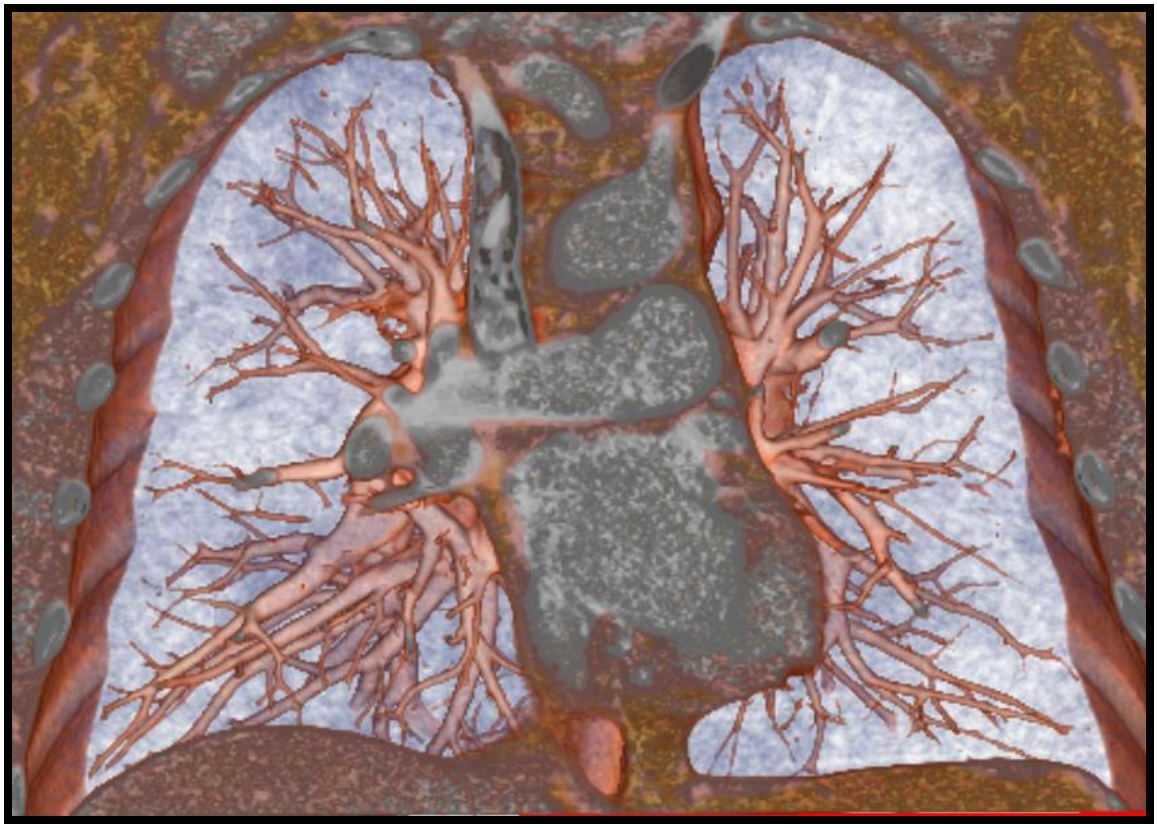
6ª REUNIÓN DE INVESTIGACIÓN EN HIPERTENSIÓN PULMONAR



3, 4 DE MARZO DE 2022

Organiza:
Línea de Investigación en Hipertensión Pulmonar
Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES)

Instituto de Salud Carlos III ciberes

Con la colaboración de: Fundación Contra la Hipertensión Pulmonar

Jueves 3 de marzo

Bienvenida 15:00-15:10

Dr. Joan Albert Barberà
Coordinador, Línea de Investigación en Hipertensión Pulmonar, CIBERES

Dr. Francisco Pérez-Vizcaíno
Coordinador, Programa de Enfermedades Respiratorias Difusas, CIBERES

Comunicaciones. Sesión 1. 15:10-16:25

Moderadores: Victor Peinado, Javier Milara, Jair Tenorio

- The Role of Notch3 pathway in pulmonary hypertension — 15:10
  Zuriñe Blasco-Iturri
  CIC biomaGUNE, Donostia

- Expanding the evidence of a semi-dominant inheritance in GDF2 and KCNK3 associated with pulmonary arterial hypertension — 15:25
  Natalia Gallego Zazo
  Instituto de Genética Médica y Molecular, Hospital Universitario La Paz, Madrid

- Characterization of the pulmonary vascular effects of URO-K10, a novel Kv7 channel activator — 15:40
  Marta Villegas-Esguevillas
  Universidad Complutense, Madrid

- Proteomics analysis of the RV of the heart in a rat model of SuHx-induced PAH treated with 2DG — 15:55
  Lucía Fadón
  CIC biomaGUNE, Donostia

- Novel loss of function KCNA5 pathogenic variants in pulmonary arterial hypertension — 16:10
  Alba Vera-Zambrano
  Universidad Complutense, Madrid

Pausa 16:25-16:30

Comunicaciones. Sesión 2. 16:30-17:30

Moderadores: Pilar Escribano, Jesús Ruiz-Cabello, Lucilla Piccari

▪ Cigarette smoke-induced oxidative stress and mitochondrial dysfunction contribute to pulmonary arterial stiffness ▪ 16:30
María José Calzada
Universidad Autónoma, Madrid

▪ Non-invasive evaluation of pulmonary vasculopathy associated with advanced heart failure ▪ 16:45
Ángel Gaitán-Simón
Hospital 12 de Octubre, Madrid

▪ Valor pronóstico de la ergoespirometría en la hipertensión arterial pulmonar de bajo riesgo ▪ 17:00
Macarena Otero
Hospital 12 de Octubre, Madrid

▪ Predictors of the response to phosphodiesterase-5 inhibitors in pulmonary arterial hypertension ▪ 17:15
Agustín García
Hospital Clínic, Barcelona

Conferencia Magistral 17:30-18:20

▪ Converging Pathways Lead to a Novel Biologic, Small Molecule and Cell Based Therapy for Pulmonary Arterial Hypertension
Prof. Marlene Rabinovitch
Stanford University School of Medicine

Viernes 4 de marzo

Comunicaciones. Sesión 3. 15:00-16:30

Moderadores: Isabel Blanco, Ángel Cogolludo, Diego A. Rodríguez

- Study of a novel hypoxia system to evaluate endothelial cell dysfunction in chronic thromboembolic pulmonary hypertension (CTEPH) — 15:00
  Ylenia Roger
  Hospital Clínic, Barcelona

- Vitamin D deficiency, a potential cause for insufficient response to sildenafil in pulmonary arterial hypertension — 15:15
  María Callejo
  Universidad Complutense, Madrid

- Computational hemodynamic studies in porcine pulmonary artery for different spectrum of ARDS (acute respiratory distress syndrome) conditions — 15:30
  Rahul Kumar
  CIC biomaGUNE, Donostia

- Balloon pulmonary angioplasty in patients with non-operable or residual chronic thromboembolic hypertension — 15:45
  Clara Martín-Ontiyuelo
  Hospital Clínic, Barcelona

- Efficacy of a home respiratory rehabilitation program for patients with pulmonary hypertension. Proyecto Respira — 16:00
  Mar Esteban
  Fisiorespi, Madrid

- Effects of a cardiopulmonary rehabilitation program in patients with pulmonary hypertension — 16:15
  Rodrigo Torres-Castro
  Hospital Clínic, Barcelona

- Fundación Contra la Hipertensión Pulmonar — 16:30

Pausa	16:40-16:45

Comunicaciones. Sesión 4	16:45-18:15

Moderadores: Remedios Otero, José Luis Izquierdo, Olga Tura-Ceide

▪ Update and management of the Spanish Pulmonary Hypertension Biobank Teresa Botta-Orfila IDIBAPS, Barcelona	▪ 16:45
▪ Presentation of the animal model tissue biobank platform in pulmonary hypertension Víctor Peinado Hospital Clínic, Barcelona	▪ 17:00
▪ New insights on the role of beta3-adrenergic receptor as a new therapeutic target for pulmonary arterial hypertension Eduardo Oliver Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid	▪ 17:15
▪ Soluble guanylate cyclase stimulators reverse in vitro the effects of cigarette smoke through normalization of the c-Jun N-terminal kinase (JNK) pathway in pulmonary artery smooth muscle cells Adelaida Bosacoma Hospital Clínic, Barcelona	▪ 17:30
▪ MicroARNs circulantes como biomarcadores para la hipertensión arterial pulmonar Mauro Lago CINBIO, Universidad de Vigo	▪ 17:45
▪ Differential proteomic profile of patients with pulmonary embolism (PE) related to events during follow-up: chronic thromboembolic pulmonary hypertension (CTEPH) or occult cancer Verónica Sánchez-López Instituto de Biomedicina de Sevilla (IBiS)	▪ 18:00
▪ IL-11 increase levels and activates circulating fibrocytes in different pulmonary hypertension in vivo animal models Inés Roger Universidad de Valencia	▪ 18:15

▪ Despedida	▪ 18:30

Organiza:
Línea de Investigación en Hipertensión Pulmonar
Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES)

Instituto de Salud Carlos III ciberes Con la colaboración de: Fundación Contra la Hipertensión Pulmonar

Comunicaciones.
Sesión 1

The Role of Notch3 pathway in pulmonary hypertension

Zuriñe Blasco-Iturri
CIC biomaGUNE, Donostia

Zuriñe Blasco-Iturri[1,2,3], Jesús Ruiz Cabello[1,2], Ana Pardo Saganta[3]
[1]Center for Cooperative Research in Biomaterial (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Paseo Miramón 182,20014 Donotia-San Sebastián, Spain. [2]CIBER Enfermedades Respiratorias, Madrid, 28029, Spain. [3]Centro de Investigación Médica Aplicada (CIMA), Universidad de Navarra, Pamplona, 31009, Spain

Pulmonary hypertension (PH) is a disease characterized by excessive vascular smooth muscle cell proliferation in small pulmonary arteries, leading to right ventricle (RV) failure and death. Notch receptor signaling is implicated in controlling pulmonary artery smooth muscle cell (PASMC) proliferation and maintaining them in an undifferentiated state. The communication between PASMC and pulmonary artery endothelial cells (PAEC) seems to have a critical role in PAH development and since Notch3ICD expression is upregulated in PAH patients, we hypothesize that Notch3 signaling mediates this intercellular communication and represent a relevant mechanism to investigate.

We first characterized the Notch3 pathway in two PH disease mouse models: a) Hipoxia + Sugen and b) Bleomycin 14 days post induction (dpi). We observed an increase in NICD3-expressing cells in PH- induced lungs and in particular in PASMC (Figure 1). These cells exhibit higher proliferation as demonstrated by Ki67 immunostaining, and they present a lower expression of the markers of the contractile phenotype Myh11, transgrelin and calponin. In addition, we detect the Notch ligands Jag1, Jag2 and Dll1 in endothelial cells at homeostasis and Jag1 and Jag2 seems to be upregulated in PAH.

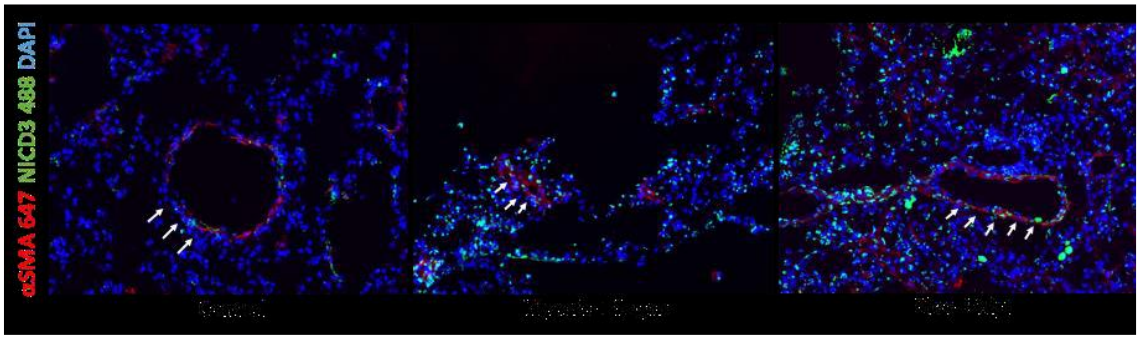


Figure 1: NICD3 activity in PA from control, Hipoxia+Sugen and Bleo 14dpi mice.

In conclusion, changes in the expression of Notch ligands in PAECs upon PH damage suggest that this may trigger the pathogenesis of PH promoting Notch3 activation in neighboring PASMCs driving the PH phenotype and therefore, these proteins emerge as therapeutic targets to prevent PH.

Expanding the evidence of a semi-dominant inheritance in GDF2 and KCNK3 associated with pulmonary arterial hypertension

Natalia Gallego Zazo
Instituto de Genética Médica y Molecular, Hospital Universitario La Paz, Madrid

Natalia Gallego[1,2,3], Alejandro Cruz-Utrilla[4,5], Inmaculada Guillén[6], Amparo Moya Bonora[7], Manuel López[8] Nuria Ochoa[4,5], Pedro Arias[1,2,3], Pablo Lapunzina[1,2,3], Pilar Escribano-Subias[4,5,9], Julián Nevado[1,2,3] and Jair Tenorio-Castaño[1,2,3].*
[1]Instituto de Genética Médica y Molecular (INGEMM), IdiPaz, Hospital Universitario La Paz, Madrid, Spain; [2]CIBERER, Centro de Investigación en Red de Enfermedades Raras, Instituto de Salud Carlos III, Madrid, Spain; [3]ITHACA, European Reference Network on Rare Congenital Malformations and Rare Intellectual Disa-bility; [4]Pulmonary Hypertension Unit, Department of Cardiology, Hospital Universitario 12 de Octubre, Madrid, Spain; [5]Centro de Investigación Biomédica en Red en Enfermedades Cardiovasculares, Instituto de Salud Carlos III (CIBERCV), Madrid, Spain; [6]Pediatric cardiology Unit, Department of Pediatrics, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [8]Lung transplant and pulmonary hypertension Unit, Hospital Vall d´Hebron, Barcelona, Spain; [9]Pediatric cardiology Unit, Department of Pediatrics, Hospital Universitario La Fe, Valencia, Spain; [8]ERN, European Reference Network Pulmonary Hypertension.

Background: Pulmonary arterial hypertension (PAH) is a severe and uncommon disease which causes right ventricle failure and potentially can lead to death if not treated. It has variable etiology and clinical expressivity, making sometimes the clinical diagnosis a challenge. The advance in massive paralleled sequencing in PAH has allowed the describing of several new causative and susceptibility genes related to PAH.

Methods: As part of the clinical routine and to stablish a genetic diagnosis, we have analyzed three patients diagnosed with primary and other associated forms of PAH trough whole exome sequencing. A custom pipeline for variant prioritization was carried out to obtain candidate variants and copy number variants. We also study segregation of the variants in available family members.

Results: We present 3 homozygous variants in *GDF2* and *KCNK3* in 3 patients diagnosed with PAH from different unrelated families. We have detected a homozygous missense variant in *GDF2* in a pediatric patient diagnosed with PAH associated with HHT and a missense variant along with a heterozygous deletion in another idiopathic PAH patient (compound heterozygous inheritance). On the other hand, an idiopathic PAH patient showed a homozygous missense variant in *KCNK3*. In order to establish variants segregation, we analyzed all available family members. In all cases, parents were heterozygous carriers for the variants, but neither is affected.

Conclusions: Our work adds evidence to the notion that variants in *GDF2* may generate a greater predisposition to develop both idiopathic PAH and a "HHT-like" syndrome. In the same way, *KCNK3* pathogenic variants are implicated in the development of PAH. Our results expand the clinical spectrum and the inheritance pattern associated with pathogenic variants detected in *GDF2* and *KCNK3* associated with PAH. These results suggest a semi-dominant pattern of inheritance, incomplete penetrance, and/or variability of expressivity.

Grants: PI18/01233, FCHP unrestricted grant

Characterization of the pulmonary vascular effects of URO-K10, a novel Kv7 channel activator

Marta Villegas-Esguevillas
Universidad Complutense, Madrid

Marta Villegas-Esguevillas, Alba Vera, Bianca Barreira, Laura Moreno, Sung Joon Kim, Francisco Pérez-Vizcaino, Belen Climent, Angel Cogolludo.

Pulmonary arterial hypertension (PAH) is a rare disease characterized by exaggerated pulmonary vasoconstriction and proliferation of pulmonary artery smooth muscle cells (PASMC). Voltage-dependent K^+ (Kv) channels are responsible for setting PASMC membrane potential, which in turn controls the opening of Ca^{2+} channels whose cytosolic concentration regulates contraction, proliferation, and hypertrophy of these cells. Recent studies have shown that Kv7 channels are novel players in the pulmonary circulation. Hence, their agonists may represent an attractive strategy for PAH treatment. In this study, we have analyzed for the first time the effects of URO-K10, a novel Kv7 channel modulator, on the pulmonary vasculature. Our data show that URO-K10 increases Kv current in control rat PASMC and in PASMC treated with channel inhibitors mimicking the characteristic ion remodeling that occurs in HAP. Additionally, this drug exerts a potent vasodilatation of pulmonary arteries that is markedly increased in arteries treated with the above-mentioned inhibitors. Noteworthy, the vasodilator efficacy of URO-K10 was considerably increased in pulmonary arteries from PAH (monocrotaline) rats. In addition, it exerts a concentration-dependent antiproliferative effect on human PASMC. URO-K10 is more effective as a vasodilator and as an antiproliferative agent than the classical Kv7 activator retigabine. Thus, compared to classical Kv7 activators, URO-K10 may represent a better alternative drug for PAH treatment.

Proteomics analysis of the RV of the heart in a rat model of SuHx-induced PAH treated with 2DG

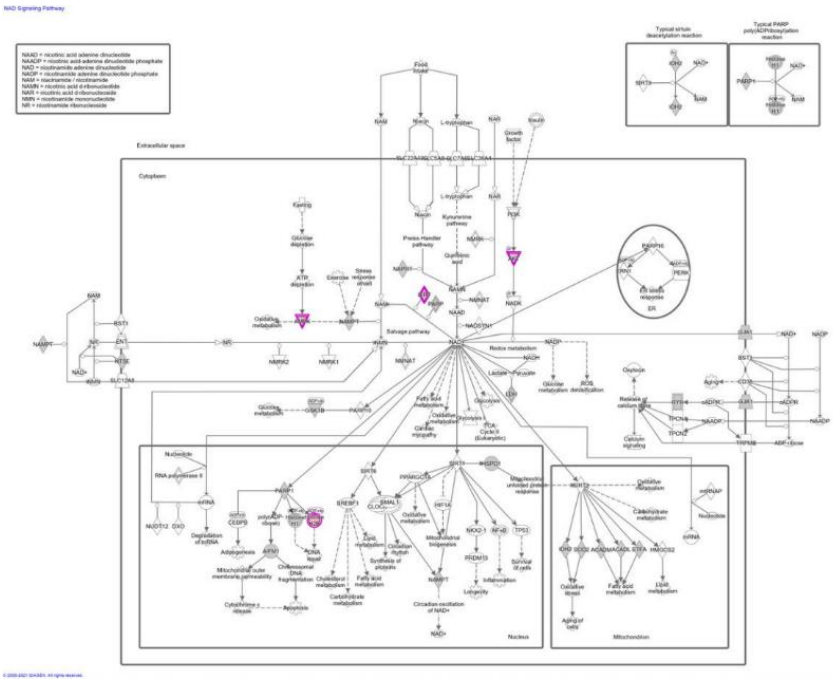
Lucía Fadón
CIC biomaGUNE, Donostia

Lucía Fadón Padilla, Zuriñe Blasco, María Jesús Sánchez-Guisado, Irati Aiestarán, Mikel Azkargorta, Félix Elortza, Ian Holt, Edurne Berra, Jesús Ruiz-Cabello

Although the underlying pathology of Pulmonary Arterial Hypertension (PAH) is highly heterogeneous, disparate manifestations appear to converge in metabolism and mitochondrial disfunction. Apoptosis resistance and excessive proliferation of PASMCs resulting from mitochondrial dysfunction is the core mechanism leading to PAH pathogenesis. On this basis, we have used 2-deoxyglucose (2-DG), a glucose analog that inhibits the glycolytic pathway, to enhance mitochondrial biogenesis and study the effects on the development of PAH.

We have previously presented how the PAH-associated vascular and RV phenotype improves in a SuHx-induced female rat model treated with 2-DG. The major manifestations of the disease, right ventricular systolic pressure (RVSP) and remodeling of the pulmonary vessels, present lower values in the treated group, which points to a beneficial effect of 2-DG in the context of PAH.

A protein expression analysis of the right ventricle (RV) of these rats has been performed by nLC-MS / MS technique and evaluated with Ingenuity Pathway Analysis (IPA) software. The results reveal that some pathways, such as the Estrogen Receptor Pathway or the Nitric Oxide Signaling in the Cardiovascular System Pathway are activated when PAH is treated with 2-DG. Interestingly, we found that the inhibition of NAD Signaling Pathway, associated with PAH in rats, is reversed in the presence of this glucose analog. These proteomic results could explain the beneficial effects of the drug. In addition, we have located some molecules, such as Sirtuin 2, which could be key in this process.



Novel loss of function KCNA5 pathogenic variants in pulmonary arterial hypertension

Alba Vera-Zambrano
Universidad Complutense, Madrid

Alba Vera-Zambrano[1,2,3], Mauro Lago-Docampo[4,5], Natalia Gallego[6,7,8], Juan Felipe Franco-González[9], Daniel Morales-Cano[10,11], Alejandro Cruz-Utrilla[12,13], Marta Villegas[1,14], Pilar Escribano-Subías[12,13], Jair Antonio Tenorio Castaño[6,7,8], Francisco Perez-Vizcaino[1,14] Diana Valverde[4,5], Teresa González[2,3], Angel Cogolludo[1,14]
[1]Department of Pharmacology and Toxicology, School of Medicine, University Complutense of Madrid, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain. [2]Department of Biochemistry, School of Medicine, Universidad Autónoma de Madrid, Madrid, Spain. [3]Instituto de Investigaciones Biomédicas "Alberto Sols" CSIC-UAM, Madrid, Spain. [4]CINBIO, Universidad de Vigo, Vigo, Spain. [5]Instituto de Investigación Sanitaria Galicia Sur (IIS Galicia Sur), SERGAS-UVIGO, Vigo, Spain. [6]Institute of Medical and Molecular Genetics (INGEMM)-IdiPAZ, Hospital Universitario La Paz-UAM, Madrid, Spain [7]CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain. [8]ITHACA, European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability, Hospital Universitario La Paz, Madrid, Spain. [10]Experimental Pathology of Atherosclerosis Laboratory, Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain. [11]Atherosclerosis Research Unit, Department of Clinical Medicine, Aarhus University, 8200 Aarhus, Denmark [12]CIBER Enfermedades Cardiovasculares (CIBERCV), Spain. [13]Unidad Multidisciplinar de Hipertensión Pulmonar, Servicio de Cardiología, Hospital Universitario 12 de Octubre, Madrid, Spain. [14]Ciber Enfermedades Respiratorias (CIBERES), Spain.

Pulmonary arterial hypertension (PAH) is a rare, debilitating and progressive disease. Kv1.5 channel was the first ion channel related to PAH. Since then, association of defective Kv1.5 channels with susceptibility to PAH has been extensively reported in both experimental and clinical PAH. However, there is no definitive consensus on its potential pathogenicity, and still considered as a gene with a low level of evidence as a causal factor. Thus, pathogenic variants in this gene remain an unvalidated causal factor mainly due to the lack of studies assessing their functional consequences.

In this study we selected seven *KCNA5* variants found in a cohort of PAH patients for functional analysis. Two additional variants were not analysed, as they encoded a truncated or aberrant proteins. We provide a detailed functional analysis of the other seven *KCNA5* variants which are all localized in the N-terminal domain and loops between the transmembrane segments of the channel.

We found that some of these variants (i.e. p.Arg184Pro and p.Gly384Arg) resulted in a clear loss of potassium channel function. In the case of Arg184Pro, the loss of Kv1.5 current appears to be due to both, a decreased channel conductance and reduced protein expression. This Kv1.5 channel dysfunction in p.Arg184Pro and p.Gly384Arg variants, which severely affected current amplitude, resulted in a decreased apoptosis of hPASMC compared with the WT, demonstrating that *KCNA5* dysfunction in both variants affects cell viability. Thus, in addition to affect channel activity, both variants were associated with a clear impairment in a key PASMC process linked to the disease.

In summary, here we demonstrate that some *KCNA5* variants found in PAH patients display a direct Kv1.5 dysfunction affecting PASMC viability. Our study is consistent with a role of *KCNA5* mutations as a risk factor for PAH and provides evidence for full consideration in genetic screening and patient management.

Comunicaciones.
Sesión 2

Cigarette smoke-induced oxidative stress and mitochondrial dysfunction contribute to pulmonary arterial stiffness

María José Calzada
Universidad Autónoma, Madrid

Sevilla-Montero J, Pino-Fadón J, Munar O, Balsa E, Choya-Foces C, Martínez-Ruiz A, Barreira B, Cogolludo A and Calzada MJ.

Background: Chronic obstructive pulmonary disease (COPD), whose main risk factor is cigarette smoking (CS), is one of the most common diseases globally. Many COPD patients also develop pulmonary hypertension (PH), a severe complication that leads to premature death. However, the effects of CS on the pulmonary vasculature are not completely understood. Evidence suggests reactive oxygen species (ROS) involvement in COPD and PH, especially regarding pulmonary artery smooth muscle cells (PASMC) dysfunction. CS might induce the expression of NADPH oxidase (NOX) complexes or mitochondrial damage, which are well-known sources of vascular ROS. Alternatively, CS could also affect antioxidant responses mediated by NRF2 pathway and diminish PASMC responsiveness to nitric oxide (NO). To test these hypotheses, we exposed human PASMC to cigarette smoke extract (CSE), analyzed ROS levels and their likely source, as well as the status of the antioxidant response. Furthermore, we aimed to analyzed if CSE effects on ROS production might also contribute to pulmonary arterial insensitivity to NO.

Results: Analysis of NRF2 and its target genes revealed insufficient activation of antioxidant defenses in hPASMC challenged with CSE. In addition to this, CSE-treated hPASMC demonstrated increased mitochondrial superoxide levels, reduced mitochondrial membrane potential and severely affected mitochondrial respiration and network morphology. Furthermore, CSE treatment decreased proteins involved in NO-signaling pathway, mainly reductase CYB5R3, this suggesting excessive sGC oxidation that would account for pulmonary arterial NO unresponsiveness. Most importantly, these effects were minimized when the PASMC and the pulmonary arteries were treated with specific mitochondrial antioxidants.

Conclusions: This study provides evidence that there might be a connection between mitochondrial ROS and altered vasodilation responses in COPD. Therefore, these studies strongly support the potential of antioxidant strategies, specifically targeting mitochondria, as a new therapy for these diseases.

Non-Invasive Evaluation of Pulmonary Vasculopathy Associated with Advanced Heart Failure

Ángel Gaitán-Simón
Hospital 12 de Octubre, Madrid

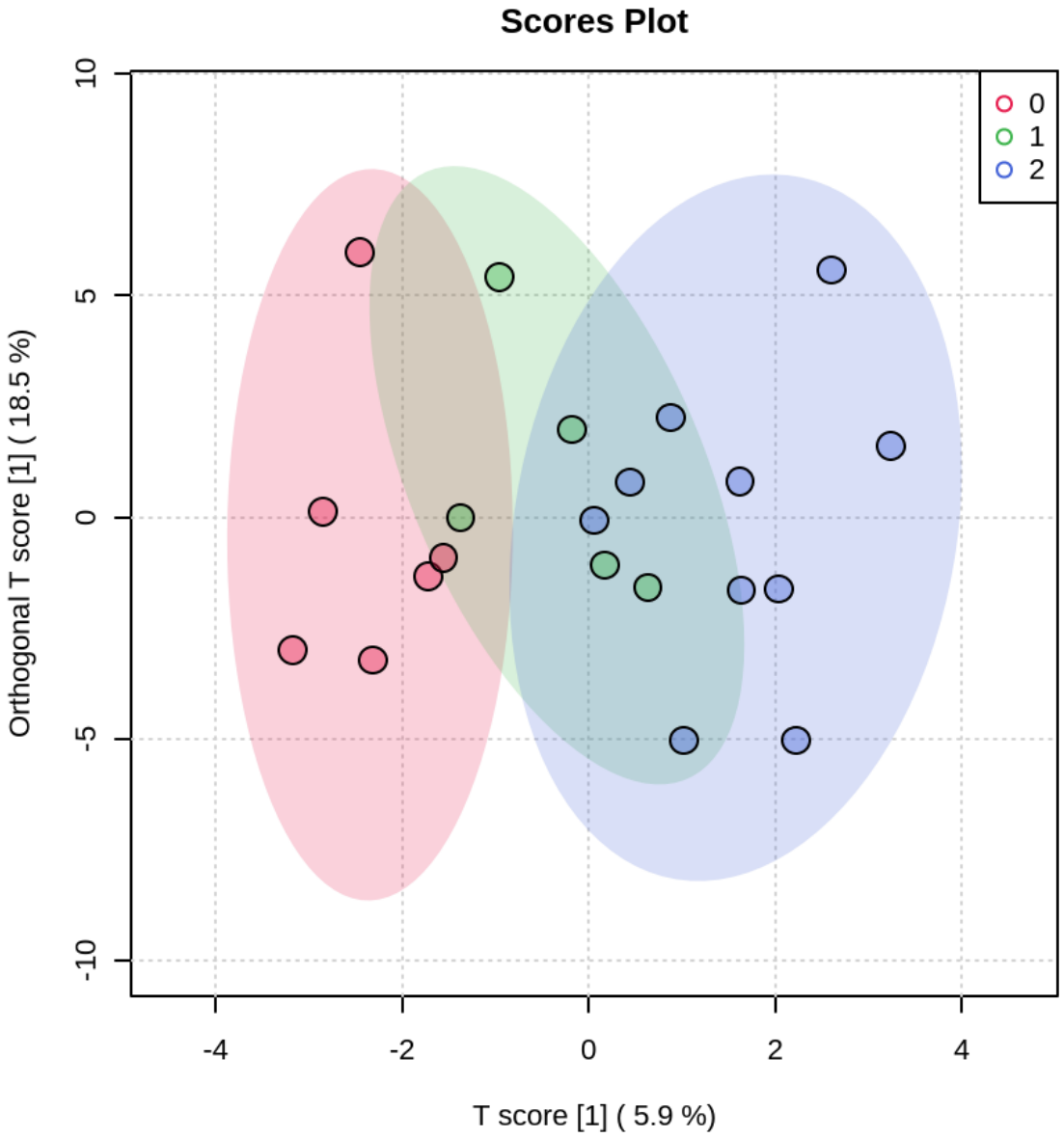
Ángel Gaitán-Simón, Jesús Ruiz-Cabello, Arnoldo Santos-Oviedo, Rahul Kumar, Jorge Nuche-Berenguer, Violeta Sánchez, Juan Delgado-Jiménez.

Introduction: Pulmonary hypertension (PH) is a chronic disease characterized by an increase in pulmonary vascular impedance and pulmonary vascular dysfunction and it eventually leads to right ventricle failure. Diagnosis must be confirmed in many cases by a right heart catheterization to measure a mean pulmonary artery pressure (mPAP) greater than 25mmHg.
We seek non-invasive diagnostic methods to characterize this disease. Cardiac Magnetic Resonance Imaging (MRI) and 4D Flow MRI (4DMRI) appear as non-invasive tools for right ventricle function and pulmonary vasculature evaluation.

Methods: More than 40 patients with PH associated with left heart disease (WHO group 2) are participating in a study from H.U. 12 de Octubre together with CNIC.
From 21 patients' 4DMRI, several flow parameters have been calculated at different points of the pulmonary artery using a semi-automatic segmentation method. Unsupervised (PCA, K-means) and supervised (sPL-DA, oSPL-DA) analysis have been performed to reduce the 67 variables calculated to the most significant ones and to classify these patients into groups.

Results: Helicity is the most significant variable given by oSPL-DA. Furthermore, it correlates with pvr ($r=0.545$, $p=0.010$) and tpg ($r=0.512$, $p=0.017$), the two variables on which the classification is based.
Figure 1 shows the oSPL-DA classification for patient classification by hemodynamic conditions: 0 for mPAP < 25mmHg; 1 for post-capillary PH; 2 for mixed PH.

Discussion & conclusion: Helicity appears as a promising biomarker which correlates with mean pulmonary artery for patients.
oSPL-DA shows a successful patient classification; hence we expect this model avoids the right heart catheterization.
10 more patients are going to be included in this analysis in order to validate the oPLS-DA model.


Scores Plot
0
1
2
Orthogonal T score [1] (18.5 %)
10
5
0
-5
-10
-4
-2
0
2
4
T score [1] (5.9 %)

Valor pronóstico de la ergoespirometría en la hipertensión arterial pulmonar de bajo riesgo

Macarena Otero
Hospital 12 de Octubre, Madrid

Macarena Otero, Raquel Luna, Teresa Segura, Alicia Ruíz, Alejando Cruz, Williams Hinojosa, Pilar Escribano.

En la hipertensión arterial pulmonar (HAP) la valoración pronóstica a través del test de la marcha de 6 minutos (TM6M) puede resultar insuficiente por la existencia de un efecto techo, especialmente en pacientes jóvenes. El objetivo de este estudio es demostrar el valor pronóstico añadido de la valoración mediante ergoespirometría en pacientes en bajo riesgo según el TM6M.

Para ello realizamos un estudio de cohortes retrospectivo en pacientes con HAP referidos a un centro entre junio 2006 y junio de 2018, que reunían los siguientes criterios: PAPm ≥20 mmHg, PCP≤ 15 mmHg, RVP >3 UW, edad>18 años y contar con una ergoespirometría realizada en la misma semana que el TM6M. Comparamos las variables no invasivas de dichos test entre los supervivientes y los no supervivientes (trasplante o muerte) a los 3a desde la ergoespirometría. Para las variables que presentaban significación estadística, se determinaron unos puntos de corte óptimos para dicha supervivencia y se valoró el poder pronóstico añadido de la ergoespirometría asociada al NT-ProBNP en pacientes de bajo riesgo según el TM6M (definido como >440 metros) mediante un análisis de supervivencia.

Incluimos un total de 205 pacientes (32% hombres) durante una mediana de 8,7 años (rango 4,4-11,9). En el seguimiento a 3 años, 38 pacientes murieron o precisaron de un trasplante pulmonar. 116 pacientes (64,8%) caminaron más de 440 metros en el TM6M, siendo este subgrupo significativamente más joven (42 ±11 -vs- 48±16 años). De dichos pacientes, 13 (11,2 %) murieron o precisaron de trasplante a los 3 años. En los pacientes que caminaban más de 440 metros, aplicamos el modelo de variables no invasivas asociado al pronóstico de acuerdo con los puntos de corte óptimos obtenidos (Equivalente de CO2 en 1er umbral-VE/VCO2U1- ≥41; consumo pico de O2 (VO2 pico) < 15 ml/kg/min, NT-ProBNP ≥950 pg/ml). El análisis de supervivencia de Kaplan–Meier demostró que los pacientes con NT-ProBNP ≥950 y VE/VCO2U1- ≥41 y/o VO2 pico <15ml/kg/min tenían un peor pronóstico a los 3 años de seguimiento, pese a caminar más de 440 metros en el TM6M.

Predictors of the response to phosphodiesterase-5 inhibitors in pulmonary arterial hypertension

Agustín García
Hospital Clínic, Barcelona

García, Agustín Roberto; Blanco, Isabel; Borrás, Roger; López-Meseguer, Manuel; Domingo-Morera, Juan Antonio; Martin-Ontiyuelo, Clara; Tura-Ceide, Olga, Escribano-Subías, Pilar; Barberà, Joan Albert.

Achievement of a low risk profile with targeted therapy is a strong predictor of long-term outcome in pulmonary arterial hypertension (PAH). However, treatment effect on modifying risk profile is variable among patients. Thus, the identification of clinical phenotypes more likely to respond to a particular class of drug, or vice versa, will help clinicians in guiding treatment decisions. This study is aimed to identify variables that might predict a favourable response to phosphodiesterase-5 inhibitors (PDE-5i).

Methods: We extracted PAH patients from the Spanish Registry (REHAP) followed for ≥1 year that started PDE5i treatment as monotherapy or add-on therapy during a period from 2007 to 2019. Initial combination treatment was excluded. Were considered responders when they met three criteria at 12 months follow-up: 1) alive; 2) no clinical events leading to treatment change; 3) improved ESC/ERS risk score or remained in low-risk.

Results: 644 patients analyzed: 213 classified as responders and 431 as non-responders. Hemodynamic severity at diagnosis were similar in both groups and not related to response. Better risk profile at baseline was associated with higher response (OR 5.55; p<0.001). In the univariate analysis, male sex 1 (OR 1.61, p=0.006) functional class I-II (OR 14.2; p<0.001), 6-minute walk distance ≥≥ 440 mts (OR 3.40; p<0.001) and NT-proBNP ≤≤ 300 pg/ml (OR 3.22; p<0.001) at baseline were associated to the favorable response to PDE-5i. Background PAH therapy (OR 1.2; p=0.265) were not significative related to the response to PDE-5i. In the multivariate analysis, women over > 50 years, diagnosed with IPAH, and with DLCO < 40% emerged as independent predictors of poorer treatment response.

Conclusion: One in three PAH patients responded favorably to starting PDE-5i therapy. Male patients under 50 years, diagnosed with portopulmonary arterial hypertension and HIV-PAH, and in low-risk category at baseline have higher likelihood of response to PDE-5i. Those patients identified as non-responders might benefit from enhancing NO pathway by using guanylate-ciclase stimulator, riociguat.

Comunicaciones.
Sesión 3

Study of a novel hypoxia system to evaluate endothelial cell dysfunction in chronic thromboembolic pulmonary hypertension (CTEPH)

Ylenia Roger
Hospital Clínic, Barcelona

Ylenia Roger, Isaac Almendros, Esther Marhuenda, Adelaida Bosacoma, Anna Sardiné, Ana Ramírez, Victor I. Peinado, Isabel Blanco, Manuel Castellà, Joan A. Barberà, Olga Tura-Ceide.

Oxygen (O_2) plays a key role in respiratory diseases and hypoxia can be essential in the progression of diseases such as CTEPH. The endurance of hypoxic conditions can contribute to a metabolic shift characterized by an abnormal cell proliferation, tissue hypertrophy and remodelling.

The aim of the study is to evaluate the effect of chronic hypoxia on endothelial cells (ECs) derived from patients with CTEPH (EC-CTEPH) compared to healthy controls and to identify potential differences when ECs are subjected to different O_2 culture conditions.

Methods: Both patient and control cells were cultured under different O_2 conditions (1%, 4%, 13% and 21% O_2) for 48h. qRT-PCR for angiogenic and metabolic genes and supernatant (SN) analysis were performed using EC-CTEPH (n=6). Healthy ECs were used as control group (n=6).

Results: Both cell populations react to hypoxia by upregulating hypoxic responsive genes such as VEGF or NIP3. Genes related to oxidative stress (NOX4, SOD2) are also significantly upregulated under hypoxia compared to normoxia in both groups.
Genes related to glycolytic pathway such as HK2 or LDHA are only upregulated in control cells under 1% O_2. These genes are not significantly upregulated in EC-CTEPH. This correlates with a lack in lactate production in EC-CTEPH. Whereas lactate production is increased in control cells under hypoxia, it is not increased in EC-CTEPH.

Conclusions: Both cell populations respond to hypoxia (1% O_2) by upregulating genes involved in different pathways.
EC-CTEPH present an altered metabolic reprogramming with a reduction in glycolytic gene expression under hypoxia compared to healthy controls.

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Vitamin D deficiency, a potential cause for insufficient response to sildenafil in pulmonary arterial hypertension

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Background: Phosphodiesterase 5 inhibitors (PDE5i), such as sildenafil are frequently used to treat pulmonary arterial hypertension (PAH). However, a sizeable proportion of PAH patients fail to maintain treatment goals with PDE5i. The results from REPLACE study show that patients remaining at intermediate risk, switching to riociguat is beneficial in terms of clinical improvement as compared to PDE5i maintenance therapy. Several reports have shown that vitamin D (vitD) regulates the NO signaling pathway in systemic arteries.

Objectives: The aim of this study is to analyze if vitD deficiency in PAH may account for the limited efficacy of sildenafil in some patients.

Methods: The vasodilator response to sildenafil and riociguat were studied in isolated pulmonary arteries (PA) from rats with PAH that had been exposed to vitD-free diet for 8 weeks with those after restoring vitD status for the last 3 weeks. We retrospectively compared the 25(OH)vitD plasma levels of PAH patients that responded (n=17) vs those who did not respond (n=13) to PDE5i. Plasma samples were obtained from the Spanish National Pulmonary Hypertension Biobank and clinical data from the REHAP.

Results: VitD deficiency led to a poor vasodilator response to sildenafil in isolated PA which can be reverted by restoring vitD levels. The response to riociguat was unaffected by the vitD status. The responders to sildenafil had significantly higher 25(OH)vitD levels than non-responders.

Conclusions: VitD deficiency causes a poor vasodilator response to PDE5i, but preserved response to riociguat in rats with PAH, indicating that vitD deficiency reduces the apparent basal NO-dependent cGMP production. Lower levels of 25(OH)vitD in non-responders to PDE5i compared to responders suggest that vitD deficiency may cause insufficient response to PDE5i in some patients with PAH.

Computational hemodynamic studies in porcine pulmonary artery for different spectrum of ARDS (Acute respiratory distress syndrome) conditions

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The clinical definition of ARDS in adults is "Rapid onset of noncardiogenic pulmonary edema", which is an abnormal accumulation of fluid in the extravascular compartments of the lungs. The hemodynamic instability is a primary factor influencing mortality and the mortality rate among COVID-19 ARDS ventilated patients is one of the most disheartening published results in 2020. The important question is, can we correlate flow alterations and ARDS and come up with the good predictors?

The purpose of this study is to characterize flow patterns and several other hemodynamic parameters using computational fluid dynamics model by combining imaging data from 4D-Flow MRI with hemodynamic pressure and flow waveforms from control and hypertensive subjects (related to acute respiratory distress syndrome).

A realistic and automated segmented geometry of pulmonary artery for mechanically ventilated conditions (Baseline, ARDS, Optimal Lung Approach, Hyperinflation and Collapse) is obtained by training a 2D convolutional neural network (CNN) on CTA scans obtained by translating MRA images. Fluid dynamics equations are solved for the subject specific pulmonary arterial meshes. This work considers the utility of computational models in providing insights into identifying abnormal flow features of the pulmonary circulation, and their application in clinically motivated studies related to ARDS. Specific attention is devoted to mutual validation of quantitative parameters generated from CFD and PC-MRI images.

So far, the analysis is done on six subjects with five ventilatory conditions (Basal, ARDS, OLA, HI, COL). Different hemodynamic parameters are analysed after in-vivo validation of velocity profiles and then important predictors is presented after statistical validation. This work mainly concerns how to facilitate bench-bedside approach using CFD tools and is a continuation of our previous work with diseased cases.

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Balloon pulmonary angioplasty in patients with non-operable or residual chronic thromboembolic hypertension

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Introduction: Balloon Pulmonary Angioplasty (BPA) has become an emerging and complementary strategy for chronic thromboembolic hypertension (CTEPH) patients who are not suitable for pulmonary endarterectomy (PEA) or present recurrent symptoms after PEA. The purpose of our study was to evaluate the efficacy and safety of BPA in patients with non-operable or residual CTEPH during the initial 5-year experience in a single national referral center.

Methods: Fifteen consecutive, non-operable or with residual CTPEH after PEA, anatomically suitable, symptomatic patients on stable medical therapy for CTEPH were identified and offered BPA between January 2017 and December 2021. Baseline assessment was performed using pulmonary hemodynamics, New York Heart Association (NYHA) functional class, 6-minute walking distance (6MWD), and N-terminal pro b-type natriuretic peptide (NT pro-BNP). Serial BPA sessions were then performed. The treatment effect was measured by comparing the same values before and 3-6 months after all BPA sessions. The Society of Interventional Radiology (SIR) adverse event classification was used to grade procedure-related complications.

Results: A total of 76 procedures were performed, with a median of 5 BPA sessions per patient (range, 4-7). Mean pulmonary arterial pressure (PAPm) (preBPA: 32+10 vs postBPA: 26+7mmHg, p=0.001), pulmonary vascular resistance (PVR) (421±193 vs 290 ±114 din/s/cm5, p=0.001) and NYHA functional class I-II (%) (60% vs 100%, p=0.05) were significantly improved. The 6MWD (475±101 vs 484±87, p=0.29) and NT pro-BNP (187± 81 vs 159 ± 46 pg/mL, p=0.65) tended to improve too. No deaths or major complications requiring invasive ventilation were reported. The most common complication was lung injury (shown as mild to moderate hemoptysis or temporarly hypoxemia).

Conclusion: In our initial experience, BPA significantly improves cardiopulmonary hemodynamics with an acceptable safety profile in patients with non-operable or residual CTEPH.

Efficacy of a Home Respiratory Rehabilitation Program for patients with Pulmonary Hypertension. – Proyecto Respira -

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Introduction: Respiratory Rehabilitation Programs (RRP) have demonstrated efficacy and benefits for patients with Pulmonary Hypertension (PH). RRP are hospital programs limited in time, usually spanning 2 to 3 months. Subsequently, patients must continue their rehabilitation on their own; however, dropout rates are often high due to a variety of reasons, including lack of resources at home and direct supervision. The Respira project aims to reinforce the knowledge acquired during outpatient hospital treatment for patients to continue the RRP at home with the resources available to them and under the supervision of a specialized physiotherapist.

Objective: To improve or maintain patients' respiratory capacity, functional capacity, exercise tolerance and quality of life achieved after outpatient RRP.

Material and methods: At the end of inpatient PRR the patient undergoes a first evaluation (Table 1). Subsequently, home RR begins (Table 2), which consists of a weekly face-to-face session at home during the first month. For the following 5 months, a weekly telephone consultation and a monthly face-to-face home reminder session is carried out.
Following the first 6 months, a second evaluation is carried out in the same way as the first one.

TABLE 1. EVALUATION

Variable	Functional Capacity	Respiratory Capacity	Muscular Strength	Physical Activity Level	Quality of life
Method of evaluation	6´Walking test	Spirometry and PIM (Photo1)	Hand Grip	IPAQ Questionnaire	Questionnaire SF-12

TABLE 2. HOME-BASED RESPIRATORY REHABILITATION PROGRAM				
Type of Exercise	**Respiratory physiotherapy techniques**	**Respiratory muscle training**	**Muscle strength (Photo 2)**	**Cardiorespiratory Endurance Exercise (Photo 3)**
Frequency	3-5 days/week	5 days/week	3-5 days per week	5-7 days/week
Duration / Mode	10 repetitions 1 set	30 repetitions 2 series	10 repetitions 3 sets	20 - 30 minutes Walking, biking or similar
Intensity	--	30-40% PIM	7-8 Borg	5-6 Borg

Results: A total of 6 patients have already been enrolled in the study. Initial measurements have been taken and data on the progress of the program are being collected.

Conclusions: Pending definitive results, we observed good subjective acceptance, which may reflect greater adherence and safety when performed under the supervision of a specialized physiotherapist.

Effects of a cardiopulmonary rehabilitation program in patients with pulmonary hypertension

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Introduction: Cardiopulmonary rehabilitation (CPR) has shown to be beneficial in multiple chronic respiratory diseases; however, it is still controversial in pulmonary arterial hypertension (PAH). The aim of the project is to evaluate the effect of physical training on markers of endothelial function and to identify those biomarkers and/or clinical characteristics associated with a better therapeutic response, in patients with PAH. Responders and non-responders will be identified for a 12-week resistance training program.

Methods: We performed a prospective, study of a 3-month CPR program in patients with PAH. The inclusion criteria were age over 18 years, and stable status for at least two months before entering the study. Subjects were classified as responders if the endurance time was greater than or equal to 200 seconds. We compared anthropometric measures, pulmonary function, hemodynamic measures, and physical capacity variables (endurance time, peak oxygen consumption (VO2peak), maximal workload (Wmax), and six-minute walk distance (6MWD) between both groups. Additionally, biological samples were taken that are pending.

Results: We recruited 27 patients. We have 4 drops out (two by pandemic, one by lung transplant, and one abandoned). Finally, 23 patients (18 female) were analysed, 19 responders and 4 non-responders. The baseline characteristics are shown in figure 1. In the responders group, the endurance time increased from 238±76 to 742±171 seconds ($p<0.001$), VO_2peak increased from 13.5±4.8 to 15.4±4.8 ($p=0.01$), Wmax increased from 82±35 to 97±40 ($p<0.001$). In the non-responders group, we do not found statistical difference between pre and post intervention in none of the exercise tolerance variables.

Conclusion: A 3-month structured program improved exercise capacity in patients with PAH. Most of the patients were rated as responders. Once the sample size is reached, we will evaluate the relationship between this response and the biomarkers from blood samples both at baseline and their change with the CPR.

Comunicaciones.
Sesión 4

Update and management of the Spanish Pulmonary Hypertension Biobank

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The Spanish Pulmonar Hypertension Biobank 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) in order to promote and facilitate biomedical research. Clinical information is available at the Spanish Pulmonary Arterial Hypertension Registry (REHAP). Recently, samples from pulmonary hypertension associated with respiratory diseases 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 of the BEHIP functioning and outcomes focusing on the sustainable collection of samples and associated data.

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

BEHIP is a consolidated and dynamic repository of biological samples from patients with pulmonary hypertension. The BEHIP has collected samples from 593 donors with 12 different diagnoses, obtained at 8 Spanish hospitals. The generated aliquots are 576 extracted DNA, 1705 normalized DNA, 5210 plasma, 4185 serum and 783 PBMCs aliquots. In addition, samples are linked to their clinical information contained in national registries.

BEHIP is the result of a well-established synergy of multidisciplinary collaborations between clinical/research centers and platforms such as HCB-IDIBAPS Biobank. The availability of samples from non-PAH patients (CTEPH and respiratory associated PAH) and the associated clinical information add a high strategic value to the collection. Finally, a data review revealed that IC record is not as fast as sample processing, which means that the Biobank should improve these records to foster efficacy in sample procurement to research projects.

The authors are deeply in debt to the sample donors and their relatives, as well as to the technical staff from Biobank HCB-IDIBAPS and professionals from the clinical centers. Founded by: ISCIII (PI12/00510), SEPAR, CIBERES and FCHP.

Presentation of the animal model tissue biobank platform in pulmonary hypertension

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Definition: The Animal Model Tissue Biobank Platform in Pulmonary Hypertension (AMTBPPH) aims to be a transversal, public and non-profit structure, promoted by the Pulmonary Hypertension area of the CIBER of Respiratory Diseases (CIBERES) in which research groups participate voluntarily providing lung tissue samples, fluids and/or tissues from other organs as well as relevant physiological and/or molecular information obtained in preclinical studies carried out in animal models of respiratory pathology.

Justification: The use of biological samples obtained in preclinical animal models of respiratory diseases is an indispensable tool in biomedical lung research. The creation of biobanks makes it possible to obtain tissue collections that research groups cannot achieve individually, either because of the technical difficulty in carrying out different preclinical models or because researchers work in institutions that lack the necessary infrastructure (animal facilities).

Target: Through a database, accessible through a web page (http://phmodelbank.org), the objective of the AMTBPPH is to act as an intermediary between the different CIBERES research groups to promote collaboration in carrying out preclinical biomedical research projects in the field of pulmonary hypertension and respiratory diseases, always within the framework of the technical requirements, legal and ethical collected in the Spanish legislation.

Strategic interest: Promote the development of research by the Ciberes groups themselves, facilitating access to biological samples of animal models of pulmonary pathology. Therefore, the actions of AMTBPPH will be to coordinate the different research groups that voluntarily agree to it. This strategy will allow: (1) to increase the number of lung tissue samples accessible to researchers; (2) guarantee the homogeneity and quality in obtaining and managing biological samples in accordance with agreed protocols and standards; (3) Promote collaboration between different Biomedical Research groups; (4) Reduce the use of animals intended for research purposes.

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New insights on the role of beta3-adrenergic receptor as a new therapeutic target for pulmonary arterial hypertension

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The role of β3-adrenergic receptor (β3-AR) in heart and vessels has been widely studied, and the use of β3-agonist has been proposed as a therapeutic strategy in certain cardiovascular diseases. Pulmonary arterial hypertension (PAH) is originated by an aberrant vascular remodelling characterized by endothelial dysfunction and vascular cell proliferation. These changes lead to an increase in pulmonary arterial pressure followed by right ventricular hypertrophy, heart failure and premature death.

To clarify the role of β3-AR in PAH and whether it may be a potential therapeutic target, we have used two animal models: hypoxia- and monocrotaline-induced PAH in mice and rats, respectively. In addition, we have analysed both β3-AR knockout and transgenic mice, with conditional cell-specific restoration of β3-AR expression in a β3-AR knockout background.

We found that loss of β3-AR aggravates the PAH phenotype while its restoration in endothelial cells (EC), but not in cardiomyocytes nor in vascular smooth muscle cells (SMCs), leads to an ameliorated pathophysiology. This amelioration is reflected in a decrease in RVSP, RV hypertrophy, arterial remodelling, SMC proliferation and a recovered endothelial dysfunction (reflected by a decreased ectopic vWF expression and normalized NO production). Accordingly, pharmacological activation of β3-AR, both in mice and rats also leads to better hemodynamic and pathophysiological parameters. In vitro experiments, with human pulmonary artery EC and SMC, demonstrate that activation of endothelial β3-AR induces NO production, which acts indirectly on SMCs to regulate vasodilation and proliferation. Concurrently, pharmacological activation of β3-AR ceases to have any beneficial effect in eNOS knockout mice exposed to hypoxia. Additionally, β3-AR regulates ROS levels and shows a role in controlling endothelial cellular stress and mitochondrial fitness.

In conclusion, β3-AR stands as a new therapeutic target for the treatment of PAH and other pulmonary vascular diseases in which endothelial dysfunction plays a relevant role.

Soluble guanylate cyclase stimulators reverse *in vitro* the effects of cigarette smoke through normalization of the c-Jun N-terminal kinase (JNK) pathway in pulmonary artery smooth muscle cells

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In a guinea pig model of COPD, we identified that emphysema and/or pulmonary hypertension (PH) induced by cigarette smoke (CS) was related to the alteration of genes of pathways like inflammation, apoptosis and cell proliferation (AJP LCMP 2019; 317: L222-34). Treatment with a soluble guanylate cyclase (sGC) stimulator induced the normalization of ≈50% of altered genes, especially those related to the MAPK pathway, while improving PH values and reversing emphysema. Even more, an in-silico analysis assessing the effect of cigarette smoke in humans, also showed that genes related with JNK pathway were altered in smokers.

The aim of the present study was to evaluate *in vitro* the effects of CS on JNK pathway in endothelial (HPAEC) and smooth muscle cells (PASMC) of human pulmonary arteries, as well as the effects of the sGC stimulator, BAY63-2521.

Commercially available human lung cells (HPAEC and PASMC) were cultured with CS extract (CSE) (diluted range 0-1/5) and BAY63-2521 (100uM) to analyse cell viability and JNK-related gene expression, by qRT-PCR.

The results showed that growth rate diminished after addition of CSE in both cell types. Besides, CSE (1/5) significantly increased the expression of *JUN* and *FOS* in both HPAEC and in PASMC as well as the genes related to apoptosis *CASP3* and *P21*. Remarkably, in PASMC cultured with CSE and treated with BAY63-2521, but not in HPAEC, the expression level of these genes was normalized to controls.

In conclusion, these results highlight the importance of PASMC in the normalization of JNK pathways by sGC stimulators and suggest a prominent role of these cells in the control of PH and CS-induced emphysema.

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MicroARNs circulantes como biomarcadores para la Hipertensión Arterial Pulmonar

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Pulmonary Arterial Hypertension (PAH) is a rare disease where the thickening of the precapillary pulmonary arteries ends up inducing right heart failure. The prognosis of PAH patients depends on multiple factors, being the time of diagnosis a critical one. Currently, diagnosis is complicated and usually delayed until performing right-heart catheterization.

In this work, we perform small RNA sequencing in plasma of idiopathic PAH patients and controls. We were able to find 29 differentially expressed microRNAs and validate 7 of them in a nationwide cohort (let-7a-5p, let-7b-5p, let-7c-5p, let-7f-5p, miR-9-5p, miR-31-5p, miR-3168). We then used classification models to analyze their potential as PAH predictors. In the first half of our cohort, we obtained a model with an AUC of 0.888. Although, this value lowered to 0.738 after adding the information of the whole cohort of patients. Also, we identified miR-3168 as a novel upregulated miRNA in PAH patients. After functional characterization we demonstrate that it targets the Bone Morphogenetic Protein Receptor type 2 (BMPR2), as validated at mRNA and protein levels. Preliminary results show that miR-3168 overexpression has no effect in apoptosis resistant but decreases angiogenesis.

In conclusion, we found novel downregulated and upregulated microRNAs in idiopathic PAH patients. We were able to develop a 3-microRNA signature for diagnosis and functionally characterized *in vitro* the effect of miR-3168 as a possible modulator of the disease.

Differential proteomic profile of patients with Pulmonary Embolism (PE) related to events during follow-up: chronic thromboembolic pulmonary hypertension (CTEPH) or occult cancer

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Introduction: In the follow-up of patients diagnosed with PE, either CTEPH or cancer can be identified. We hypothesized that cancer and CTEPH might share pathogenic pathways such as inflammation, cell proliferation, or apoptosis.

Aim: Analyze the proteomic profile of patients with CTEPH, uncomplicated PE and PE patients with occult cancer to identify proteins dysregulated in PE group compared to those with occult cancer and CTEPH.

Methods: Fourteen patients with CTEPH, 6 with venous thrombosis disease (VTD) who were subsequently diagnosed with cancer, and 7 with PE who did not present neither CTEPH nor occult cancer after 2 years follow-up were evaluated. Citrated plasma was obtained from all of them and used for protein quantification in a mass spectrometer by iTRAQ© labeling. After quality control and normalization, differential expression analysis was performed using the Kruskal-Wallis test with the Benjamini-Hochberg (BH) correction for multiple testing.

Results: A total of 382 proteins were determined in all groups, 28 were found to be differentially expressed in the 3 groups of patients. We selected five of them based on their under- or overexpression in the PE group, compared to the groups that developed cancer or CTEPH (Table 1).

Conclusion: The circulating proteins identified could help to differentiate patients with PE who are at risk for presenting subsequent cancer or CTEPH.

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Table 1. Protein ratio in PE, CTEPH and VTD+cancer groups

	PE (N=7)	CTEPH (N=14)	VTD+cancer (N=6)
Zinc finger protein 850	0.94(0.32)	0.60(0.14)	0.91(0.22)
Apolipoprotein M	*0.83(0.08)*	*1.03(0.09)*	*0.85(0.12)*
Plasminogen	*0.81(0.07)*	*0.97(0.05)*	*0.82(0.10)*
Lumican	*0.81(0.14)*	*1.16(0.18)*	*0.83(0.05)*
Coagulation factor XII-Mie	*0.77(0.12)*	*1.07(0.17)*	*0.78(0.08)*

Results were expressed as mean (SD). Cursive letter: protein under-expressed in PE group compared to CTEPH and VTD+cancer. Normal letter: protein over-expressed in PE group compared to CTEPH or VTD+cancer group.

IL-11 increase levels and activates circulating fibrocytes in different pulmonary hypertension *in vivo* animal models

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Background: Fibrocytes are described as circulating bone-marrow-derived cells with the ability to adapt to myofibroblast-like phenotype contributing to lung fibrosis and pulmonary artery remodeling and hypertension. Recent works indicate that IL-11 system promotes lung fibrosis and pulmonary hypertension (PH), but cell target and mechanism is poorly understood.

Objective: To analyze the role of IL-11 on circulating fibrocytes in *in vitro* and *in vivo* models relevant to PH.

Methods: Human fibrocytes were isolated from whole blood and stimulated with IL-11, soluble (s)IL-11Rα or its combination for 48h. Fibrocyte to myofibroblast-like transition and fibrocyte adhesion to pulmonary artery endothelial cells (HPAECs) were evaluated measuring the increase of myofibroblast markers and using video-microscopy flow chamber respectively.
The contribution of IL-11 to circulating fibrocytes was evaluated in three animal models. Transgenic Tie2-endothelial-GFP reporter mice were subjected to daily subcutaneous injections of recombinant mouse IL-11 or saline for 20 days. Sprague-Dawley rats and transgenic Tie2-endothelial-GFP reported mice received an intratracheal single installation of monocrotaline at 60 mg/kg or bleomycin at 1.5U/Kg respectively. IL-11 siRNA or non-targeting control siRNA were administered to the rats by intranasal and intravenous delivery every other day during 21 days or 14 days respectively. The circulating fibrocytes were measured by flow cytometry.

Results: IL-11 and the combination with sIL-11Rα induce the transformation of circulating fibrocytes to myofibroblasts and participate in their recruitment and adhesion to the HPAECs. Furthermore, IL-11 mice infusion promotes pulmonary fibrosis and PH and increased the number of circulating and bronchoalveolar fibrocytes with parallel increased levels of CXCL12 in serum. In rats or mice treated with monocrotaline or bleomycin respectively, there was an increase in the number of circulating fibrocytes and the protein levels of CXCL12. Animals transiently transfected with siRNA-IL-11 decreased circulating fibrocytes in both animal models

Conclusions: IL-11 promotes an increase of circulating fibrocytes, pulmonary artery endothelial adhesion and phenotypic switching to myofibroblast-like cells.

Organiza:
Línea de Investigación en Hipertensión Pulmonar
Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES)

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