce human error, and highlight a preclinical application of this technology.

CMR was performed using a 7 Tesla Bruker Biospec 70/30 USR scanner and reconstructed using retrospective gating information. For assessing cardiac function, short-axis images were obtained from the apex to the base, covering both ventricles. All cine MR images were divided according to trigger times corresponding to each phase of the cardiac cycle. Endocardial and epicardial contours of both ventricles were manually segmented in all frames, including papillary muscles and trabeculations, using ITK-SNAP software.

These manually segmented datasets were processed on a workstation equipped with an NVIDIA RTX A4000 GPU (6144 CUDA cores, 16 GB RAM) to train an artificial intelligence (AI) model based on nnU-Net, designed to automatically segment ventricular MRI images. The model's performance was evaluated using the DICEscore coefficient by comparing results with two independent manual segmentations. DICE scores were higher when compared to the analyst who generated the training data that to a second independent analyst, reflecting inter-observer variability. On average, the DICE scores were 0.924±0.075(N=300) for the LV and 0.841±0.159 (N=300) for the RV. This is expected given its complexity. Remarkably, the AI processed 300 images in only 318 seconds, proving both accuracy and speed. The AI-assisted approach offers a reliable, time-efficient alternative for preclinical cardiac MRI analysis. As far as we know there is no software available to do this preclinical data analysis.

Non-Invasive Risk Stratification in Severe Pulmonary Hypertension: Derivation and Validation of the NIRV Score

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Introduction: Severe pulmonary hypertension (PH) is a clinical syndrome in which right ventricular (RV) dysfunction determines prognosis. Based on established markers of RV failure, we operationally defined a High-Risk Phenotype (HRP) as meeting ≥2 of three pre-specified criteria: RV–pulmonary artery uncoupling (TAPSE/PSAP ≤0.31 mm/mmHg), severe myocardial stress (NT-proBNP ≥1400 pg/mL), or advanced functional limitation (WHO-FC III–IV). We sought to derive and validate a non-invasive NIRV Score to identify HRP and support specialized triage.

Methods: We analyzed a cross-sectional cohort of 490 outpatients with echocardiographic suspicion of severe PH recruited from specialized clinics. Using Recursive Partitioning (CRT), we constructed the NIRV Score. CRT identified optimal data-driven thresholds (distinct from clinical definitions): TAPSE/PSAP ≤0.321 (3 points), WHO-FC II–IV (2 points), and NT-proBNP >1377 pg/mL (1 point). Performance was assessed in this derivation cohort using the Area Under the Curve (AUC) and Hosmer–Lemeshow calibration.

Results: In this predominantly elderly cohort, HRP was present in 63.5%. High-risk patients were significantly older (87.0 vs. 84.0 years; $p<0.001$) and had worse lung diffusion capacity (DLCO 47.0% vs. 57.0% predicted; $p=0.028$). They exhibited marked mechanical uncoupling (TAPSE/PSAP 0.30 vs. 0.40; $p<0.001$) and substantially higher neurohormonal stress (NT-proBNP 2682 vs. 743 pg/mL; $p<0.001$). The NIRV Score achieved an AUC of 0.924 (95% CI: 0.897–0.951) with robust calibration ($p=0.44$). A cut-off of ≥4 points (maximal Youden index) yielded 88.1% sensitivity, 86.8% specificity, and 87.6% accuracy, with a clear dose-response gradient across score levels.

Conclusions: The NIRV Score is a highly accurate, non-invasive tool for stratifying RV risk. By integrating mechanical uncoupling, clinical severity, and biological stress, it provides a practical strategy to prioritize High-Risk Phenotype patients for specialized management.

Distinct translational dysregulation in endothelial cells reveals a unique molecular signature of chronic thromboembolic pulmonary hypertension

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Background: Pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH) are rare, severe forms of pulmonary hypertension marked by elevated mean pulmonary arterial pressure (mPAP) and poor survival outcomes, with a 20% mortality rate at 3 years. While PAH is characterized by progressive obliterative vasculopathy, CTEPH results from persistent obstruction of pulmonary arteries by fibrotic thrombotic material, triggering vascular remodeling, right ventricular hypertrophy, and eventual heart failure.

Aims: This study aimed to identify disease-specific molecular and ultrastructural alterations in endothelial colony-forming cells (ECFCs) derived from PAH and CTEPH patients compared with healthy controls, focusing on transcriptomic profiles and mitochondrial architecture. We further evaluated the effect of Riociguat, the only approved treatment for inoperable or persistent post-surgical CTEPH.

Methods: Transcriptomic profiling via gene expression analysis and ultrastructural examination by transmission electron microscopy (TEM) were performed on ECFCs from PAH, CTEPH patients, and controls. The effect of Riociguat on gene expression and mitochondrial ultrastructure was assessed in CTEPH-ECFCs.

Findings: Distinct gene expression patterns were observed across the groups. Notably, CTEPH-ECFCs exhibited a significant upregulation of genes involved in protein translation, including ribosomal proteins and elongation factors (RPS11, RPS2, RPL23, RPL23A, RPL12, RPL4, RPL21, EEF2, RPS4Y1), which was absent in PAH-ECFCs and controls-ECFCs. TEM revealed structural abnormalities in mitochondria-associated endoplasmic reticulum membranes (MAM) in CTEPH-ECFCs including changes in shape, size, mitochondria–ER distance, caveolae, and glycogen particles. Riociguat treatment did not reverse these transcriptional or mitochondrial structural alterations.

Conclusion: These findings reveal dysregulated protein biosynthesis together with alterations in mitochondria-associated membranes (MAMs) as key pathophysiological hallmarks distinguishing CTEPH from PAH. This unique molecular signature highlights the limitations of current Riociguat therapy and underscores the need for novel therapeutic strategies targeting endothelial alterations, particularly those involving translational and mitochondrial dysfunctions, in CTEPH. These insights enable the development of CTEPH-specific biomarkers and therapies.

Impact of intermittent hypoxia on venous thrombosis: a combined murine model of deep vein thrombosis

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Background: Epidemiological studies report high venous thromboembolism (VTE) prevalence in obstructive sleep apnea (OSA). Recent research, including unpublished data from our group, suggests OSA contributes to persistent perfusion defects after pulmonary embolism (PE).

Aim: To assess the role of intermittent hypoxia (IH) in thrombus formation and development.

Methods: We combined a murine deep vein thrombosis (DVT) model with IH exposure. Thrombi were induced by infrarenal inferior vena cava (IVC) ligation (stasis). DVT mice were assigned to IH or normoxia. Thrombi were examined by histology (hematoxylin–eosin, Gomori's trichrome) and immunohistochemistry. Elastic net logistic regression was used to discriminate thrombus proteomic signatures between IH and normoxia; a heatmap visualized model-selected protein expression patterns.

Results: Thrombi from normoxic mice showed greater collagen content than those exposed to IH (Figure 1; Table 1), indicating differences in thrombus remodeling. Mechanistic proteomic data are currently being generated (Figure 2).

Conclusion: IH modulates thrombus composition, particularly extracellular matrix remodeling, with potential implications for thrombus persistence and resolution in OSA.

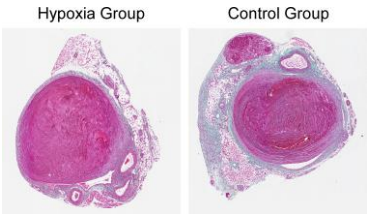

Figure 1 Representative hematoxylin and eosin (H&E)–stained thrombus sections from mice subjected to inferior vena cava (IVC) ligation under normoxia (control) and intermittent hypoxia (IH)

Collagen category	Normoxia (control), n=27	Intermittent hypoxia (IH), n=29	Total, N=56
Detected	6 (22.2%)	18 (62.1%)	24 (42.9%)
Moderate or higher	21 (77.8%)	11 (37.9%)	32 (57.1%)
Total	**27 (100.0%)**	**29 (100.0%)**	**56 (100.0%)**

Table 1. Collagen content by exposure group (n, % within group). Pearson's chi-square test: p = 0.003.

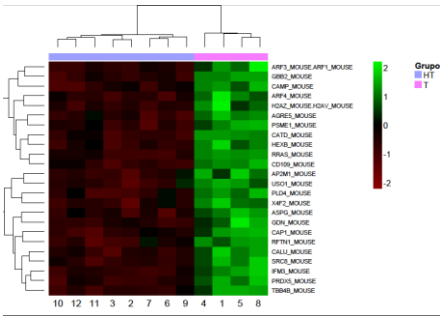

Figure 2. Heatmap of the thrombus proteomic signature selected by the elastic net logistic regression model. Rows represent model-selected proteins and columns represent individual samples. Protein abundances were normalized (z-score) and visualized from low (red) to high (green) values (scale −2 to +2). Unsupervised hierarchical clustering is shown for both proteins and samples. The top annotation bar indicates experimental group: intermittent hypoxia (HT) and normoxia control (T).

Session: Pulmonary Arterial Hypertension

Chairs:
María Lázaro, Jesús Ruiz-Cabello

Parathyroid hormone as an independent prognostic biomarker in pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is associated with reduced vitamin D levels, a major cause of secondary hyperparathyroidism. Herein we investigated the prognostic value of parathyroid hormone (PTH) in PAH (Clinicaltrials.gov NCT06872710).

Serum intact PTH (iPTH) was measured in a discovery cohort of 116 patients with idiopathic, heritable, drug-induced or associated PAH followed for a median [IQ range] of 964 days [452-1048] after sampling. The validation cohort of 169 PAH patients was followed for 2778 [2239-3795] days. Results were compared with respective control cohorts of 99 and 46 healthy subjects, respectively. Samples were obtained from the Spanish Biobank of Pulmonary Hypertension and clinical parameters were from the Spanish Registry of PAH (REHAP).

Serum iPTH levels were significantly higher in PAH patients compared to controls in both the discovery (69.6 [44.7–95.5] vs. 40.3 [29.6–53.9] pg/mL) and validation cohorts (70.5 [47.2–103.4] vs. 36.7 [27.2–61.8] pg/mL; $P < 0.0001$ for both).
ROC analysis yielded AUC values of 0.75 [95% CI 0.68-0.82] and 0.77 [0.69-0.86], respectively. iPTH remained significantly elevated in all subgroups of 25OH-vitamin D (< 10, 10-20, 20-30 and > 30 ng/ml). Calcium levels did not differ significantly between PAH patients and controls.

Notably, high iPTH values (> 90 pg/ml) were associated with significantly reduced survival, with a hazard ratio of 8.28 [95% CI: 2.45-27.95], and 3.39 [1.67-6.88], in the discovery and the validation cohorts, respectively ($P < 0.001$ for both). iPTH had also prognostic value in the subgroup of patients with 25OH vitamin D > 20 ng/ml ($P < 0.01$).

In conclusion, we confirmed elevated iPTH in PAH patients and report for the first time that iPTH has a strong and independent prognostic value for mortality.

Transcriptomic Profiling of Pulmonary Endothelial Cells Harvested from Pulmonary Artery Catheter in Pulmonary Arterial Hypertension

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Endothelial dysfunction plays a central role in the development and progression of pulmonary arterial hypertension (PAH). Characterizing transcriptional alterations in pulmonary arterial endothelial cells (PAECs) and their modulation by therapy may provide insight into disease mechanisms and identify therapeutic targets and biomarkers of treatment response.

This study aimed to analyze the transcriptomic profile of PAECs obtained from pulmonary artery catheter tips used in diagnostic right heart catheterization and to explore treatment-associated transcriptional changes.

A total of 29 PAH patients and 24 subjects without pulmonary hypertension (no-PH) were included. PAH patients were classified as idiopathic (44.8%), systemic sclerosis-associated (27.6%), or other etiologies (27.6%), while the no-PH group comprised individuals without systemic sclerosis (58.3%) and with systemic sclerosis (41.7%). Eighteen PAH patients underwent post-treatment assessment after four months of double or triple combination therapy. Low-input bulk RNA sequencing was performed in 25 PAH patients at baseline, 14 PAH patients after treatment, and 19 no-PH subjects. Single-cell RNA sequencing was conducted in 8 matched PAH patients pre- and post-therapy and 8 no-PH subjects.

PAH patients were predominantly female (82.8%) with a mean age of 59.7±15.1 years, comparable to no-PH subjects (79.2% female; 62.6±12.7 years). Pulmonary arterial pressure (PAP) at baseline in PAH patients was 42.1±13.9 mmHg and pulmonary vascular resistance (PVR) 8.65±4.87 Wood Units (WU), and in no-PH subjects, 14.9±3.9 mmHg and 1.78±0.83 WU, respectively. NT-proBNP levels were substantially elevated in PAH patients (864.6±1242 pg/mL vs 282.2±478.7 pg/mL), while 6-minute walk distance was modestly reduced (414.4±120 vs 424±120.5 m). After PAH treatment, improvements were observed in PAP (−12.11±7.41 mmHg; $p<0,0001$), PVR (-4.77±3.86 WU; $p<0,0001$), and NT-proBNP (-884±1312 pg/mL; $p=0,0005$).

Preliminary single-cell RNAseq analyses confirmed endothelial identity across groups. Exploratory transcriptomic profiling revealed enrichment of endothelial homeostatic and immune-related pathways in controls, while PAH samples exhibited activation of cell cycle, hypoxia, inflammatory, and endothelial-mesenchymal transition pathways. Additional transcriptomic analyses are under way and will be presented at the meeting.

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A Novel Partial *TBX4* Duplication Causing Small Patella Syndrome and Pulmonary Arterial Hypertension

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We report a 24-year-old male patient with early-onset pulmonary arterial hypertension (PAH) and subtle skeletal anomalies consistent with small patella syndrome (SPS), also known as ischiocoxopodopatellar syndrome (ICPPS). The patient experienced recurrent respiratory infections and failure to thrive during infancy, and was diagnosed with idiopathic PAH at three years of age. Whole-genome sequencing (WGS) of peripheral blood identified a de novo heterozygous partial duplication of *TBX4*, classified as pathogenic.

To assess the functional impact of the duplication, a skin biopsy was performed, primary dermal fibroblasts were cultured, and RNA sequencing (RNA-seq) revealed altered *TBX4* expression, supporting a functional effect of the variant. Clinically, the patient exhibited a variable disease course, with stabilization under combination therapy, and skeletal manifestations became more apparent over time.

This case expands the mutational and phenotypic spectrum of *TBX4*, highlighting that partial gene duplications can underlie SPS with early-onset PAH, and emphasizes the importance of considering *TBX4* copy number variants in pediatric PAH patients with subtle skeletal features. Additionally, RNA-seq proved to be a valuable tool to validate the pathogenicity of structural variants.

Metformin fails to reverse key features of pulmonary arterial hypertension: evidence for sex-dependent metabolic effects

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Pulmonary arterial hypertension (PAH) is marked by a metabolic shift towards glycolysis in pulmonary artery smooth muscle cells, endothelial cells and right ventricle (RV) cardiomyocytes, which is believed to drive disease progression (PMID: 29987825). Although reversing this "glycolytic switch" seems a logical therapeutic target, metformin, which enhances glucose uptake and glycolysis, has paradoxically been proposed for PAH treatment (PMID: 37389952). We hypothesized that metformin would exacerbate PAH's metabolic dysfunction in the clinically relevant Sugen5416/hypoxia model, combining vascular injury with hypoxic stress.

Mice received 0,25% w/v metformin in drinking water, PET/CT and MRI were performed after 21 days. Although PAH-metformin treated animals (PAH+Met), increased glycolysis in the RV, there were no hemodynamical benefits, as RVSP remained elevated equally in PAH and PAH+Met across both sexes, with no reduction in RV hypertrophy. PET/CT imaging revealed the following myocardial [^{18}F]-FDG uptake in females: Ctrl < Ctrl+Met < PAH < PAH+Met. In the female RV, the SUVmean rose from 1.7±0.3 (PAH) to 2.4±0.8 (PAH+Met), representing a 45% increase compared with PAH alone ($p<0.01$). Conversely, in males, the RV SUVmean was 21% lower in the PAH+Met group than PAH alone.

Cardiac MRI revealed a decline in function among PAH mice treated with metformin, as evidenced by a reduced ejection fraction and increased end-diastolic volumes compared to PAH alone. Not only did metformin fail to enhance cardiac function, MRI cardiac parameters worsened in the PAH+Met group.

Clinical trials have associated metformin with improved RV function (PMID: 33167773), despite the metabolic shifts, suggesting that the increased glucose uptake enhances substrate availability to support the energy-deprived burden in females points to sex- specific differences in RV metabolic adaptation, which should be considered in trial design.

These findings raise concerns as the applicability of metformin as a metabolic intervention in PAH, highlighting the importance of considering sex as a biological variable.

Effects of Physical Training in Patients with Pulmonary Arterial Hypertension

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Introduction: Pulmonary arterial hypertension (PAH) is a rare and progressive disease characterized by elevated pulmonary arterial pressure and right ventricular overload, ultimately leading to right heart failure and death. Low-intensity physical training (PT) has emerged as a safe, effective, and cost-efficient non-pharmacological intervention. PT has been associated with improvements in endothelial function in this vascular disease. Circulating endothelial progenitor cells (EPCs) and endothelial microparticles (MPCs) are two promising non-invasive biomarkers, which may reveal endothelial function status and endogenous repair capacity. However, its impact on pulmonary vascular integrity and endothelial homeostasis remains incompletely understood. This study aimed to assess the effects of a structured PT program on circulating EPCs and MPCs in patients with PAH before and after 40 PT sessions (3 months).

Methods: A prospective study was conducted in 34 consecutive PAH patients who successfully completed 40 supervised PT sessions. Circulating EPCs ($CD34^+CD45^{\wedge}dim$) and MPCs ($CD31^+CD42b^-$) were quantified by flow cytometry before and after the intervention.

Results: Low-intensity PT significantly increased specific EPC markers, including KDR ($p = 0.0083$) and $CD144^+$ ($p = 0.0223$). Conversely, PT was associated with a reduction in MPC parameters, including total MPC count ($p = 0.0003$), $CD62E^+$ ($p = 0.0489$), $CD62E^+CD31^+$ ($p = 0.0341$), and $CD31^+$ ($p = 0.0399$).

Conclusion: These findings indicate that low-intensity PT exerts beneficial effects on endothelial function in patients with PAH, enhancing vascular repair mechanisms and potentially contributing to improved pulmonary arterial homeostasis.

Genomic Architecture of Drug Response in Pulmonary Arterial Hypertension: Refining the Clinical Gold Standard via Precision Medicine

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While guidelines emphasize multimodal risk stratification in Pulmonary Arterial Hypertension (PAH), therapeutic selection remains predominantly empirical. This limitation is critical for the subgroup selected by acute hemodynamic response, where the phenotype is often transient and ~50% of patients experience secondary failure within one year. We explored the genomic architecture of drug response to identify markers ensuring mechanistic stability superior to current functional criteria.

We leveraged Whole Genome Sequencing (WGS) coupled with deep clinical phenotyping to systematically interrogate the PAH pharmacogenomic landscape. We analyzed pharmacokinetic and pharmacodynamic loci across the entire therapeutic spectrum, prioritizing clinically translatable signals.

Our profiling revealed distinct stratifiers for dosage and safety. *PFAS* variants drove a "high-clearance" phenotype in treprostinil therapy, requiring 35% higher maintenance doses. Similarly, *SLCO1B1* emerged as a determinant for ambrisentan tolerability. Notably, within the Calcium Channel Blocker (CCB) arm—characterized by pharmacological purity—*CACNA1C/D* carriers exhibited a maintained response rate of 83.3%. This contrasts with the ~54% historical success rate of hemodynamic selection alone, validating the genetic signal as a robust predictor of durability.

These findings illustrate that pharmacogenomics is pivotal for patient management. We identify CCB therapy as the ideal "clinical gateway" for implementing pharmacogenetics in PAH, advocating for a selection model that integrates functional reactivity with genomic integrity to deliver true Precision Medicine.

β3-Adrenergic Receptor Agonists Attenuates Pulmonary Inflammation and Vascular Remodelling in Pulmonary Hypertension

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Pulmonary hypertension (PH) is a serious and progressive disease in which elevated pulmonary vascular resistance leads to right ventricular (RV) overload and dysfunction. A key factor in the pathobiology of PH is endothelial dysfunction, frequently caused by hypoxia or chronic inflammatory stimuli. Under these conditions, endothelial cells increase the expression of adhesion molecules such as E-selectin (CD62E) and vascular cell adhesion molecule 1 (VCAM-1), which promotes leukocyte recruitment, inflammation, and vascular remodelling by inducing markers such as smooth muscle actin (α-SMA) and growth differentiation factor 15 (GDF15).

In this study, we evaluated the therapeutic potential of the β3-adrenergic receptor (β3-AR) agonists—Mirabegron and Vibegron—both clinically approved for overactive bladder. Using the hypoxia+Sugen mouse model, we analysed hemodynamic parameters together with inflammatory and remodelling markers. Western blot and immunofluorescence analyses revealed that treatment with β3-AR agonists tended to reduce the expression of E-selectin, VCAM-1, GDF15, and α-SMA in lung tissue. These molecular changes were accompanied by improvements in RV systolic pressure and hypertrophy.

These findings are consistent with our previous studies, where we reported endothelial-protective effects of Mirabegron and identified circulating biomarkers associated with clinical response. Complementary magnetic resonance imaging (MRI) and positron emission tomography (PET) imaging in treated animals further indicated improvements in heart structure and in pulmonary and cardiac metabolic activity.

Altogether, our findings suggest that β3-AR agonists may improve key pathological processes in PH, such as endothelial dysfunction, inflammation, and vascular remodelling, while also may improve cardiopulmonary metabolic function. Biomarkers such as E-selectin, VCAM-1, and GDF15 could be useful for monitoring disease progression and guiding personalized therapeutic strategies, supporting the repurposing of β3-AR agonists as promising candidates for the treatment of PH.

Identification of Structural Variants in *BMPR2* by whole-genome sequencing in patients with pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is a severe disease characterized by elevated pulmonary artery pressure, leading to heart failure and premature death if left untreated. Genetic factors play a major role in PAH, and several genes have been implicated in its pathogenesis. Among them, *BMPR2* is the gene most frequently mutated in patients with both idiopathic and heritable PAH. The aim of this study was to identify structural variants (SVs) affecting *BMPR2* in patients with idiopathic or heritable PAH who had previously undergone inconclusive genetic testing.

We analyzed a cohort of 158 individuals (101 PAH patients and 57 relatives) using whole-genome sequencing (WGS). Variants were prioritized using a custom in-house pipeline and classified according to ACMG guidelines.

We identified six patients with idiopathic PAH (5.95%) carrying clinically significant SV encompassing the *BMPR2* gene, affecting either coding or non-coding regions. The detected SVs included two duplications and four deletions.

These findings highlight the increased diagnostic yield achieved by whole-genome sequencing, which enables the detection of SV that may be missed by standard-of-care approaches such as exome sequencing. In addition, we developed an in-house pipeline specifically optimized for SV analysis from WGS data in patients with PAH.

One Genome, Multiple Answers: Diagnostic and Predictive Value of Whole-Genome Sequencing in PAH and PVOD

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Pulmonary veno-occlusive disease (PVOD) is a rare subtype of pulmonary arterial hypertension (PAH) with distinct clinical, histological, and genetic features, often misdiagnosed as idiopathic PAH. Its hallmark is intimal fibrosis of pulmonary venules, leading to luminal obstruction, severe hypoxemia, and unpredictable response to PAH-specific therapies. Definitive diagnosis requires histology or detection of EIF2AK4 mutations, though high-probability diagnosis relies on clinical, radiological, and functional findings. PVOD carries a particularly poor prognosis, with survival markedly shorter than PAH. Response to therapy is heterogeneous: while some patients show transient benefit, others develop pulmonary edema and rapid deterioration. A founder mutation (*EIF2AK4*:c.3344C>T;p.Pro1115Leu) was identified in gipsy PVOD patients, revealing strikingly different outcomes despite the same genotype, underscoring the need for biomarkers of treatment response. Genetic association studies, including GWAS, may provide such predictors and enable precision medicine.

Based on these observations, our objective was to conduct a comprehensive genomic association study (GWAS) of treatment response in patients with PVOD. Specifically, we aimed to compare individuals who tolerate PAH-specific therapy with those who are intolerant, in order to identify potential genetic determinants of therapeutic response. To this end, we performed whole-genome sequencing (WGS) in a cohort of 50 PVOD patients for whom detailed clinical data on vasodilator response had been systematically collected. Using genome-wide association study approaches, we investigated whether specific genetic variants could serve as biomarkers to predict treatment response in this highly vulnerable and clinically challenging patient population. This poster presents the results obtained. Identifying such biomarkers would represent a major step toward precision medicine in PVOD, enabling early stratification of patients and guiding therapeutic decision-making to improve outcomes.

Poster

Update and management of the Spanish Pulmonary Hypertension Bank

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The Spanish Pulmonary Hypertension Biobank (BEHIP) was established in 2013 with the aim to collect a well-characterized repository of biological samples from patients with pulmonary arterial hypertension (PAH) or chronic thromboembolic pulmonary hypertension (CTEPH) to promote and facilitate biomedical research. Clinical information is available through the Spanish Pulmonary Arterial Hypertension Registry (REHAP). Additionally, samples from pulmonary hypertension (PH) associated with respiratory diseases (Group 3, G3PH) have been incorporated to the repository, with linked clinical information available at the Spanish Registry of Pulmonary Hypertension Associated with Respiratory Diseases (REHAR). In this communication, we provide an update on the functioning and outcomes of BEHIP, focusing on sample collection and efforts to increase sample procurement.

Donors are sourced from centers participating in the REHAP or REHAR registries. Fresh blood samples are processed and stored at the HCB-IDIBAPS Biobank to obtain backup total blood, DNA, plasma, serum, and peripheral blood mononuclear cells (PBMCs). These samples are coded and linked to the clinical data registries for subsequent traceability and clinical characterization.

At the end of 2025, BEHIP has collected samples from 896 patients (86 during 2025) across 8 Spanish hospitals; 507 with PAH, 292 with CTEPH, and 97 with G3PH. PAH subtypes include: idiopathic PAH (n=182), hereditary PAH (n=16), drug-induced/toxic PAH (n=13), Scleroderma (n=66), PAH associated with connective tissue diseases (n=59), PAH associated with HIV infection (n=43), PAH associated with portal hypertension (n=39), PAH associated with congenital heart disease (n=61), and other forms of PAH (n=14). 14 patients have multifactorial PH. The available aliquots include: 858 extracted DNA, 1705 normalized DNA, 634 back-up total blood, 7974 plasma, 5402 serum, and 1960 PBMCs. Notably, by the end of 2025, 1512 aliquots have been procured to 12 requests (353 plasma, 123 DNA, 1036 serum), representing 54% of total donors (485/898). 137 patients have genetic testing data from 171 analyses, performed using Sanger or next-generation sequencing. Genetic variants were identified in 29 patients, including benign (n=2), variants of uncertain significance (n=12), likely pathogenic (n=2), and pathogenic variants (n=13).

BEHIP is the result of a well-established synergy of multidisciplinary collaborations between clinical/research centers and the HCB-IDIBAPS Biobank. Finally, enhancing sample procurement for researchers is essential to foster biomedical research.

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