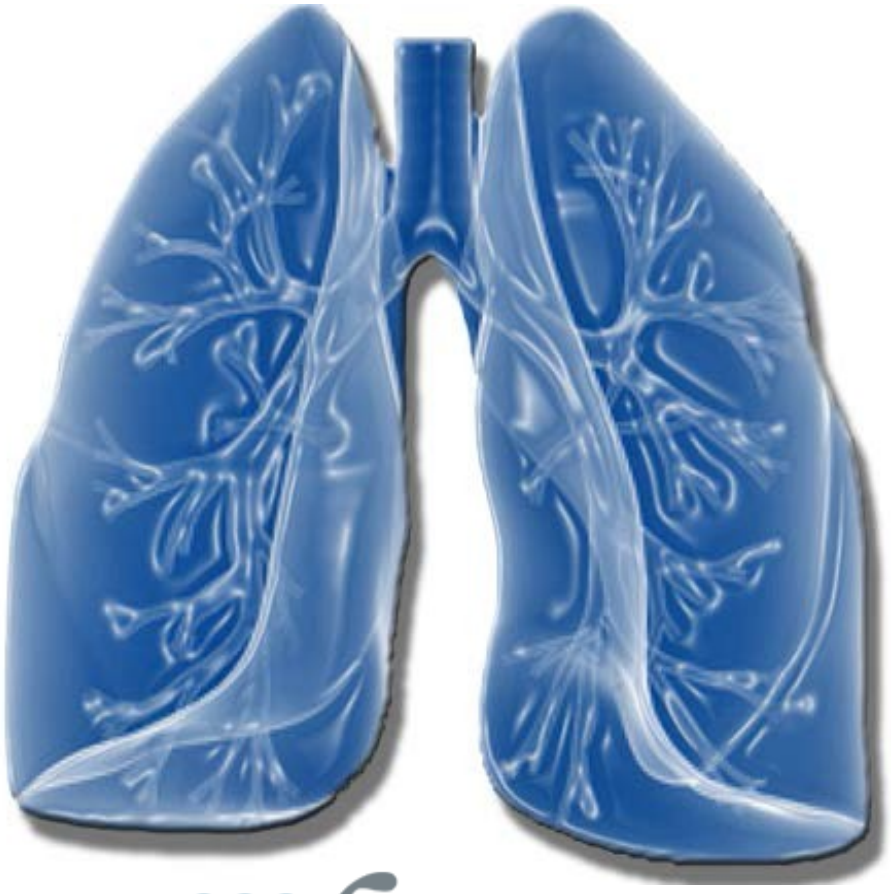
2ª Reunión de Investigación en Hipertensión Pulmonar



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Viernes 2 de Febrero de 2018
9:30 - 17:30 horas
Aula de conferencias. Departamento de Farmacología
Facultad de Medicina
Universidad Complutense Madrid
2ª planta Pabellón 3
MADRID

2nd Research Meeting on Pulmonary Hypertension

Organized by:

Line of Research in Pulmonary Hypertension

Biomedical Research in Respiratory Diseases

Network (CIBERES)

Madrid, February 2nd, 2018

9:30 - 17:30 h

Venue / Sede

Conference room 2nd floor. Department of Pharmacology

Faculty of Medicine, University Complutense of Madrid

Fundación Contra la Hipertensión Pulmonar

MSD

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Welcome

On behalf of the organizing committee, we are pleased to welcome you to the second Research Meeting on Pulmonary Hypertension organized by The Line of Research in Pulmonary Hypertension from the Biomedical Research in Respiratory Diseases Network (CIBERES). The meeting aims at disseminating recent research advancements in the field of pulmonary hypertension, offering researchers the opportunity to present their novel results and to foster collaborative actions. The scientific program includes two invited lectures by Prof. Martin Wilkins (Department of Medicine, Imperial College London) and by Prof. Ralph Schermuly (Universities of Giessen and Marburg Lung Center, Giessen, Germany), as well as short oral communications.

We look forward to seeing you in Madrid.

Dr. Joan Albert Barberà. *Coordinator, Line of Research in Pulmonary Hypertension (CIBERES)*
Dr. Francisco Pérez-Vizcaíno, Dr. Jesús Ruiz-Cabello, Dr. Ángel Cogolludo. *Organizing and Scientific Committee.*

Bienvenida

En nombre del comité organizador, nos complace darle la bienvenida a la segunda Reunión de Investigación en Hipertensión Pulmonar organizado por la Línea de Investigación en Hipertensión Pulmonar del Centro de Investigación Biomédica en Red Enfermedades Respiratorias (CIBERES). La reunión tiene como objetivo difundir los avances recientes en investigación en el campo de la hipertensión pulmonar, ofreciendo a los investigadores la oportunidad de presentar sus resultados novedosos y fomentar acciones de colaboración. El programa científico incluye dos conferencias invitadas del Prof. Martin Wilkins (Department of Medicine, Imperial College, London, UK) y del Prof. Ralph Schermuly (Universities of Giessen and Marburg Lung Center, Giessen, Germany), así como comunicaciones orales.

Esperamos verte en Madrid.

Dr. Joan Albert Barberà. *Coordinador, Línea de Investigación en Hipertensión Pulmonar (CIBERES)*
Dr. Francisco Pérez-Vizcaíno, Dr. Jesús Ruiz-Cabello, Dr. Ángel Cogolludo. *Comité Organizador y Científico.*

Program / Programa

09:00-09:30 *Registration / Recogida de documentación*

09:30-09:40 *Opening / Apertura*

- ***Dr. Joan Albert Barberà.*** *Coordinator, Line of Research in Pulmonary Hypertension (CIBERES)*
- ***Dr. Francisco Pérez-Vizcaíno, Dr. Jesús Ruiz-Cabello, Dr. Ángel Cogolludo.*** *Organizing and Scientific Committee.*

09:40-10:15 *Invited Lecture / Conferencia Invitada*

- *"Elucidating and exploiting heterogeneity in pulmonary arterial hypertension"* ***Prof Martin Wilkins.*** *Department of Medicine, Imperial College London.*

10:15-11:45 *Oral communications. Session 1 / Comunicaciones Orales. Sesión 1*
Chairs / Moderadores: Ana Obeso / Marco Filice

- *Clinical phenotype and prognosis of PAH patients with TBX4 mutations.* **Ignacio Hernández González.** Hospital Universitario Doce de Octubre.

- *Hemodynamic and cardiac magnetic resonance effect of pulmonary artery denervation in a translational model of chronic pulmonary hypertension.* **Inés García-Lunar.** Centro Nacional de Investigaciones Cardiovasculares (CNIC).

- *Heterogeneity in Lung 18F-FDG uptake in precapillary pulmonary hypertension.* **Jeisson Osorio.** Hospital Clínic de Barcelona.

- *Pulmonary artery 4d flow evaluation on a porcine model of acute pulmonary hypertension caused by acute lung injury.* **Angel Gaitán Simón.** Universidad Complutense de Madrid.

- *Predicting bronchopulmonary dysplasia in very low weight preterm babies: Are molecular biomarkers better than clinical risk factors?* **María Álvarez Fuente.** Hospital Ramón y Cajal.

- *Conflict between mitochondrial DNA variants.* **Ana Victoria Lechuga-Vieco.** Centro Nacional de Investigaciones Cardiovasculares (CNIC).

11:45-12:05 *Coffee Break / Café*

12:05-14:05 *Oral communications. Session 2 / Comunicaciones Orales. Sesión 2*
Chairs / Moderadores: Laura Moreno / Víctor Peinado

- *Pulmonary arterial hypertension is aggravated by vitamin D deficiency.* **María Callejo.** Department of Pharmacology, School of Medicine, University Complutense of Madrid.

- *Hypoxia and Pulmonary Arterial Hypertension: revisiting HIF2 function in the pathogenesis of PAH and associated heart failure.* **Silvia Martin-Puig.** Centro Nacional de Investigaciones Cardiovasculares (CNIC).

- *Dysfunctional endothelial cells in patients with chronic thromboembolic pulmonary hypertension.* **Valerie Smolders.** IDIBPAS – Clinic, Universidad de Barcelona.

- *Estrogens and age influence on pulmonary hypertension in female rats.* **Elena Olea.** Universidad de Valladolid.

- *Expression of HIV-1 proteins induces endothelial dysfunction and K^+ channel impairment in the pulmonary vasculature.* **Gema Mondéjar-Parreño.** Department of Pharmacology, School of Medicine, University Complutense of Madrid.

- *A frameshift variant in CRIPAK is associated with endothelial dysfunction and small vessel loss in pulmonary arterial hypertension.* **Jair A. Tenorio Castano.** Instituto de Genética Médica y Molecular – Hospital Universitario La Paz.

- *Thrombospondin-1 role in pulmonary adventitial fibroblasts myodifferentiation during vascular remodeling in pulmonary diseases.* **María José Calzada García.** Universidad Autónoma de Madrid.

- *Riociguat and (5z)-7-oxozeanol treatments attenuate the metabolic reprograming induced by pulmonary arterial hypertension in right ventricle and lung tissue.* **José Luis Izquierdo-García.** Centro Nacional de Investigaciones Cardiovasculares (CNIC).

14:05-15:00 *Lunch / Almuerzo*

15:05-15:35 *Invited Lecture / Conferencia Invitada*

- *"Tyrosine kinase inhibitors as a treatment of pulmonary hypertension: Lessons learned and future challenges"* ***Prof. Ralph Schermuly.*** *Universities of Giessen and Marburg Lung Centre, Giessen, Germany*

15:35-17:20 *Oral communications. Session 3 / Comunicaciones Orales. Sesión 3*
Chairs / Moderadores: Pilar Escribano / Mª Jesús del Cerro

- *Update and Management of the Spanish Biobank of Pulmonary Hypertension.* **Joan Albert Barberá.** Hospital Clínic-IDIBAPS, Barcelona.

- *Preliminary results of a National Registry for Pulmonary Hypertension due to Respiratory Diseases (Registro Español de Hipertensión pulmonar Asociada a enfermedad Respiratoria, REHAR).* **Diego A. Rodríguez.** Hospital del Mar-IMIM (Barcelona).

- *Pulmonary gas exchange in severe pulmonary hypertension associated with COPD.* **Lucilla Piccari.** Servicio de Neumología, Hospital Clínic de Barcelona – IDIBAPS.

- *Balloon pulmonary angioplasty for inoperable patients with chronic thromboembolic pulmonary hypertension. Results and complications of the therapy.* **Maite Velázquez Martín.** Hospital Universitario 12 de Octubre.

- *Diseño y aplicación de un panel de secuenciación masiva (NGS) para el estudio de la Hipertensión Arterial Pulmonar (HAP): Panel_HAP_v1.2.* **Jair Tenorio.** Instituto de Genética Médica y Molecular – Hospital Universitario La Paz.

- *In vivo molecular imaging of lung inflammation with neutrophil-specific tracers.* **Fernando Herranz.** Centro Nacional de Investigaciones Cardiovasculares (CNIC).

- *Endothelial ß3-adrenergic receptor prevents hypoxia-induced pulmonary hypertension.* **Eduardo Oliver Pérez.** Centro Nacional de Investigaciones Cardiovasculares (CNIC).

17:20-17:30 *Conclusions. Closing ceremony / Conclusiones. Acto de Clausura*

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

Oral communications / Comunicaciones orales

O1- Clinical phenotype and prognosis of PAH patients with TBX4 mutations

Ignacio Hernández González[1] , Carlos Andrés Quezada[2], Jair Tenorio[3,4] , Nuria Ochoa Parra[1] , Paula Navas[5] , Amaya Martínez Meñaca[6] , Pablo Lapunzina [3,4] , Pilar Escribano[1,7]

1. Unidad multidisciplinar de Hipertensión Pulmonar, Servicio de cardiología, Hospital Universitario 12 de Octubre, Madrid. 2. Servicio de Neumología. Hospital Universitario Ramón y Cajal. Madrid. 3. Instituto de Genética Médica y Molecular (INGEMM). Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid. 4. CIBERER, Centro de Investigación en Red de Enfermedades Raras, ISCIII, Madrid. 5. Servicio de Cardiología. Hospital Gregorio Marañón. Madrid 6. Servicio de Neumología. Hospital Marqués de Valdecilla. Santánder 7. CIBER en Enfermedades Cardiovasculares, ISCIII, Madrid

Background: mutations in TBX4 gene are an uncommon cause of PAH, typical in childhood forms and usually associated to skeletal disorders. Previous studies have suggested a good prognosis in TBX4 mutations carriers.

Methods: since November 2011, genetic testing is offered to all patients with idiopathic o hereditable PAH or PVOD included in the Spanish Registry of PAH.

Results: in this period of time, we have studied 395 patients with idiopathic or hereditable PAH and PVOD. 4 pathogenic or likely pathogenic mutations were observed in 5 patients and 2 variant of unknown significance in 3. After familial screening, mutations were found in 6 relatives: 5 asymptomatic carriers and 1 diagnosed with early stage PAH.

- c.G432T:p.M144I: 2 patients had this mutation. Both cases shared distinguishing features: low DLCO, severe respiratory insufficiency and CT scan pattern suggestive of PVOD (excluded in the histological study after lung transplantation)
- c.1351A>G;p.M451V: 3 members of a family had this mutation. The index case was a 59-year- old family diagnosed after several years of fatigue. 2 daughters had the mutation: 1 asymptomatic carrier and 1 diagnosed with PAH in screening.
- c.1423A>C;p.N475H: 2 males in a family had this mutation. The index case was diagnosed in childhood and has had a benign course for decades. A brother of the patient is asymptomatic carrier.
- c.1351A>G;p.M452V: 4 members of a single family carry this mutation. El index case is a 40-year- old female diagnosed in childhood. A distinguishing feature of the patient has an adequate response to CCB and has had a child without complication during pregnancy or birth. Her father and 2 brothers are asymptomatic carriers.

Conclusions: PAH patients with TBX4 mutations have a wide spectrum of phenotypes. In our study, reduced CO diffusion is a distinctive finding and might determine prognosis.

Index case	Mutation	Status	Age at diagnosis	Follow Up (Y)	DLCO (%)	PVR (WU)
Patient 1	c.G432T:p.M144I	Death	62	1,5	32	5
Patient 2	c.G432T:p.M144I	Trasplantation	28	9	55	21,4
Patient 3	c.1351A>G;p.M451V	Alive	59	3,5	29	7,5
Patient 4	c.1423A>C;p.N475H	Alive	5	49		12
Patient 5	c.1351A>G;p.M452V	Alive	10	30		3,5

O2- Hemodynamic and cardiac magnetic resonance effect of pulmonary artery denervation in a translational model of chronic pulmonary hypertension.

Inés García-Lunar, Daniel Pereda, Evelyn Santiago, Nuria Solanes, Joaquim Bobí, Jorge Nuche, Ana Paula Dantas, María Ascaso, Montserrat Rigol, Manel Sabaté, Borja Ibáñez y Ana García-Álvarez

Introduction: Pulmonary hypertension (PH) is a prevalent condition with few available therapeutic strategies. Pulmonary artery denervation (PADN) is a novel technique currently under investigation as a potential treatment for PH, but the number of studies assessing its efficacy is limited and controversial. We aimed to perform a proof-of-concept study of PADN using surgical bipolar radiofrequency ablation clamps in a translational model of chronic PH.

Methods: Nineteen 3-month old Large-White pigs with chronic PH induced by surgical banding of the inferior pulmonary venous confluent were randomized to either PADN of the pulmonary trunk and both branches or sham procedure. They underwent hemodynamic and cardiac magnetic resonance (CMR) evaluation at 2 and 3 months follow-up. Continuous variables between groups were compared with Mann-Whitney U and ANCOVA tests.

Results: Twelve animals completed the study protocol (n=6 in each group). There were no significant differences in baseline parameters between PADN and sham animals. Mean blood pressure decreased in PADN animals at 2 and 3 months follow-up compared to controls. There were no other significant hemodynamic changes at follow-up. On CMR, PADN animals displayed a trend towards greater biventricular volumes and masses at both time points, being the differences in the indexed left ventricular end-diastolic volume significant at the end of follow-up.

Conclusion: In a large-animal model of chronic post-capillary PH, PADN with surgical bipolar radiofrequency clamps was not associated with a beneficial effect in terms of hemodynamics and cardiac performance. Moreover, our results suggest a trend towards biventricular dilatation in PADN-treated animals. More studies are needed to evaluate the effect of this therapy in chronic PH.

O3- Heterogeneity in Lung 18F-FDG Uptake in Precapillary Pulmonary Hypertension

Jeisson Osorio[1], Olga Tura-Ceide[2], Javier Pavia[3], Francisco Lomeña[3], Iván Vollmer[3], Eduard Agustí[3], Isabel Blanco[1], Jesús Ruiz-Cabello[4], Samuel España[5], Manel Castellà[6], Yolanda Torralba[1], Laura Sebastián[1], Lucilla Piccari[1], Cristina Bonjoch[2], Víctor Peinado[2], J. A. Barberà[1].

1Department of Pulmonary Medicine, Hospital Clínic-IDIBAPS, University of Barcelona; Biomedical Research Networking Center on Respiratory Diseases (CIBERES), Barcelona, Spain, 2Biomedical Research Networking Center on Respiratory Diseases (CIBERES), Barcelona, Spain, 3Center for Diagnostic Imaging, Hospital Clínic-IDIBAPS, University of Barcelona, Barcelona, Spain, 4Biomedical Research Networking Center on Respiratory Diseases (CIBERES); Complutense University of Madrid, Madrid, Spain, 5National Center for Cardiovascular Research (CNIC), Madrid, Spain, 6Department of Cardiovascular Surgery, Department of Cardiovascular Surgery, Hospital Clínic-IDIBAPS, University of Barcelona, Barcelona, Spain.

INTRODUCTION:

Proliferative changes in pulmonary vessels may contribute to the development of pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH). Previous studies using [18F]-fluoro-D-glucose positron emission tomography (PET-FDG) imaging have shown increased cellular metabolism in pulmonary artery (PA), lung parenchyma (LP) and right ventricle (RV) in patients with PAH. Little is known on these metabolic changes in CTEPH. The present study aimed to evaluate cellular metabolism in LP, PA and RV by means of PET-FDG in patients with PAH or HPTEC, compared to healthy subjects.

METHODS:

In this preliminary analysis 4 groups were evaluated (n=6, each): patients with idiopathic PAH (iPAH); patients with HPTEC with distal lesions non tributary to pulmonary endarterectomy (PEA) (CTEPH-distal); patients with CTEPH with proximal lesions, candidates to PEA (CTEPHproximal); and healthy subjects (control). The mean values of standardized 18F-FDG uptake of predefined regions of interest in LP (7 planes per lung in defined anatomical points), PA, RV and left ventricle (LV), were computed and normalized to hepatic uptake (SUVR). SUV ratios in patients were related to functional class (FC), exercise tolerance, hemodynamic parameters and serum BNP.

RESULTS:

Results are shown in the Table. The 18F-FDG uptakes in LP and PA were significantly correlated, and the RV/LV ratio of 18F-FDG uptake correlated with both pulmonary vascular resistance (PVR) and BNP.

CONCLUSIONS:

We conclude that patients with precapillary pulmonary hypertension show increased FDG uptake in the right ventricle, particularly those with iPAH. No consistent changes in FDG uptake of pulmonary arteries or lung parenchyma were observed neither in iPAH or CTEPH.

Funded by grants from Fondo de Investigación Sanitaria (PI15/00582) and Fundación Contra la Hipertensión Pulmonar (FCHP).

Table

	iPAH	CTPEH-distal	CTEPH-proximal	Control
FC, I-II/III-IV (%)	83/17	83/17	33/67	100/0
mPAP (mmHg)	49±14	47±14	48±7	ND
CI (L/min/m2)	2.39±0.35	2.68±0.49	1.93±0.29	ND
PVR (dyn·s·cm-5)	794±429	418±206	809±179	ND
6MWD (m)	584±53	424±85*	442±102*	650±34
BNP (ng/L)	39±25*	39±34*	161±141*	8±5
LP SUVR	1.32±0.17	1.05±0.15	1.00±0.15*	1.30±0.28
PA SUVR	3.03±0.32	2.32±0.47*	2.59±0.33	3.06±0.34
SUV RV/LV	1.11±0.26*	0.73±0.28	1.05±0.44	0.59±0.19

*p<0.05 compared with control subjects

O4- Pulmonary artery 4d flow evaluation on a porcine model of acute pulmonary hypertension caused by acute lung injury

Ángel Gaitán Simón, Arnoldo Santos, Ignacio Rodríguez, Ehsan Yazdanparast, Rubén Mota and Jesús Ruiz-Cabello.

Acute lung injury has been recognized as a cause of pulmonary vascular dysfunction leading to acute pulmonary hypertension and to right ventricle failure. It is clinically relevant as patients in mechanical ventilation that develops pulmonary vascular dysfunction show worth outcomes. However it is also relevant from a physiological point of view in the study of pulmonary hypertension, as it is model for fast development of complex hemodynamic phenomena relevant in this disease.

In this study we evaluated by means of 4d flow magnetic resonance imaging complex phenomena happening in the pulmonary artery in a porcine model of acute lung injury. In particular we studied if vortex phenomena were related with the development of acute respiratory distress syndrome and the mechanical ventilation settings.

4 pigs were submitted to lung saline lavages until reaching a PaO2/FiO2 ratio below 200mmhg followed by 1.5 hours of injurious mechanical ventilation. Then an alveolar recruitment manoeuvre followed by a decremental PEEP titration were performed. Three mechanical ventilation were then evaluated randomly according to the PEEP level related to the point of maximal airway dynamic compliance: Optimum 2cmH2O above, Overdystension 6 cmH2O above and Collapse 6 cmH2O below. The rest of mechanical ventilation were similar (control volume ventilation with 6-8ml/kg tidal volume). A MRI acquisition for 4d flow of the main pulmonary artery was acquired after 30minutes stabilization period in baseline and after lung injury (both with PEEP 8cmH2O) and in three PEEP levels. Vortex was defined as flow streamlines creating circular patterns in at least two frames in sagital slide encompassing the whole main pulmonary artery.

Vortices were detected in the 75% of the cases in the situation immediately after lung injury and in 50% of the cases in the collapse situation. The localization of the vortex was always at the mid level of main pulmonary artery coinciding with the point in which the pulmonary artery shows the maximum curvature.

These results could help to understand the complex pulmonary hemodynamics that occurs in a lung injury and also are promising as a diagnostic tool in pulmonary hypertension.

O5- Predicting bronchopulmonary dysplasia in very low weight preterm babies: Are molecular biomarkers better than clinical risk factors?

María Álvarez Fuente, Laura Moreno Gutiérrez, Paloma López-Ortego, Luis Arruza, Andrea Martínez Ramas, Carlos Zozaya, Alejandro Ávila, Marta Muro, María Jesús del Cerro Marin.

Introduction: Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease in childhood and the most common cause of pulmonary hypertension (PH) secondary to pulmonary disease. It is characterized by a pulmonary developmental arrest, in which extrinsic factors are involved. Moderate and severe BPD have a worst outcome and are related more frequently with PH. The prediction of BPD development in extremely premature newborns is vital to implement preventive strategies.

Hypothesis: Molecular biomarkers are superior to clinical variables to predict BPD development in very low birth weight preterm newborns.

Material and Methods: Prospective longitudinal study of a cohort of preterm newborns (gestational age under 28 weeks and weight under 1250 grams). Weekly clinical and echocardiographic variables as well as molecular biomarkers (IL-6, IL-1, IP10, uric acid, HGF, endothelin-1, VEGF, CCL5) in blood and tracheal aspirate, were analyzed from birth to week 36 (post menstrual age)

Results: Between November 2014 and February 2016 we included 50 patients with a median gestational age of 26 weeks (IQR 25-27) and weight of 871 g (SD 161.0) (range 590-1200g). Three patients were excluded due to an early death (first four days).

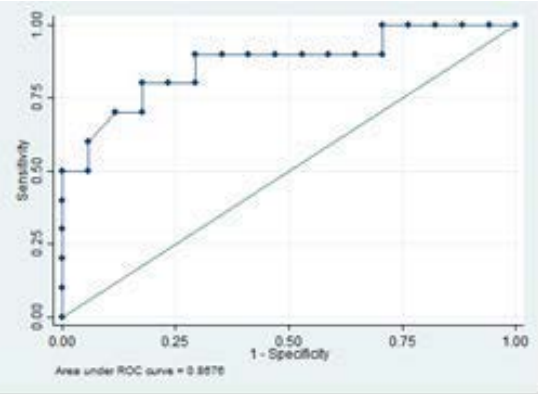
Thirty-six of the 47 patients (76.6%) developed BPD (mild n=14, moderate n=15, severe n=7). In the serial echocardiographic studies performed, 51.1% of the patients had signs of PH. We performed a logistic regression in order to identify risk factors for moderate or severe BPD. With the following results:

BPD development	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
MV day 7	18.27971	23.00283	2.31	0.021	1.551793	215.3301
IVS bowing day 7	7.619599	9.281129	1.67	0.095	.7000314	82.93669
Endothelin-1 day 7	.2708498	.1938921	-1.82	0.068	.0665859	1.101729
_cons	.8688042	1.368204	-0.09	0.929	.0396679	19.0285

MV= mechanical ventilation, IVS= interventricular septum

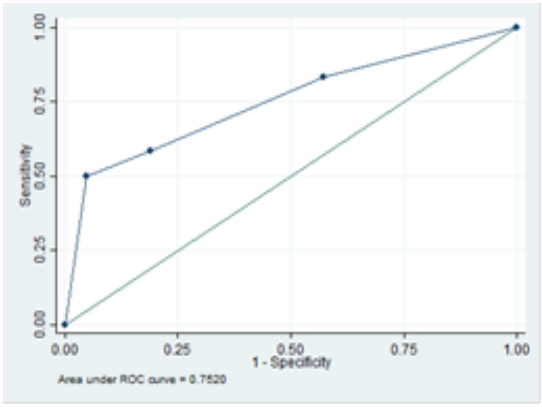
We compared two predicting models: 1) including MV, IVS bowing and endothelin-1, 2) only with clinical variables (MV and IVS), with the following results:

Model 1:



Sensitivity	Pr(+| D)	70.00%
Specificity	Pr(-|~D)	82.35%
Positive predictive value	Pr(D| +)	70.00%
Negative predictive value	Pr(~D| -)	82.35%

Model 2:



Sensitivity	Pr(+| D)	50.00%
Specificity	Pr(-|~D)	95.24%
Positive predictive value	Pr(D| +)	85.71%
Negative predictive value	Pr(~D| -)	76.92%

Conclusions: The combination of endothelin-1 with clinical and echocardiographic variables increases the prediction power for BPD diagnosis, although the role of endotelin-1 and its possible therapeutic implications in BPD and BPD-related PH requires further research.

O6- CONFLICT BETWEEN MITOCHONDRIAL DNA VARIANTS

Ana Victoria Lechuga-Vieco[1,2], Ana Latorre-Pellicer[1], Iain G Johnston[3], Enrique Calvo[1], Juan Pellico[1,2], Jesús Vázquez[1], Nick S Jones[3], Jesús Ruíz-Cabello[1,2,4,*] and José Antonio Enríquez[1,5,6,*].

[1] Centro Nacional de Investigaciones Cardiovasculares Carlos III; Madrid (SPAIN) [2] CIBERES: C/ Melchor Fernández-Almagro 3, 28029 Madrid, [3] School of Biosciences, University of Birmingham, United Kingdom. [4] Universidad Complutense de Madrid [5] Departamento de Bioquímica y Biología Molecular y Celular. Universidad de Zaragoza. Zaragoza (SPAIN) [6] CIBERFES: C/ Melchor Fernández-Almagro 3, 28029 Madrid,

All mitochondrial DNAs (mtDNA) of a given cell in our organism are essentially identical, a situation named homoplasmy. Heteroplasmy refers to the presence of more than one variant of mtDNA co-existing in the same cytoplasm. Heteroplasmy is actively combated by several mechanisms, including degradation of the paternal mtDNA upon fertilization. Heteroplasmy may be naturally generated by mutagenesis during mtDNA replication, but also can be caused by novel medical technologies, as oocyte rejuvenation and mitochondrial replacement in pulmonary pathologies. Animal models with identical nuclear genomes but with different mtDNA haplotypes generate functionally different OXPHOS systems that shape the organismal metabolism[1], supporting the conclusion that different mtDNA wild type haplotypes are phenotypically relevant. Pulmonary disorders are one of the most frequents causes of death worldwide. Until recently, mitochondrion was a cellular organelle which its principal function was the production of energy. During the past years, it has been recognised new roles during processes of apoptosis and inflammation, demonstrating its importance as a nutrient and oxygen sensor. Dilucidating the role of mitochondria in different pulmonary diseases, such as Pulmonary Hypertension (PH), is a challenging task. There is an unsolved controversy regarding the possible functional consequences of different physiological haplotypes of mtDNA and heteroplasmy in pulmonary physiology. To address this issue, we have characterised conplastic and heteroplasmic mice throughout their lifespan through transcriptomic, metabolomic, biochemical, physiological and phenotyping studies. We have also focused on *in vivo* imaging techniques for non-invasive assessment of cardiac and pulmonary energy metabolism. The existence of intrinsic mismatch between mtDNA and nDNA reveals lifelong metabolic consequences and alterations in cardiac and pulmonary performance. Also, we found that the co-existence of two wild type mtDNA variants can cause profound influence on animal physiology. The metabolic theory is increasingly seen as one of the key factors for understanding of PH, placing the role of mitochondria at the center stage for the development of novel diagnostic and therapeutic tools.

[1] Latorre-Pellicer, A. et al. Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. Nature **535,** 561–565 (2016)

O7- Pulmonary arterial hypertension is aggravated by vitamin D deficiency

Callejo M[1], Morales-Cano D[1], Mondéjar-Parreño G[1], Barreira B[1], Esquivel-Ruiz S[1], Moreno L[1], Barberá JA[2], Cogolludo A[1] and Pérez-Vizcaíno F[1].

[1] Department of Pharmacology, School of Medicine, University Complutense of Madrid, Spain. CIBERES. Insto Investigación Sanitaria Gregorio Marañón (IISGM.). [2] Hospital Clinic Barcelona-IDIBAPS, Barcelona, Spain. CIBERES.

Multiple studies have reported increased incidence of vitamin D hipovitaminosis. Epidemiological studies also suggest a relationship between vitamin D deficiency and cardiovascular diseases. Pulmonary arterial hypertension (PAH) is a severe and progressive vascular disease characterized by vasoconstriction, arterial remodelling and thrombosis. Interestingly, calcitriol, the active form of vitamin D, modulates several signalling pathways affected in PAH. The aims of this study were to examine the vitamin D levels in PAH patients and to investigate if vitamin D deficiency may predispose to PAH.

25(OH)-vitamin D and parathormone (iPTH) plasma levels from 67 PAH-patients were measured by immunoassay. Male Wistar rats were fed a standard or a vitamin D-free diet for five weeks. Then, rats were further divided into two groups: controls or PAH. PAH was induced by a single dose of SU5416 (20 mg/Kg) followed by exposure to chronic hypoxia (10% O_2) for 2 weeks.

PAH patients presented a severe deficiency of 25(OH)-vitamin D and higher levels of iPTH. PAH animals maintained without vitamin D showed an increase in pulmonary pressure and increased pulmonary artery muscularization compared to PAH animals with standard diet. Vitamin D deficiency produced endothelium-dysfunction measured as acetylcholine-induced relaxation and higher contraction to serotonin. Myocytes isolated from pulmonary arteries had a reduced potassium current density in vitamin D deficient rats.

These findings suggest a possible pathophysiological role of vitamin D deficiency in PAH.

O8- Hypoxia and Pulmonary Arterial Hypertension: revisiting HIF2 function in the pathogenesis of PAH and associated heart failure

Villalba M, Escobar B, Santos A, Izquierdo-García JL, Ruiz-Cabello J and **Martin-Puig S**

Low ambient oxygen concentration results in Pulmonary Arterial Hypertension (PAH) and increased right ventricular systolic pressure (RVSP); hence chronic hypoxia exposure is one of the experimental protocols used to induce PAH in small animal models.

Cellular response to hypoxia depends on the stabilization of Hypoxia Inducible Transcription Factors (HIFs) that mediate the transcriptional adaptation to low oxygen inducing target genes like Glut1, Epo, Vegf or VE-Cadherin among others. Although HIF1α deletion alleviates vascular remodeling associated with chronic hypoxia, it cannot prevent cardiac defects in low oxygen conditions. In contrast, vascular endothelial HIF2α signaling has been implicated in pulmonary vasoconstriction in response to hypoxia by controlling Arginase 1 and Endothelin 1 expression. However, the role of HIF2 in non-endothelial compartments of the lung has not been fully explored.

Wilms tumor 1 (Wt1) is a marker of embryonic mesodermal progenitors that give rise to the epicardium in the heart and in the lung contribute to the pleura and different cellular components of the parenchyma, especially bronchial and vascular smooth muscle cells, as well as bronchial and adventitial fibroblasts. We have generated a new mouse model of HIF2 deletion in the Wt1 lineage to evaluate the role of non-endothelial HIF2 signaling in the progression of PAH and heart failure (HF). Preliminary echography and histological analysis show that both control and HIF2-deficient mice presented pulmonary damage, certain level of cardiac hypertrophy and diastolic dysfunction after 21 days of hypoxia exposure (10% O_2). However, HIF2 mutant mice display increased severity and mortality, with significant dilation of the descending pulmonary artery, acute pulmonary congestion, remarkable dilation of the right atrium and right ventricle as well as severe left ventricular hypertrophy and systolic dysfunction. Furthermore, HIF2 deficient lungs presented numerous hemorrhages with collapsed microvasculature and increased macrophage content, together with enhanced arterial remodeling of the adventitia. We are currently characterizing the molecular mechanisms behind these structural and functional differences and determining the impact of HIF2 deletion in RVSP. These results indicate the importance of HIF2 signaling in non-endothelial lung cells for adaptation to chronic hypoxia and may help to understand new aspects of PAH pathogenesis.

O9- Dysfunctional endothelial cells in patients with chronic thromboembolic pulmonary hypertension

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Rationale: Material obtained at pulmonary endarterectomy (PEA) offers the unique opportunity to unravel pathophysiological mechanisms underlying chronic thromboembolic pulmonary hypertension (CTEPH). Enhanced proliferation and an apoptosis-resistant phenotype, that could be involved in vascular changes occurring in CTEPH, might be linked to metabolic dysregulation of CTEPH endothelial cells (CTEPH-EC). The aim of this study was the development of an *in vitro* model of EC pathology using primary cultures of patient derived EC to assay cell metabolism in CTEPH.

Methods: Cells isolated from PEA specimens (N=12) were confirmed as being EC. Cell migration was evaluated using scratch migration assay. Metabolic changes in CTEPH-EC are being studied using RT-PCR, Western-Blot and colorimetric enzyme activity assays. Findings in CTEPH-EC were compared with human pulmonary arterial endothelial cells (HPAE).

Results: CTEPH-EC showed endothelial cobblestone morphology accompanied with the expression of endothelial markers, such as CD31, vWF and VE-CAD. Migration capacity of CTEPH-EC was lower than HPAE ($p<0.0001$). Lactate dehydrogenase (LDH) activity in cell pellet was higher in CTEPH-EC than in HPAE (median, 167 mU/mL [IQR, 131-197] vs 125 mU/mL [88-135]). No significant differences in the LDH activity were found in the supernatant of the same cultures. Expression of PFKFB3, LDH and other glycolytic enzymes as well as hexokinase activity was not significantly different between CTEPH-EC and HPAE.

Conclusions: Our results show that CTEPH-EC present a functional impairment compared to control endothelial cells. Similarities with the known metabolic profiles of rapid growing cells, enhanced glycolytic rates and reduced oxidative metabolism, suggest the existence of a Warburg effect in CTEPH-EC based on the enhanced LDH activity compared to controls.

O10- Estrogens and age influence on pulmonary hypertension in female rats

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In spite of the protective effect of estrogens on the systemic and pulmonary vasculature, female sex has been shown to be a risk factor for pulmonary arterial hypertension (PAH), namely, the estrogen paradox.

Previous work in young rats showed that chronic sustained hypoxia (CH) during 2 weeks causes a faster progression of PAH and right ventricular hypertrophy in young female rats with respect to their male counterparts, although the vascular injury was larger in males, suggesting a sexual dimorphism.

Our aim was to study the estrogens role in PAH development in young (CH3m) and old female rats (CH24m) exposed to a normobaric hypoxic atmosphere (10%O2; $pO_2 \approx 70$ mmHg, 14 days) respect to their control females (C3m and C24m).

PAH measured by right heart catheterization was lower in CH24m vs. CH3m (18.2±1.4mmHg, n=7 vs. 22.9±1.0mmHg, n=6; *p<0.05). CH increased the hematocrit similarly in old and young female rats but the Fulton index only increased in the CH3m (p<0.001). CH didn´t cause significant endothelial damage in pulmonary arteries (PA) in young rats, measured using small vessels myography, as 3□M carbachol induced relaxation. However, endothelial dysfunction was observed in old female rats (72.6±5.8% n=15 vs. 93.8±2.9%, n=11 in controls, **p<0.01), in which CH didn´t cause further damage. 17-β-estradiol (30□M) induced relaxation in PA increased in C24m vs. C3m (60.0±6.2%, n=11 vs. 42.2±5.9%, n=12,* p<0.05), with no changes in CH either in young or old animals. This effect of 17-β-estradiol could be mediated by estrogens receptors remodeling in old female animals.

We conclude that hemodynamics and vasomotor effects of CH were ameliorated in CH24m compared to CH3m rats. Old animals showed endothelial damage in PA and CH didn´t worse it. In CH3m no significant damage was observed. The decreased estrogen levels in 24 months old female rats could be the responsible of the observed results.

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O11- Expression of HIV-1 proteins induces endothelial dysfunction and K^+ channel impairment in the pulmonary vasculature

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Introduction: Pulmonary arterial hypertension (PAH) is a severe disease that results in increased pulmonary vascular resistance, right heart failure and death. Endothelial dysfunction and downregulation of K^+ channels are considered common abnormalities in most forms of PAH. Thus, reduced expression and/or activity of Kv1.5 and TASK1 channels have been reported in heritable and other forms of PAH (1). Human immunodeficiency virus (HIV) infection is an established risk factor for PAH, however the pathogenesis of HIV-related PAH remains unclear. Our aim was to analyse if the expression of HIV proteins is associated with impairment of endothelial or K^+ channel function in the pulmonary circulation.

Methods: HIV transgenic mice (HIV) expressing seven of the nine HIV viral proteins and wild type (Wt) mice were used in this study. Right ventricular systolic pressure and systolic, diastolic and mean pulmonary arterial pressures were measured in open chest mice. Vascular reactivity was studied in endothelium-intact pulmonary arteries (PA) mounted in a wire myograph. K^+ currents were recorded in freshly isolated PA smooth muscle cells (PASMC) using the patch-clamp technique. K^+ channel gene expression was assessed using rt-PCR. Data are expressed as mean ± sem.

Results: Hemodynamic parameters were similar in HIV and Wt mice. PA from HIV mice showed impaired endothelium-dependent relaxation (11±5% vs 33±5% relaxation to Acetylcholine 1μM; n=6 $p<0.05$). Likewise, PA and PASMC derived from HIV mice had preserved Kv1.5 channel activity but decreased Kv7 channel activity assessed by vascular reactivity and patch-clamp experimental approaches. This was associated with reduced expression of Kv7.1 and Kv7.4 channels. Moreover, TASK currents were abolished in PASMC from HIV mice.

Conclusion: Our data indicate that HIV proteins induce endothelial dysfunction and K channel impairment, which could contribute to the vascular dysfunction in HIV-associated PAH.

Boucherat O, et al. Potassium channels in pulmonary arterial hypertension. Eur Respir J. 2015;46(4):1167-77.

O12- A frameshift variant in *CRIPAK* is associated with endothelial dysfunction and small vessel loss in pulmonary arterial hypertension

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Introducción: La Hipertensión Arterial Pulmonar (HAP) es una enfermedad poco frecuente cuya etiología es muy variada. La disfunción endotelial es una de las características principales que contribuyen a la patología vascular en pacientes con HAP. Así, el objetivo de este trabajo fue el de aplicar la técnica de secuenciación de exoma completo (WES) con el fin de encontrar variantes en genes que pudieran estar relacionados con la fisiopatología de la enfermedad, y realizar una caracterización funcional de estas variantes. La proteína codificada por *CRIPAK,* es el principal regulador de la expresión de PAK1, que participa en la vía de señalización de VEGF

Material y métodos: De una cohorte de 35 pacientes con HAP idiopática, hereditaria y secundaria a metanfetamina y tras el análisis de las variantes, se detectaron aquellos genes que pudieran tener una implicación en la desregulación endotelial. Se realizó un análisis bioinformático y mediante programas in sillico con el fin de poder detectar variantes cuya frecuencia estuviera incrementada en pacientes con PAH. La validación funcional de las variantes detectadas se realizó en células endoteliales microvasculares pulmonares (PMVECs) extraídas de donantes sano y pacientes. El análisis funcional del gen detectado se realizó mediante estudios de formación de tubos, y ensayo de motilidad células en PMVECs transfectadas con un RNA de interferencia específico

Resultados: Se detectó una mutación en el gen *CRIPAK*, cuya frecuencia es mucho mayor en pacientes con HAP comparado con la frecuencia de la población general. Los niveles proteicos de CRIPAK estaban significativamente reducidos en pacientes con HAP en comparación con los niveles en controles de donadores sanos. Por el contrario, los niveles de fosfo-PAK1 (forma activa de la proteína) estaban incrementados. Además, las células PMVECs transfectadas con el ARNsi mostraban alteraciones en el patrón de formación tubos y la respuesta motor de las células en cultivo

Conclusión: El estudio mediante WES ha permitido la identificación de una variante en el gen *CRIPAK*, que podría ser un gen potencialmente modificador del fenotipo en HAP. La reducción de los niveles de CRIPAK podría contribuir a la aparición de un fenotipo asociado a HAP mediante la reducción de la viabilidad endotelial, promoviendo la alteración en la formación de pequeños vasos y acelerando el remodelado vascular.

O13- Thrombospondin-1 role in pulmonary adventitial fibroblasts myodifferentiation during vascular remodeling in pulmonary

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Pulmonary vascular remodelling is the main cause of increase in pulmonary artery pressure. This is thought to result from the combined effects of hypoxia, inflammation and loss of capillaries in severe emphysema. The matricellular protein thrombospondin-1 (TSP-1) is elevated in patients with pulmonary arterial hypertension (PAH) and our results from *in vivo* models indicate that TSP-1 aggravates some of the symptoms when upregulated in the hypoxic lung. We aimed to analyze whether TSP-1, through transforming growth factor (TGF)-β activation, plays a role in myofibroblast differentiation and proliferation that together with the proliferation of smooth muscle cells is considered an important event during pulmonary artery muscularization. We used human pulmonary artery adventitial fibroblasts subjected to hypoxia and studied the expression of TSP-1 and its effects on the expression of myofibroblast markers such as α-smooth muscle actin (SMA) and myosin light chain 9 (MYL9). We also tested TSP1 effects on cell proliferation in the presence or absence of LSKL, a specific inhibitor of TSP-1-dependent activation of TGF-β. Additionally, we optimized traction force microscopy (TFM) to evaluate the functional relevance of muscularization and contractility markers in these models.

O14- Riociguat and (5z)-7-oxozeanol treatments attenuate the metabolic reprograming induced by pulmonary arterial hypertension in right ventricle and lung tissue

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Objective: Pulmonary arterial hypertension (PAH) is characterized by a wider metabolic reprogramming in right ventricle (RV) and lung tissue. The aim of this study is to identify novel metabolic biomarkers for monitoring the evolution of the disease and the response to short-term vasodilator or antiproliferative therapies in a rat model of PAH.

Methods: PAH was induced in male Wistar rats by a single injection of the VEGF receptor antagonist SU5416 (20mg/Kg) followed by exposure to hypoxia (10%O_2) for 21 days. Two weeks after SU5416 administration, vehicle (HPX), riociguat (3mg/kg/day) (RIO), the TAK-1 inhibitor (5z)-7-oxozeaenol (3mg/kg/day) (OXO) or both drugs combined (OXO-RIO) were administered for 7 days. Normoxic control (NMX) rats were kept in a regular oxygenated room. Lung and RV tissue were analyzed by magnetic resonance spectroscopy. Principal Component Analysis was performed to determine the differences between groups.

Results: Metabolomic profiling of RV and lung samples discriminated between NMX and HPX groups. We identified 10 metabolic biomarkers of PAH in RV tissue, but more importantly, a metabolic shift was also observed following each pharmacological treatment. Thus, a significant trend to metabolic normalization was observed after OXO treatment, attenuation of glucose, glycerophosphocholine (GPC) and phosphocholine (PC) alterations; RIO treatment, attenuation of inosine, glucose, creatine and PC alterations; and OXO-RIO treatment, attenuation of previous metabolic biomarkers and glutamine. In lung tissue, we identified 15 PAH metabolic biomarkers, but the metabolic response to each treatment was more limited. OXO treatment significantly attenuated the glutamine and α-hydroxybutyrate metabolic shifts; RIO treatment attenuated the myo-Inositol, leucine and α-hydroxybutyrate metabolic shifts and the combined treatment with OXO-RIO induced an additional normalization of glucose concentration.

Conclusions: We demonstrated that (i) the PAH rat model reproduces the metabolic abnormalities observed in PAH patients; (ii) Identified potential metabolic markers of therapeutic response to riociguat and (5z)-7-oxozeanol in PAH.

O15- Update and *Management of the Spanish Biobank of Pulmonary Hypertension*

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Introduction. In 2013, the Spanish Biobank of Pulmonar Hypertension (*Biobanco Español de Hipertensión Pulmonar, BEHIP*) was established with the aim to collect a well-characterized repository of biological samples from patients with pulmonary arterial hypertension (PAH) or chronic thromboembolic pulmonar hypertension (CTEPH) in order to promote and facilitate biomedical research in these diseases. The clinical information linked to these biological samples is available at the Spanish Pulmonary Arterial Hypertension Registry (*Registro Español de Hipertensión Arterial Pulmonar, REHAP*), according to an agreement established in 06/26/2013. The objective of the communication is to provide an update of the results obtained during the 4 years of existence of the BEHIP.

Material and methods. Donors come from hospitals that participate in the REHAP registry. Blood samples (20 mL) are collected to obtain plasma, serum and DNA. In 2017, blood was also collected for the obtention of lymphocytes (PBMC). Fresh blood samples are sent to the BEHIP, located at the Biobank HCB-IDIBAPS, Barcelona, where they are processed and stored. The samples are coded and linked to the REHAP registry code for subsequent traceability and clinical characterization.

Results. Since the start of operation of BEHIP until today, samples from 296 donors have been procured. Origin of the samples are: H. Clínic, Barcelona, 202; H. 12 de Octubre, Madrid, 34; H. Virgen del Rocío, Sevilla, 21; H. Vall d'Hebron, Barcelona, 20; H. del Mar, Barcelona, 11; H. La Fe, Valencia, 5; H. Virgen de la Salud, Toledo, 3. In the last year a great effort has been made to recruit the maximum possible number of participating national hospital centers in order to increase the value of the collection.

The diagnoses of the available samples are shown in Table:

Pulmonar arterial hypertension	**206**	**70%**
Idiopathic	76	26%
Hereditary	8	3%
Drug-induced or toxic	2	1%
Associated with connective tissue diseases	50	17%
Associated with HIV infection	28	9%
Associated with portal hypertension	16	5%
Associated with congenital heart disease	17	6%
Other forms of PAH	9	3%
Associated with congenital heart disease	**85**	**29%**
Multifactorial	5	2%

From these samples, there are more than 1600 aliquots of normalized DNA, 3000 aliquots of plasma, 2000 aliquots of serum and 30 aliquots of lymphocytes. Seven samples requests have been made, including 5 national and 2 international research projects.

Conclusions. The BEHIP is a consolidated biobank of biological samples from patients with pulmonary hypertension. Their most outstanding characteristics are its link to the clinical information contained in national registry REHAP, and the availability of samples from patients with CTEPH. These characteristics give to the collection a high strategic value for its use in research projects in pulmonary hypertension, both at the national and international levels.

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O16- Preliminary results of a National Registry for pulmonary hypertension due to respiratory diseases (Registro Español de Hipertensión pulmonar Asociada a enfermedad Respiratoria, REHAR)

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REHAR is a prospective voluntary reporting of previously diagnosed and incident pulmonary hypertension due to respiratory diseases (PH-RD). The main objective was to define the clinical and epidemiological characteristics of these patients, as well as to assess their response to PH treatment.

Data will be collected by means of electronic data capture (eDC), starting at the initial baseline assessment reported in the medical records. Patients diagnosed before 2017 will be entered retrospectively, and prospectively thereafter. Patients with newly or previously diagnosed PH-RD will be eligible for enrolment if they meet the definition of chronic obstructive pulmonary disease (COPD) (GOLD), interstitial lung disease (ILD) (ATS/ERS guidelines) or combined pulmonary fibrosis/emphysema (CPFE) (ATS/ERS guidelines) and a RHC must have been performed before the study entry with the follow criteria: mild–moderate PH-RD defined as mean Pap ≥ 25 mmHg and ≤ 35 mmHg, and severe PH-COPD defined by mean Pap > 35 mmHg or Pap ≥25 mmHg in the presence of a low cardiac output (CI <2.5 L/min, not explained by other causes). In addition to RHC information, will be included the following data: clinical, anthropometric and demographic data, pulmonary function, and echocardiography, detailed blood testing and high-resolution computed tomography.

During October and November 2017 we included the first 24 cases [Males, 75%; age, 60 (7); never smokers, 15%]. Preliminary data indicates that ILD was the most frequent group (62%) followed by COPD (30%) and CPFE (8%). The majority of patients were in functional class (FC) III at the time of diagnosis. Co-morbidities were common such as systemic hypertension (25%) and diabetes (17%). The hemodynamic profile reveal: mPAP: 37 (11) mmHg; CI: 2.6 L/min (0.7); PVR: 16 UW; and pulmonary capillary wedge pressure (PCWP): 10 (3) mmHg. Sildenafil was the first agent used (50%) and Bosentan was the second (only two patients).

O17- Pulmonary gas exchange in severe pulmonary hypertension associated with COPD

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Introduction: A great percentage of patients with chronic obstructive pulmonary disease (COPD) suffer from pulmonary hypertension (PH), which is usually mild or moderate. A small subgroup presents instead with severe PH and distinct clinical and functional characteristics, among which there is notably a marked impairment of gas exchange. Whether the pulmonary vascular derangement is the cause or the consequence of the gas exchange impairment in these patients is currently unknown. The objective of this study was to analyse the determinants of arterial oxygenation (PaO_2) in patients with COPD.

Methods: We analysed ambispectively 169 patients with COPD who underwent right heart catheterisation; a subgroup of 69 of them also underwent multiple inert gases elimination technique (MIGET). Patients were divided into three groups: COPD without PH (n=98); COPD with mild-moderate PH (mPAP 25-34 mmHg) (n=30); and COPD with severe PH (mPAP ≥35 mmHg, or mPAP ≥25 mmHg and CI <2.5 L/min/m²) (n=41). We compared functional parameters, arterial and mixed venous respiratory gases, pulmonary and systemic hemodynamics and ventilation-perfusion (V_A/Q) ratio distributions.

Results:

	COPD without PH (n=98)	COPD with moderate PH (n=30)	COPD with Severe PH (n=41)
Age (yrs)	63 ± 9	62 ± 7	66 ± 7#
Sex (% M)	94	90	92
FEV_1 (%pred.)	41 ± 17	36 ± 14	57 ± 21*#
FEV_1/FVC (%)	42 ± 13	41 ± 12	54 ± 12*#
TLC (%pred.)	117 ± 20	119 ± 27	99 ± 14*#
DL_{CO} (%pred.)	57 ± 24	53 ± 26	34 ± 19*#
mPAP (mmHg)	17 ± 4	28 ± 3*	43 ± 10*#
CI (L/min/m²)	3.28 ± 0.88	3.28 ± 0.48	2.19 ± 0.65*#
PVR (dyn·s·cm^{-5})	181 ± 79	271 ± 103*	751 ± 377*#
PAWP (mmHg)	5.5 ± 3.6	9.1 ± 4.2*	9.0 ± 4.7*
PaO_2 (mmHg)	71 ± 11	64 ± 11*	57 ± 13*#

$PaCO_2$ (mmHg)	39 ± 6	45 ± 5*	36 ± 8#
Aa PO_2 (mmHg)	32 ± 9	32 ± 9	49 ± 19*#
PvO_2 (mmHg)	35 ± 2	35 ± 2	33 ± 4
SvO_2 (%)[a]	59 ± 11	69 ± 13*	62 ± 9
Shunt, %QT [a]	2.60 ± 1.10	2.45 ± 2.44	4.25 ± 2.84
Low V/Q, %QT[a]	0.92 ± 2.48	2.65 ± 4.80	2.37 ±3.03
Mean Q [a]	0.80 ± 0.40	0.60 ± 0.35	1.22 ± 0.50#
LogSD Q[a]	0.91 ± 0.20	0.94 ± 0.22	1.06 ± 0.31
High V/Q, $\%V_A$ [a]	5.04 ± 8.83	1.98 ± 3.37	4.48 ± 5.51
Dead Space, $\%V_A$ [a]	30.92 ± 11.37	28.44 ± 13.65	23.41 ± 18.68
Mean V [a]	2.14 ± 1.07	1.63 ± 0.54	3.02 ± 0.72#
LogSD V [a]	0.98 ± 0.30	1.05 ± 0.16	0.88 ± 0.31
DISP R-E* [a]	13.4 ± 6.8	15.0 ± 5.3	13.3 ± 4.2

Values expressed as mean ± SD. (*) p <0.05, compared with the group Without PH; (#) p <0.05, compared with the group with Moderate PH. [a] 69 patients (44 without PH, 20 with moderate PH, 5 with severe PH).

Patients with COPD and severe PH show milder ventilator impairment and worse gas exchange, including lower PaO_2 and $PaCO_2$, with no significant differences in the V_A/Q ratio distributions, compared to the other groups.

Conclusions: In patients with COPD and severe PH, the impairment of gas exchange is explained by the amplified effect of lower PvO_2, due to the reduced cardiac output, on the V_A/Q mismatch.

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O18- Balloon Pulmonary Angioplasty for Inoperable Patients with Chronic Thromboembolic Pulmonary Hypertension. Results and Complications of the Therapy.

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Introduction: Balloon pulmonary angioplasty (BPA) for inoperable chronic thromboembolic pulmonary hypertension (CTEPH) is becoming widely accepted. Procedure refinement has reduced complications, mainly reperfusion pulmonary edema (RPE). Our primary objective was to analyse clinical status, hemodynamics, biomarkers and complications of the first BPA programme of a pulmonary hypertension reference center in our country.

Methods: Observational, prospective series that included all consecutive BPA procedures performed in inoperable CTEPH patients between May 2013 and Dec 2017 at one institution. We analysed improvement after ≥ 3 procedures, RPE and mortality.

Results: We performed 221 BPA sessions in 60 patients. The cause of inoperability was distal involvement in 56 patients, comorbidities in 2 patients and individual choice in 2 patients. One patient died due to RPE. In 3 patients therapy was interrupted, 2 due to no improvement after 3 procedures and one unable to walk due to invalidating arthrosis. The remaining 56 patients are in active BPA programme or have completed it. In 38 patients in which we have performed ≥ 3 BPA procedures to date, pulmonary vascular resistance was reduced by 50% (10.36±5.1 vs 5.18±1.99 W.U. $p<0.001$), mean pulmonary arterial pressure was reduced by 29.1% (50.6±12.7 vs 35.87± 8.7 mm Hg, $p<0.001$), cardiac index rose 11% (2.43±0.8 vs 2.7±0.5 L/min/m^2 p=0,05), NT pro-BNP levels were reduced by 80.4% (1336±1329 vs 261±326 pg/dl, $p<0.001$) and six-minute walk distance improved 69 metres (394,3±109 vs 463.8±102 m, p=0,001). After ≥3 procedures 90% patients were in WHO class I or II vs 93% of them in WHO class III or IV at baseline ($p<0.001$). RPE of any degree developed after 14 BPA sessions (6.4%) although only one patient required mechanical ventilation. This RPE was refractory to any invasive treatment causing the death of the patient (mortality 1.6%).

Conclusions: Current refined BPA for inoperable CTEPH has became a safe and effective therapy which improves hemodynamics, functional status and biomarkers with low severe peri-procedural complication rate and mortality.

O19- Diseño y aplicación de un panel de secuenciación masiva (NGS) para el estudio de la Hipertensión Arterial Pulmonar (HAP): Panel_HAP_v1.2.

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Objetivos: La hipertensión arterial pulmonar (HAP) es una enfermedad infrecuente de pronóstico ominoso en ausencia de tratamiento. La HAP puede presentarse en diferentes formas, entre ellas la HAP idiopática (HAPI), hereditaria (HAPH) y una forma más grave denominada veno-oclusiva. Gracias a la secuenciación masiva, se han descrito una elevada cantidad de genes relacionados con la HAP en los últimos años.

Material y métodos: Se seleccionó una cohorte de 168 pacientes con HAPI, HAPH o venooclusiva, para la realización de un panel de genes relacionados con HAP, tanto genes ya descritos en la literatura como genes candidatos propuestos por otros grupos. Se diseñó un panel de genes mediante la plataforma Nimbledesign de Roche, captura SeqCap EZ, genoma de referencia hg19, análisis bioinformático mediante un script propio y análisis de datos mediante diferentes herramientas bioinformáticas.

Resultados: Tras el análisis bioinformático se encontró un 20% de pacientes con mutaciones patogénicas y un 5,8% de variantes de significado incierto. Un 8,3% de las muestras se rechazaron tras el análisis de los parámetros de calidad establecidos. Además, se encontraron dos variantes en un mismo paciente, ambas relacionadas con HAP, cuyo análisis in silico demuestra la posible patogenicidad de ambas. Una variante en BMPR2, c.961C>T; p.Arg321*) ya descrita previamente y otra variante en un gen que codifica un canal de potasio y que se ha relacionado en estudios de NGS con HAP

Conclusiones: Las mutaciones encontradas en HAP en la cohorte de pacientes analizada demuestran la elevada variabilidad genética de esta enfermedad. La existencia de dos variantes en un mismo paciente podría demostrar la posibilidad de la existencia de herencia digénica en la HAP, un hecho que no se había planteado hasta el momento.

O20- *In vivo* molecular imaging of lung inflammation with neutrophil-specific tracers.

Juan Pellico, Jesús Ruiz-Cabello, **Fernando Herranz**

Non-invasive quantitative detection of lung inflammation is highly desirable for assessing pathogenic processes in the lung. Inflammatory-cell activation is currently assessed by combining anatomical imaging with information obtained from invasive lung biopsy, histopathology and bronchoalveolar lavages. This time-consuming approach explains the numerous attempts to produce a reliable probe for non-invasive *in vivo* diagnosis. Neutrophils are an essential part of the inflammatory cascade. However, neutrophil invasion can cause major tissue damage. There is mounting evidence on the role of neutrophils in the development of pulmonary hypertension. From the importance of neutrophil extracellular traps (NETs) in pulmonary arterial hypertension[1] to the generation of reactive oxygen species[2] or as a diagnosis tool of the disease.[3] As a consequence we focused our attention on the development of *in vivo* tools able to detect the presence of neutrophils.

Here, we have developed a new tracer able to non-invasively detect neutrophil recruitment.[4] This new nano-radiotracer[5] has been used for non-invasive *in vivo* detection of acute and chronic inflammation in the lungs with very high *in vivo* labelling efficiency, i.e. a large percentage of labelled neutrophils. Furthermore, we demonstrated that the tracer is neutrophil-specific and yields images of neutrophil recruitment of unprecedented quality.[4]

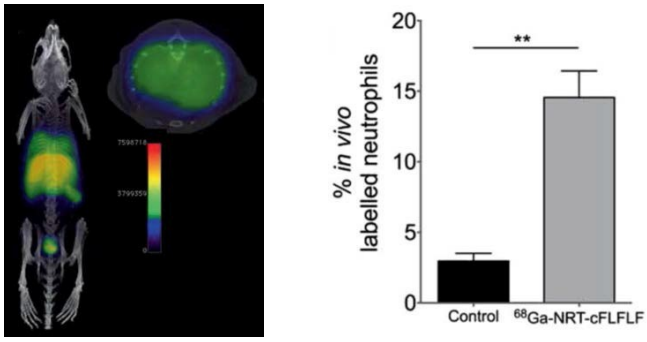

Figure 1. PET/CT imaging of neutrophils recruitment in the lungs and (right) percentage of labelled neutrophils *in vivo*.

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O21- Endothelial ß3-adrenergic receptor prevents hypoxia-induced pulmonary hypertension

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ß3-adrenergic receptor (AR) agonists have been proved to reduce pulmonary vascular resistances and increase right ventricular (RV) function in a porcine model of post-capillary Pulmonary Hypertension (PH) (1). However, its use in other types of PH and its precise mechanism is yet to be fully elucidated. The main objectives of the current work are: to discriminate which cellular compartment is the target of ß3-AR agonist to prevent PH (pulmonary vasculature or RV), and to further understand its intracellular mechanism. To achieve these objectives, we used two transgenic mice models developed in our lab over-expressing the human ß3-AR within the endothelium or within the heart, and exposed them to chronic hypoxia. Results showed that specific endothelial ß3-AR overexpression, and not cardiomyocyte-restricted overexpression, exerts a protective effect by attenuating the development of PH. Also, we demonstrated that ß3-vasodilatary effect is mediated by endothelium in porcine pulmonary arteries – partially due to the release of NO – where ß3-AR is demonstrated to be the major ß-AR according to immunohistochemistry. In order to unravel the molecular pathways, we used both human pulmonary artery endothelial cells and segments from porcine pulmonary arteries incubated with ß3-agonists, and performed western blot analysis, enzyme immunoassays and immunocytochemistry. We found that ß3-AR agonists induced: (1) an increase in ERK1/2 phosphorylation; (2) an increase in the cGMP levels – which were reduced in the presence of the endothelial NO synthase inhibitor L-NAME – with a slight increase in the cAMP levels; and (3) a reduction in the ROS production in cells exposed to hypoxia. Therefore, the beneficial ß3-adrenergic effect is twofold: a vasodilation mediated by pulmonary endothelium via NO/cGMP but also AC/cAMP pathways; and a reduction of ROS, which could prevent endothelial dysfunction and smooth muscle proliferation. Further research will clarify this mechanism and the therapeutic potential of ß3-AR agonist in PH.

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