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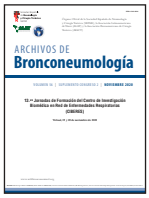

Abstracts

13.as Jornadas de Formación del Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES)

Virtual, 19 y 20 de noviembre de 2020

19F-NMR STRATEGIES TO CHARACTERIZE MONOSACCHARIDE SELECTIVITY OF C-LECTINS. THE CASES OF DC-SIGN AND MGL

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Introduction: Carbohydrates are key players in many cell-pathogen and cell-cell communication processes. Calcium dependent C-type lectins are carbohydrate receptors important in immunity and play diverse roles in host defense. DC-SIGN, example of this lectin type, has been identified as a receptor for HIV-1, HCV, Ebola virus, CMV, dengue virus, and the SARS coronavirus. Nuclear Magnetic Resonance (NMR) is a powerful technique that has been applied extensively to study carbohydrate-molecular recognition by lectins.
Objectives: To develop a screening strategy based on 19F-NMR in order to discriminate monosaccharide selectivity by C-type lectins. The cases of DCSIGN (CD209) expressed in dendritic cells and Macrophage Galactose-type Lectin (MGL, CD301) will be studied. Comparison with other lectins will be performed.
Methods: Fluorine observed NMR will be applied from the point of view of the ligand. A collection of different mono fluorinated monosaccharides will be used. Experiments based on the perturbation of the 19F-transvesal relaxation (T2) due to the binding with the lectins will be performed. The simplicity and resolution of fluorine NMR allows to use mixtures of fluorinated derivatives of Galactose, Glucose, Mannose and Fucose to identify binders and characterize the monosaccharide selectivity of the lectins.
Results: Monosaccharide selectivity and essential hydroxyl groups for binding by DCSIGN and MGL have been determine by applying NMR-T2 filtering strategy. For DCSIGN mannose and fucose are identify as the best binders while MGL is selective to galacoside derivatives. Interestingly, while DCSIGN has very broad selectivity, MGL only recognized fluorinated galactose. Furthermore, these methods allowed to identify the essential OH groups to coordinate the Ca2+. The specificity was confirmed by displacement experiments with known ligands.
Conclusions: A simple NMR strategy to identify monosaccharide binders to lectins and to inform of what hydroxyl groups can be modified in the ligand without compromising the lectin binding capacities has been developed.

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A PERIPHERAL BLOOD MICRORNA SIGNATURE THAT IS ASSOCIATED WITH COVID-19 SEVERITY

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Introduction: Around 20-30% of the hospitalized patients with COVID-19 develop a severe phenotype that is associated with high mortality rate. There is a clinical demand of reliable tools to monitor the patient's status. The non-coding transcriptome, and more specifically microRNAs (miRNAs), has emerged as a potential tool for clinical decision-making. The association between the circulating miRNA profile and COVID-19 has not been previously explored.
Objectives: To evaluate the alterations in the circulating miRNA signature linked to COVID-19 severity and its potential in decision-making.
Methods: Plasma samples were collected from 54 COVID-19 patients (23 patients hospitalized at clinical wards and 31 patients admitted to the ICU). A panel of 41 miRNAs associated with immune/inflammatory response, lung damage, myocardial damage and coagulation was analyzed using RT-qPCR. Quality control was performed using spike-ins and hemolysis tests. Differences between groups were evaluated by relative quantification using linear models for arrays. Discrimination was evaluated using the area under the ROC curve (AUC).

A variable selection process based on random forest was used to construct a predictive model of ICU stay.
Results: Fifty-three samples passed the quality control. Four deregulated miRNAs; miR-16-5p (viral acute respiratory infection), miR-92-3p (immune response), miR-451a (immune response) and miR-486-5p (antiviral defense), were identified in ICU patients compared to ward patients. Both miR-451a and miR-486-5p showed a good discrimination value (AUC = 0.78 for miR-451 and 0.77 for miR-486-5p). Random forest analyses identified a signature of 3 miRNAs (miR-27a-3p, miR-92a-3p and miR-451a) that displayed an optimal discrimination accuracy (AUC = 0.84).
Conclusions: Our findings reveal for the first time a profile of miRNAs that is altered in plasma of patients with a severe phenotype of the disease. This circulating miRNA signature may be useful for the management of the COVID-19 patient.
Funding: Supported by ISCIII (CIBERESUCICOVID, COV20/00110), co-funded by ERDF, "Una manera de hacer Europa".

A RAPID TEST FOR ENVIRONMENTAL DETECTION OF PIGEON ANTIGEN

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Introduction: Bird-related hypersensitivity pneumonitis is an interstitial lung disease induced by avian proteins, specially pigeon antigens. Complete avoidance to inhaled antigens is an essential step to ameliorate respiratory symptoms and prevent disease progression.
Objectives: The aim of the present study was to develop and compare a sandwich enzyme link immunoassay (ELISA) and a immunochromatographic test (ICT) to detect pigeon antigens in environmental samples.
Methods: An amplified sandwich ELISA using pigeon serum as a calibration standard and a ICT using gold labelled anti-pigeon serum antibodies for the rapid detection of pigeon antigens in environmental samples were developed. Twenty-two different airborne samples were collected and analysed in parallel by both methods. Strip density values obtained with ICT were calculated and compared with the concentration determined by the standardized ELISA method for pigeon antigens. Strips results were also visually analysed by five independent evaluators.

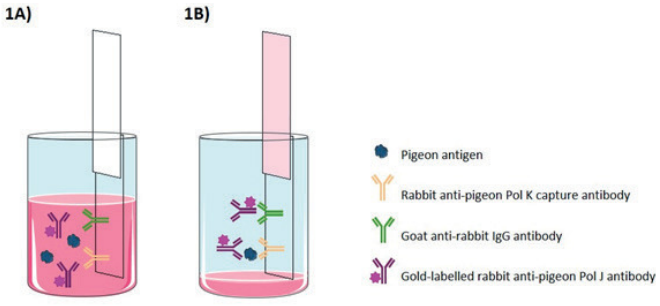

Figure 1. Schematic diagram of the strip assay. 1A) Dipping of the strip with capture antibody and Goat anti-rabbit IgG into the sample solution; 1B) Binding of gold-labelled anti-pigeon pol J antibody and gold-labelled **anti-pigeon pol J antibody-pigeon antigen** complex to control and test line, respectively.

Results: ELISA method had a lower and upper limit of pigeon antigen quantification between 58.4 and 10,112.2 ng/ml. ICT assay was able to detect a range of concentrations from 420 to 3,360 ng/ml of pigeon antigen. A kappa index of 0.736 ($p < 0.0001$) was obtained between the observers evaluating the strip results. A correlation was observed between the concentration determined by the ELISA and the relative density results of the ICT (rs: 0.935; $p < 0.0001$). Bland-Altman plot also confirmed a good agreement between both methods (mean difference: -1.626; $p < 0.0001$).
Conclusions: There was a good correlation between results obtained with ELISA and ICT assays. The ICT described is rapid, simple, highly consistent with the validated ELISA and does not require expensive equipment or specific skills.
Funding: Study funded by ISCIII (PI15/01954), FEDER and FUCAP.

ASSOCIATION ANALYSIS OF LOCAL GENETIC ANCESTRY OF TADA2B AND GRPEL1 WITH THE PRESENCE OF EXACERBATIONS IN PUERTO RICANS

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Introduction: Asthma is a chronic inflammatory disease of the airways. Asthma patients may experience episodic flare-ups, known as exacerbations, that can be life-threatening. In addition to the high prevalence and morbidity of asthma, the Puerto Rican population has genetic peculiarities that make it valuable in disease gene mapping. This population not only results from the recent admixture of Amerindian, African, and European ancestries, but it also presents genetic variation that is rare in other populations. Recently, a genome-wide association study described the involvement of four single nucleotide polymorphisms in the intergenic region of the TADA2B and GRPEL1 genes in asthma exacerbations among Puerto Ricans.
Objectives: To assess whether the association of polymorphisms of the TADA2B and GRPEL1 genes described in Puerto Ricans is due to differences in the genetic ancestry of the chromosomal region containing those loci (local ancestry).
Methods: A total of 1,132 Puerto Ricans with asthma (838 with exacerbations and 302 without exacerbations) were analyzed. Local ancestry was estimated with ELAI v1.C and global ancestry were obtained as the average of the genome. Logistic regression analyses were performed for each ancestry (European, Native American, and African local genetic ancestry) analyzing the presence or absence of exacerbations in the last year and adjusting for age, sex, and global genetic ancestry as covariates. These analyzes were carried out with R 3.6.3.
Results: At global level, the individuals analyzed showed 24% African admixture, 62% European, and 14% Native American, which not differed between cases and controls ($p > 0.05$). Regarding local ancestry, none of the ancestries (European, African or Native American) was associated with asthma exacerbations ($p > 0.05$).

Conclusions: The association of TADA2B and GRPEL1 genetic polymorphisms described in Puerto Ricans is not affected by local genetic ancestry of these loci.
Funding: This study was funded by the Spanish Ministry of Science, Innovation, and Universities and the European Regional Development Funds from the European Union (MICIU/AEI/FEDER, UE, SAF2017-83417R). EH-L was funded by a fellowship from the MICIU (PRE2018-083837). MP-Y was supported by the Ramón y Cajal Program by the MICIU (RYC-2015-17205).

CARDIOPULMONARY RESPONSE TO HYPOXIA. WHAT IS THE ROLE OF HIF2?

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Introduction: 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. Vascular endothelial HIF2α signaling has been implicated in pulmonary vasoconstriction in response to hypoxia. However, the role of HIF2α in non-endothelial compartments of the lung has not been fully explored. Wilms tumor 1 (Wt1) is a marker of mesodermal progenitors contributing to embryonic mesothelium that give rise to the epicardium in the heart and in the lung to the pleura and different cellular components of the parenchyma, especially bronchial and vascular smooth muscle cells, as well as bronchial and adventitial fibroblasts and vascular endothelium of arteries and microvasculature.
Objectives: Elucidate the role of HIF2 in heart and lung after exposure to hypoxia in other context than endothelial cells.
Methods: We have generated a new mouse model of HIF2α deletion in the Wt1 lineage to evaluate the role of HIF2α signaling in the progression of PAH and heart failure (HF) under exposure to hypoxia for 2-3 weeks (10% O2).
Results: These mice develop cardiomegaly and dilatation of the heart associated with an increase in proliferation of endothelial cells of the microvasculature. In addition, HIF2 mutants display increased alveolar wall thickness, perivascular fibrosis and inflammatory infiltration by alveolar macrophages. These histological analysis correlate with the results obtained by echocardiography and lung ultrasound studies.
Conclusions: Deletion of HIF2 in Wt1 positive cells under hypoxia, results in an increase in cardiac mass due to capillary proliferation. In lung, there is an increase in perivascular fibrosis with reduced alveolar space, and a rise in the number of alveolar macrophages.
Funding: UFV-CNIC.

CIRCULATING MICRORNA PROFILE ASSOCIATED WITH OBSTRUCTIVE SLEEP APNEA IN ALZHEIMER'S DISEASE

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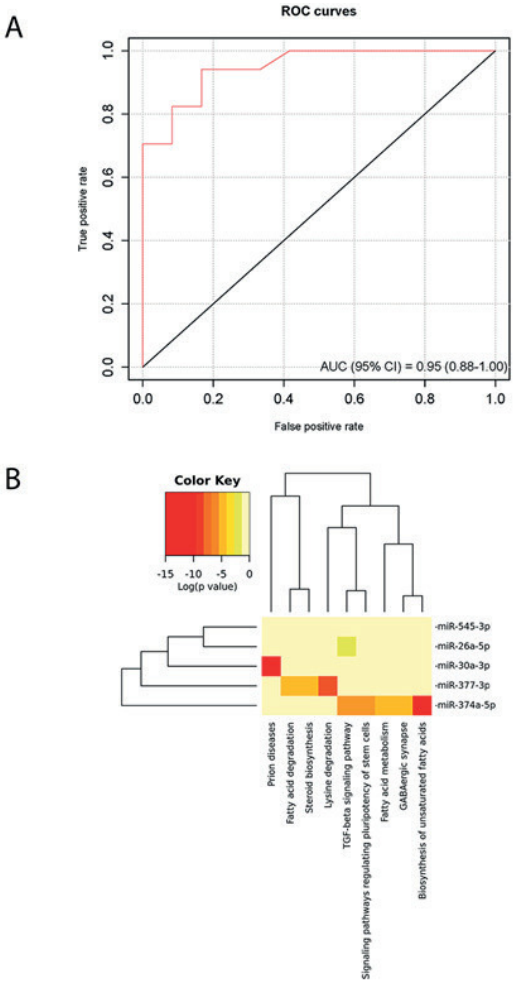
Introduction: The diagnosis of obstructive sleep apnea (OSA) in Alzheimer's disease (AD) by polysomnography (PSG) is challenging due to the required collaboration of the patients. In addition, screening questionnaires have demonstrated limited usefulness with this subpopulation.
Objectives: To investigate the circulating microRNA (miRNA) profile associated with OSA in AD patients.
Methods: This study included a carefully selected cohort of females with mild-moderate AD confirmed by biological evaluation (n = 29).

Table 1

	Differential expression		AHI correlations	
miRNA	Fold change	p-value	rho	p-value
miR-101-3p	0.667	0.025	-0.42	0.024
miR-26a-5p	0.638	0.029	-0.41	0.028
miR-30a-3p	3.980	0.008	0.43	0.02
miR-325	0.192	0.032	-0.29	0.141
miR-32-5p	0.412	0.033	-0.38	0.042
miR-337-3p	3.222	0.046	0.21	0.288
miR-374a-5p	6.142	0.039	0.43	0.02
miR-377-3p	0.456	0.023	-0.42	0.023
miR-451a	0.466	0.030	0	0.985
miR-483-3p	6.925	0.020	0.44	0.03
miR-496	0.182	0.009	-0.23	0.246
miR-545-3p	0.524	0.027	-0.36	0.05
miR-645	6.464	0.0008	0.67	0.001
miR-665	3.810	0.004	0.57	0.002
miR-95-3p	0.240	0.046	-0.3	0.175



The individuals were submitted to one-night PSG to diagnose OSA (apnea-hypopnea index ≥ 15/h) and the blood was collected in the following morning. The plasma miRNA profile was evaluated using RT-qPCR.
Results: The patients had a mean (SD) age of 75.8 (5.99) years old with a body mass index of 28.6 (3.83) kg·m^{-2}. We observed a subset of 15 miRNAs differentially expressed between OSA and non-OSA patients, of which 10 were significantly correlated with the severity of OSA (Table 1). Based on this, we built a prediction model that generated an AUC (95%CI) of 0.95 (0.88-1.00) including 5 of the differentially expressed miRNAs that correlated with OSA severity: miR-26a-5p, miR-30a-3p, miR-374a-5p, miR-377-3p, and miR-545-3p (Figure 1A). Pathway analysis demonstrated that these miRNAs were related to distinct molecular pathways associated with prion diseases, fatty acids, GABAergic synapse, among others (Figure 1B).
Conclusions: Our preliminary results suggest a plasma miRNA signature associated with the presence of OSA in AD patients. Further studies will be necessary to validate these findings.
Funding: Generalitat of Catalonia, Department of Health (PERIS 2019 SLT008/18/00050) and "Fundació La Marató TV3" (464/C/2014) to GPR. Co-financed by FEDER funds from the European Union ('A way to build Europe'). IRBLleida is a CERCA Programme/Generalitat of Catalonia. FD was supported by Agency for Management of University and Research Grants (FI_B100153).

CLINICAL IMPACT OF CLUSTERS OF DOUBLE CYCLING AND INEFFECTIVE EFFORTS IN CRITICALLY ILL PATIENTS

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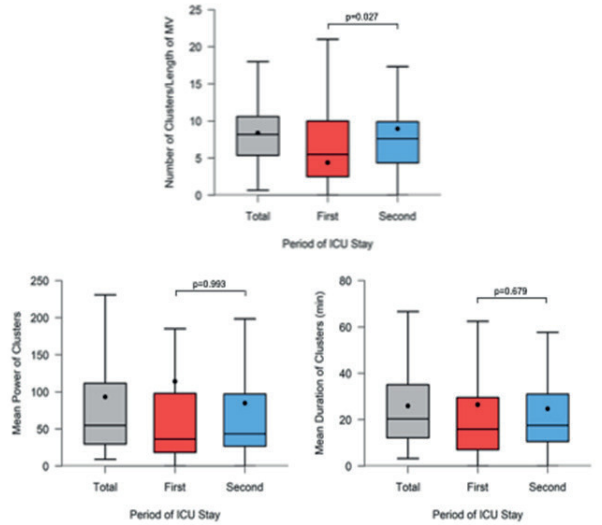
Introduction: Asynchronies during invasive mechanical ventilation can occur in clusters and are related to clinical outcomes. The presence of clusters is completely unnoticed by healthcare personnel, since none of monitoring systems adverts about them.
Objectives: To characterize clusters of double cycling and ineffective inspiratory efforts throughout mechanical ventilation, and investigate their associations with outcomes.
Methods: We included adults requiring mechanical ventilation (MV) > 24 hours with ≥ 70% of ventilator waveforms available. We characterized clusters of double cycling and ineffective inspiratory efforts in terms of power and duration. Fine-Gray's model for competing risk data was used to analyze cluster-related variables' effects on ICU discharge status (dead or alive); and generalized linear models investigated cluster's effects on duration of MV and ICU stay.
Results: We analyzed 58,625,796 breaths from 180 patients. All patients had clusters [mean/day, 8.2 (5.4-10.6); mean power, 54.5 (29.6-111.4); mean duration, 20.3 (12.2-34.9) minutes]. Clusters were less frequent during the first 48 hours of ventilation [5.5 (2.5-10) vs. 7.6 (4.4-9.9) in the remaining period (p = 0.027)]. Mean power and mean duration were similar in the two periods. The number of clusters was not associated with increased risk of death, but was associated with increased probability of being discharged alive. Higher mean power during MV and especially in the first 48 hours was associated with increased probability of death. Mean power and duration were also associated with longer MV and ICU stay.



Boxplots of cluster characteristics in each of the three periods of mechanical ventilation analyzed. Black dots represent means, lines within boxes represent medians, and boxes include the 25th through 75th percentiles. Note that outliers have been omitted to facilitate visualization.

Conclusions: Clusters of double cycling and ineffective inspiratory efforts could be considered a common unrecognized phenomenon of patient-ventilator interaction, however increased power and duration are associated with worsen clinical outcomes.
Funding: This work was funded by projects PI16/01606, integrated in the Plan Nacional de R+D+I and co-funded by the ISCIII- Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER). RTC-2017-6193-1 (AEI/FEDER UE). CIBER Enfermedades Respiratorias.

COGNITIVE PHENOTYPES ONE MONTH AFTER ICU DISCHARGE IN MECHANICALLY VENTILATED PATIENTS: A PROSPECTIVE OBSERVATIONAL COHORT STUDY

S. Fernández-Gonzalo[1], G. Navarra-Ventura[2], N. Bacardit[3], G. Gomà[4], C. de Haro[5], C. Subirà[6], J. López-Aguilar[2], R. Magrans[7], L. Sarlabous[4], J. Aquino-Esperanza[2], M. Jodar[8], M. Rué[9], A. Ochagavía[5], D. Palao[10], R. Fernández[11] and L. Blanch[5]

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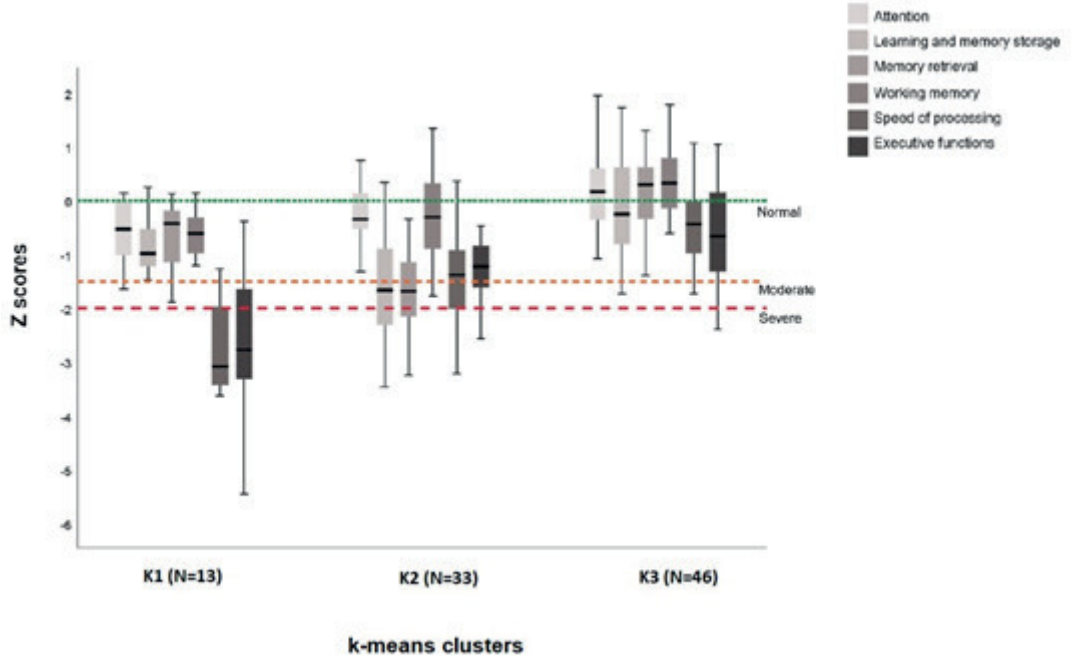
Introduction: ICU patients undergoing invasive mechanical ventilation (MV) experience cognitive deficits associated with their critical illness and its management. Early detection of different cognitive phenotypes

could reveal the involvement of various pathophysiological mechanisms and help clarify the role of precipitating and predisposing factors.
Objectives: To identify cognitive phenotypes in critically ill survivors one month after ICU discharge using an unsupervised machine learning (UML) method and to contrast them with the classical approach of cognitive impairment assessment.
Methods: One hundred and fifty-six critically ill patients undergoing MV from two ICUs were studied prospectively. Patients with previous cognitive impairment or neurological/psychiatric disorder were excluded. Clinical variables were registered during the stay in the ICU and 100 patients completed the cognitive evaluation 1-month after ICU discharge. The UML k-means clustering algorithm was applied to detect cognitive phenotypes. Precipitating and predisposing factors for cognitive impairment were explored.
Results: K-means testing identified three clusters (K) of patients with different cognitive phenotypes (Figure 1): K1 patients had severe cognitive impairment in processing speed and executive function; K2 patients had moderate-to-severe deficits in learning/memory, memory retrieval, processing speed and executive function; in K3 most patients had a normal cognitive profile. Using the classical approach, cognitive

Demographic and clinical characteristics of the sample according to cluster group

	K1 (N = 13)	K2 (N = 33)	K3 (N = 46)	p
Age, years a, c	59 (52-64)	72 (66-78)	60 (50-69)	< 0.001
Female gender (%) a, c	3 (23)	21 (64)	11 (24)	0.001
Cognitive reserve, z-score b, c	-0.07 (-0.94 - -0.05)	-0.11 (-0.45 - -0.23)	0.31 (-0.08 - 0.68)	< 0.001
Diagnosis (%)				0.507
Medical	12 (92)	27 (82)	35 (76)	
Surgical	1 (8)	5 (15)	4 (9)	
Polytrauma	0 (0)	1 (3)	7 (15)	
APACHE II at ICU admission	15 (12 - 18)	18 (15.50 - 22.50)	17 (12 - 20.25)	0.082
SOFA at ICU admission	8 (5.50 - 10.50)	7 (4 - 10)	7 (5 - 9)	0.719
SOFA slope	-1.1 (-1.80 - -0.35)	-0.9 (-1.50 - -0.25)	-0.75 (-1.40 - -0.12)	0.536
Charlson Index at ICU admission a, c	3 (2 - 4)	5 (3 - 6)	3 (1 - 4)	0.001
Length of MV, days	5 (4 - 10)	5 (3 - 8)	7 (4 - 11)	0.222
MV days ratio	0.67 (0.45 - 0.73)	0.64 (0.38 - 0.71)	0.63 (0.44 - 0.80)	0.492
Length of delirium, days	0 (0 - 1)	1 (0 - 2)	0 (0 - 2)	0.451
Delirium ratio	0 (0 - 0.12)	0.08 (0 - 0.22)	0 (0 - 0.10)	0.367
Accumulated dose of sedatives c	4.29 (0.29 -16.38)	1.35 (0.35 - 6.20)	3.85 (1.92 - 10.62)	0.050
Accumulated dose of opioids b	0 (0 - 2.33)	0.83 (0.29 - 2.10)	1.45 (0.63 - 2.52)	0.039
Days with sedatives	4 (1 - 7)	3 (1 - 6.50)	4 (3 - 7.25)	0.178
Days with sedatives ratio	0.44 (0.13 - 0.64)	0.28 (0.12-0.47)	0.40 (0.22-0.60)	0.163
Days with opioids a, b	0 (0-5)	4 (2 - 7)	5 (3 - 9)	0.016
Days with opioids ratio a, b	0 (0 - 0.37)	0.35 (0.22 - 0.61)	0.5 (0.33 - 0.64)	0.007
Length of ICU stay, days	11 (7 - 12.50)	9 (7 - 18.50)	11 (8 - 16.25)	0.604
Length of hospital stay after ICU discharge, days	15 (9 - 30)	17 (11 - 49)	17.50 (9 - 31.50)	0.591

Note: Data are expressed as n (%) or median (IQR), as appropriate. IQR: Interquartile range; APACHE: Acute Physiology and chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; MV: Mechanical Ventilation. Statistics: a: Significant difference between K1 vs K2; b: Significant difference between K1 vs K3; c: Significant difference between K2 vs K3.



The six cognitive indexes are represented according to each patient cluster. Z-scores between 0 and -1.5 SDs are considered normal, between -1.5 and -2 SDs moderate deficit, and below -2 SDs severe deficit.

impairment was observed in 47% of patients and. the k-means method accurately classified the 86%. The Table summarizes the demographic and clinical characteristics of patients in each cognitive cluster. Exploratory analysis showed that female gender (OR = 2.81; 95%IC: 1.01-7.84; p = 0.048), older age (OR = 1.05; 95%IC: 1.00-1.01; p = 0.048) and lower cognitive reserve (OR = 0.37; 95%IC: 0.16-0.83; p = 0.016) were relevant predisposing factors for cognitive impairment.
Conclusions: One month after ICU discharge, the k-means approach identified three groups of patients with different cognitive phenotypes improving the classical classification of cognitive impairment in ICU survivors. Gender, age, and cognitive reserve might be considered predisposing factors for cognitive impairment in ICU patients.
Funding: This work was co-funded by the projects PI13/02204, and PI16/01606, of the Instituto de Salud Carlos III (ISCIII) and the European Regional Development Fund (ERDF). CIBER de Enfermedades Respiratorias, Fundació Parc Taulí-I3PT (Catalonia, Spain).

CULTURING ALVEOLAR EPITHELIAL CELLS ON LUNG DERIVED HYDROGEL SCAFFOLDS

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Introduction: Most respiratory diseases affect to the lung epithelium, which is composed by type I alveolar cells (ATI), involved in gas exchange, and type II alveolar cells (ATII), ATI precursors. The study of alveolar diseases has been limited by the impossibility of maintaining alveolar cells culture due to the fact that type I do not proliferate and type II quickly differentiate into type I. In this work we study the potential improvement of culturing ATII cells on lung-derived hydrogel scaffolds.
Objectives: The objective of this study is to culture ATII cells until confluence preserving their phenotype and their proliferating ability by using lung-derived hydrogels.
Methods: Primary ATII cells were isolated from rat lungs and seeded over porcine lung-derived hydrogels. Cells were grown until confluence and subsequently trypsinized reseeded on hydrogel scaffolds and plastic dishes. ATI and ATII populations were identified by qPCR and immunofluorescence.
Results: Cells seeded on the developed scaffolds showed not only renewal and proliferation ability but also maintained their phenotype along the time. Also, they successfully proliferated when they were reseeded to another hydrogel scaffold or culture dish.
Conclusions: Data show that the use of lung-derived hydrogel scaffolds could overcome certain important limitations when studying alveolar diseases in vitro.
Funding: This work is partially funded by the Spanish Ministry of Economy and Competitiveness.

DEVELOPMENT AND MULTIMODAL CHARACTERIZATION OF A DISEASE MODEL FOR THE COPD FREQUENT BACTERIAL EXACERBATOR PHENOTYPE

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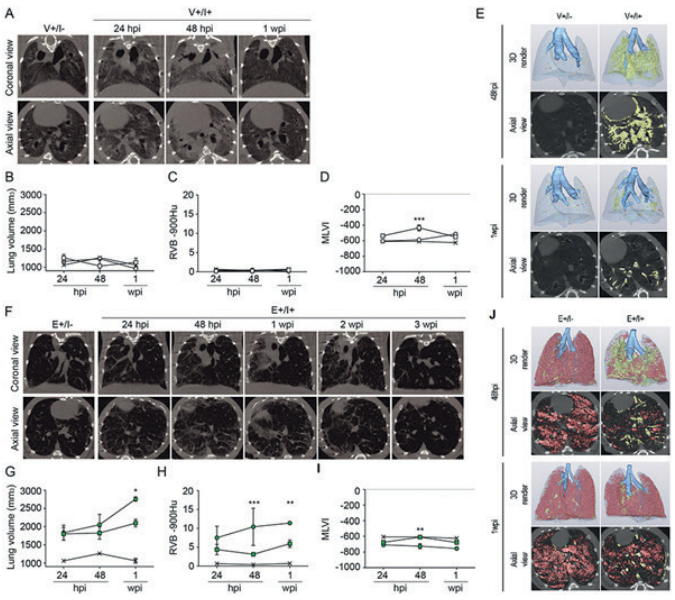
Introduction: Chronic obstructive pulmonary disease (COPD) patients undergo infectious exacerbations whose frequency identifies a clinically meaningful phenotype. Mouse models are used both to evaluate experimental infections and to model COPD. We currently lack an in vivo/preclinical model system for the COPD frequent exacerbator phenotype.
Objectives: (1) To establish a model of bacterial exacerbation by nontypeable Haemophilus influenzae (NTHi) infection on a murine model of lung emphysema. (2) To emulate the frequent exacerbator phenotype by performing two recurrent-consecutive episodes of NTHi infection on the emphysematous murine lung.
Methods: We combined noninvasive in vivo imaging and ex vivo techniques to perform a multimodal characterization of the disease model, obtaining longitudinal information about the extent of the developing lesions, host responses and bacterial load.
Results: Infection promoted inflammation, and bacterial load was reduced over time, disappearing 48 h post-infection (hpi). However, such reduction did not match with lung function recovery measured using tests of pulmonary function or disappearance of lung lesions as revealed by micro-computed X-ray tomography, which occurred 3 weeks post-infection (wpi). Then, to emulate the frequent exacerbator phenotype, we performed two recurrent episodes of NTHi infection on the emphysematous murine lung. After the second infection, bacterial load reduction was observed 96 hpi, but lung function recovery and disappearance of lesions on anatomical lung images did not happen until 12 wpi. Finally, as a proof of principle, we showed that the antibiotic azithromycin successfully cleared the recurrent infection, confirming this macrolide usefulness to ameliorate infectious exacerbation.



Conclusions: In conclusion, we have developed and characterized a mouse model of recurrent bacterial infection of the emphysematous lung, to facilitate investigating the COPD frequent exacerbator phenotype by providing complementary dynamic information of both infectious and inflammatory processes.
Funding: This work has been funded by grants from MINECO (UE FEDER) SAF2015-66520-R and RTI2018-096369-B-I00, from Health Department, Regional Navarra Govern, Spain, reference 03/2016, and

from SEPAR 31/2015 to J.G., and from MINECO (UE FEDER) RTI2018-094494-B-C222 to C.O.S. CIBER is an initiative from Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

DEVELOPMENT OF AN EFFICIENT IN VITRO HIGH-THROUGHPUT METHOD TO DISCOVER NEW COMBINATORIAL THERAPIES AGAINST NON-TUBERCULOUS MYCOBACTERIA

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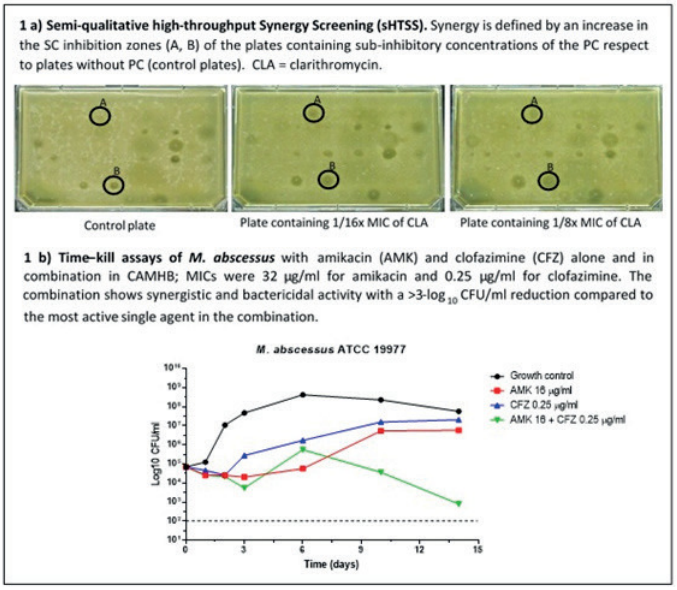
Introduction: Non-tuberculous mycobacteria (NTM) are emergent pathogens in cystic fibrosis (CF) patients, causing difficult to treat lung infections. Current treatment is based on long and costly combinatorial therapies, with low clinical success. An effective treatment remains challenging.
Objectives: To develop a robust high-throughput synergy screening methodology for efficient triage of novel synergistic drug interactions against NTMs.
Methods: This methodology allows the identification of synergistic partners of a Primary Compound (PC). Compounds from chemical libraries are transferred using a pin replicator onto bacterial lawns of Mycobacterium abscessus in presence/absence of sub-inhibitory concentrations of PC. After incubation, synergy is determined by comparing growth inhibition zones between drug-free and drug-containing agar plates (Figure 1).
A chemical FDA-library containing most clinically approved drugs (n = 1,430) was screened against amikacin and clarithromycin (PC), first-line antibiotics.
Results: The optimization process required extensive modifications of the experimental conditions (Table). Homogeneous bacterial growth and clear edges of the inhibition zones were required for image processing readout. Optimal conditions were achieved using a 1.6 mm pin replicator from 100 µL-deep 96-wells onto agar plates containing 10^6 cell/mL from an exponential pre-inoculum. Hit rates obtained in the FDA-synergy screening were 1.12% and 1.54% for amikacin and clarithromycin respectively. Thirteen priority combinations were further validated by time-kill kinetics studies (Figure), obtaining a 50% of synergistic correlation with the combinations studied so far with amikacin (3/6). Next experiments include the translational-potential evaluation against clinical isolates. Among the synergistic interactions identified, clofazimine with amikacin is a promising combination already studied for the NTM-treatment (doi: 10.1128/AAC.01505-12), which validates our screening method.



Experimental assays of identification and validation of the priority synergistic interactions.

Conclusions: Our methodology is robust and adaptable to other bacterial species for the identification of synergistic drug combinations from large chemical libraries. This technique is easily implemented in synergy screening programs, allowing the identification of novel strategies to improve the treatment of NTM-infections in CF patients.
Funding: MG is supported by a fellowship from the Government of Aragon (Gobierno de Aragón, Spain), and SRG by a grant from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID Research Grants) and a grant from the Government of Aragon, Spain (Ref. LMP132_18) ("Gobierno de Aragón y Fondos Feder de la Unión Europea "Construyendo Europa desde Aragón").

Modifications performed in the optimization process of the synergy screening method against M. abscessus ATCC 19977

Experimental condition	Experimental modifications		Result	Optimal condition
Inoculum size	10^5 cell/ml		Insufficient growth	No
	10^6-10^7 cell/ml		Uniform growth	Yes
Metabolic state of the inoculum	Stationary growth		Heterogeneous growth	No
	Exponential growth		Homogeneous growth	Yes
Inoculation method	Swab		Bacterial growth strain dependant, edges not defined	No
	Top agar		Bacterial growth strain independent, clear edges	Yes
SC stock concentrations	0.1-1 mM		Defined inhibition zones	Yes
	10 mM		High-density inhibition zones, deconvolution needed	No
Volume SCs stock concentrations	50 µl		Optimal volume with both pin diameter	Yes
	100 µl		Inhibition zones wider when using a wider replicator pin diameter	Yes
Sub-inhibitory PC concentrations[a]	Amikacin	Clarithromycin	Bacterial growth partially inhibited	No
	1/2xMIC	1/4xMIC		
	1/4xMIC	1/8xMIC	Bacterial growth not affected by inhibition effect	Yes
	1/8xMIC	1/16xMIC	Bacterial growth not affected by inhibition effect	Yes
Replicator pin diameter	3 mm		Uncertain inhibition zones due to pin diameter	No
	1.6 mm		Inhibition zones readout precise, clear edges	Yes

[a]Optimal subinhibitory concentrations were determined by 2-fold serial dilutions of the drugs under the same HTSS growing conditions.

DISUSE MUSCLE ATROPHY WORSENS THE LOSS OF MUSCLE MASS AND FUNCTION INDUCED BY LUNG CANCER-ASSOCIATED CACHEXIA THROUGH ENHANCED PROTEOLYSIS AND IMPAIRED REGENERATION IN MICE

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Introduction: Lung cancer (LC) cachexia is characterized by body weight loss and muscle wasting. Prolonged periods of immobilization due to the underlying condition of cancer, lead to a further decline in muscle function and mass. Elucidation of to what extent cancer cachexia or disuse muscle atrophy contributes to the wasting process of muscle mass remains to be elucidated.
Objectives: Thus, we hypothesized that in mice with lung cancer LC-induced cachexia, periods of immobilization of the hindlimb (7 and 15 days) may further aggravate the process of muscle mass loss and function.
Methods: Mice were divided into 7 groups (N = 10/group):): 1) non-immobilized control mice, 2) 7 days unloaded mice (7-day I), 3) 15 days unloaded mice (15-day I), 4) 21 days LC-cachexia group (LC 21-days), 5) 30 days LC-cachexia group (LC 30-days), 6) 21 days LC-cachexia group and besides 7 days of unloading (LC 21-days + 7-day I), 7) 30 days LC-cachexia group and besides 15 days of unloading (LC 30-days + 15-day I). Physiological parameters, body weight, muscle and tumor weights, phenotype and morphometry (immunofluorescence), muscle damage abnormalities (Hematoxylin-eosin), plasma levels of troponin-I (muscle damage, ELISA), proteolytic (ubiquitin ligases, proteasome and protein ubiquitination, immunoblotting) and autophagy (beclin-1, p62 and LC3B, immunoblotting) markers, muscle regeneration cells (quiescent and activated satellite cells, myoblasts and myocytes, immunofluorescence) and profile of muscle regeneration markers (MyoD and myogenin, immunoblotting) were identified in gastrocnemius muscle.
Results: In LC-induced cachexia mice exposed to hindlimb unloading, gastrocnemius weight, limb strength, fast-twitch myofiber cross-sectional area, and muscle regeneration markers significantly decreased, while tumor weight and area, muscle damage (troponin-I), and proteolytic and autophagy markers increased.
Conclusions: In gastrocnemius of cancer- cachectic mice exposed to unloading, severe muscle atrophy and impaired function was observed along with increased muscle proteolysis and autophagy, muscle damage, and impaired muscle regeneration.
Funding: CIBERES, FIS 18/00075 (FEDER), SEPAR 2018 and SEPAR 2020 and Scholarship from Universitat Pompeu Fabra.

ELECTRON MICROSCOPY MAPPING OF PULMONARY ARTERIES IN PULMONARY HYPERTENSION

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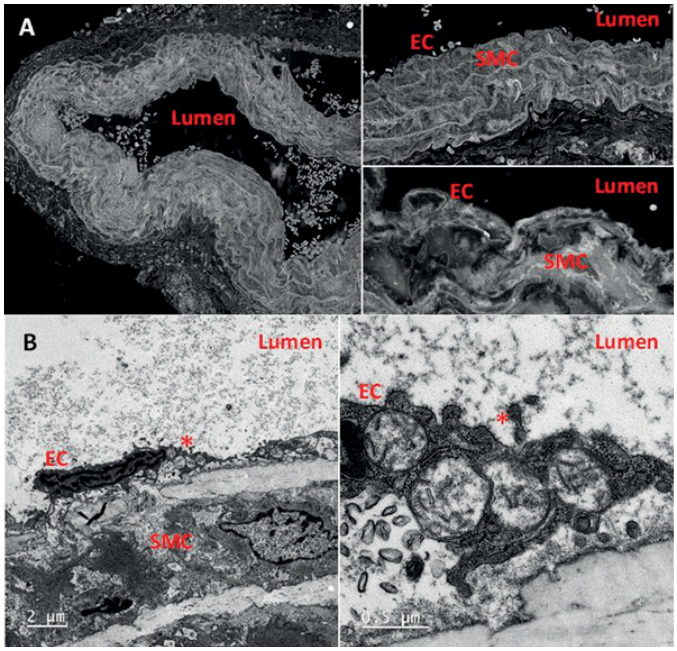
Introduction: The vascular remodelling responsible for pulmonary hypertension (PH), the endothelial-to-mesenchymal transition or the change of mitochondrial morphology due to the metabolic shift from oxidative phosphorylation to glycolysis, known as the Warburg effect, can be characterized with electron microscopy. Therefore, electron microscopy can provide key information about the effect of novel therapies against PH such as 2-Deoxy-D-Glucose (2-DG), a powerful glycolysis inhibitor.
Objectives: We attempt to implement giga pixel imaging analysis obtained with scanning electron microscopy (SEM) and combine it with transmission electron microscopy (TEM) of cell organelles to obtain a complete characterization of pulmonary arteries of murine models of PAH subjected to a 2-DG treatment. Our final goal is evaluating the effect of this glycolysis inhibitor in terms in reverting vascular remodelling, mitochondrial morphology changes and endothelial-to-mesenchymal transitions in the endothelial cells.
Methods: TEM, SEM and image software were used for characterization and analysis. PH was induced in rats under normobaric hypoxia with 10% O2 for 3 weeks, and subjected to an IP injection of Sugen each week (3 injections in total). 2-DG was administered in the drinking water (0.4%). The studied groups have been: a) normoxia, b) normoxia + 2-DG and c) hypoxia + 2-DG.
Results: Preliminary results showed that it is possible to obtain full section giga pixel SEM images of pulmonary arteries that allowed for statistical analysis of the endothelial cell orientation. This information was complemented with a detailed TEM study of mitochondria in endothelial and smooth muscle cells. Results showed differences in the endothelial cell orientation, invaginations in the intima and mitochondria morphology and location within the cytosol.



SEM giga pixel image of a pulmonary artery at different magnifications (A). TEM image of the same pulmonary artery at different magnifications (B). EC = endothelial cell and SMC = smooth muscle cell.

Conclusions: We demonstrate that electron microscopy imaging and giga pixel image analysis are powerful tools towards a complete evaluation of treatments against PH.

Funding: SCR is supported by the Ministerio de Ciencia e Innovación (PID2019-106139RA-100). JRC is supported by grants from the Ministerio de Economía, Industria y Competitividad (MEIC) (SAF2017-84494-C2-R), and from the Gobierno Vasco, Dpto. Industria, Innovación, Comercio y Turismo under the ELKARTEK Program (Grant No. KK-2019/bmG19). JRC received funding from the BBVA Foundation (Ayudas a Equipos de investigación científica Biomedicina 2018) and from La Caixa. CIC biomaGUNE is supported by the Maria de Maeztu Units of Excellence Program from the Spanish State Research Agency - Grant No. MDM-2017-0720.

EMERGENT NON-PCV13 SEROTYPES CAUSING INVASIVE PNEUMOCOCCAL DISEASE IN SPAIN: PAN-GENOME ANALYSIS

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Introduction: Streptococcus pneumoniae is a major human pathogen causing invasive disease (IPD) associated with morbidity and mortality. The introduction of conjugated vaccines (PCVs) changed the epidemiology of IPD mainly due to serotype replacement.
Objectives: We aim to analyze by whole genome sequencing (WGS) the pangenome of the most frequent non-PCV13 serotypes 5 years after PCV13 introduction.
Methods: Adult invasive episodes from 2008-2009 (pre-PCV13), 2012-2013 (early-PCV13) and 2015-2016 (late-PCV13) were collected from 6 Spanish hospitals. After serotyping and genotyping (PFGE and/or MLST), 10 representative of major clonal complexes (CC) of emergent non-PCV13 serotypes (S) [S8-CC53, S8-CC404, S12F-CC989, S9N-CC67 and S22F-CC433] were selected for WGS analysis (n = 50).
Results: A total of 2,187 IPD episodes were collected. The most frequent non-PCV13 serotypes in the last period were S8 (n = 92, 15.6%), S12F (n = 48, 7.8%), S9N (n = 33, 5.4%) and S22F (n = 25, 4.1%). WGS analysis reveled that the capsular operon was identical in all serotypes. Regarding acquired resistance genes, six isolates had cat gene and seven isolates the tet(M). No amino acids changes were observed in PBPs, ParC, ParE, GyrA and DHFR with the exception of one S12F strain presenting T338A in PBP2X and another S12F with I100L in DHFR. Pan-genome analysis revealed a total of 3,022 genes, of them 1,537 were present in all genomes. Among accessory genome, 21 genes were only present in S8-CC53, 21 in S8-CC404, 50 in S12F-CC989, 62 in S9N-CC67 and 51 in S22F-CC433. The zinc metalloprotease C (ZmpC) was found only in S8-CC53 whereas neuraminidase B (NanB) was absent only in S8-CC404. Within clonal complexes, the core-genome accounted for 94% of genome for S8-CC53 and S8-CC404; and 87% for S12F-CC989, S22F-CC433 and S9N-CC67. The core-genome of S9N-CC67 had the highest number of SNPs (2,449 SNPs).
Conclusions: Clonal complex of emerging non-PCV13 serotypes were genetically highly homogenous, a hallmark of highly invasive clones. The S9N-CC67 presented the highest diversity.
Funding: This study was supported by grants from Fondo de Investigaciones Sanitarias de la Seguridad Social (PI14/00627;PI18/00339,INT15/0186;INT16/0117) and from Centro de Investigacion Biomedica en Red (CIBER) de Enfermedades Respiratorias (CIBERES) (CB06/06/0037), an initiative of the Instituto de Salud Carlos III, Madrid, Spain; and from MINECO (SAF2017-83388-R). It was co-funded by the European Regional Development Fund/European Social Fund (ERDF/ESF, "Investinginyourfuture"). We thank CERCA Programme/Generalitat de Catalunya for institutional support.

EPIDEMIOLOGY, MANAGEMENT AND EVOLUTION OF PATIENTS WITH SEVERE SARS COV-2 INFECTION ADMITTED TO THE ICU OF A 2ND LEVEL HOSPITAL

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Hospital Nuestra Señora de Gracia, Zaragoza, Spain.

Introduction: The infection with the new and pandemic coronavirus is generating great interest around the world. It is a priority to know their behavior and the response to the different treatments tested
Objectives: To know the epidemiology and complications of Covid-19 patients admitted to our ICU.
Methods: Prospective descriptive study of characteristics, complications and treatment guidelines used in Covid-19 patients admitted during the period March-May 2020.
Results: 12 patients (75% men, 25% women). Average age 66 years. 75% local cases and 25% imported. Time to negative PCR 21d. Comorbidities: 75% HTA, 25% DM, COPD and obesity. All presented fever and dyspnea, 83.3% dry cough, 33.3% arthromyalgia, 25% diarrhea, and 8.3% headache and rash. Time to health contact 4.3d, and 9.8d to ICU admission. All had ARDS, which required MV for 21.3d. 50% required prone decubitus.100% presented lymphopenia and elevated LDH, ferritin and DD. Complications: severe polyneuropathy and mucous plugs 50%, atelectasis and NAVM 25%, delirium and bleeding 16.6%, AMI and DVT 8.3%. Average ICU stay 28.3d. Mortality in ICU 33.3%. Post-ICU hospitalization time: 11.5d. Total days of admission: 42.9. Treatment administered: azithromycin 5d + hydroxychloroquine 6-7d (suspended in 2 patients for hepatorenal ADR) + lopinavir/ritonavir 8-9d + corticosteroids (50% dexamethasone 20 mg/24h 5d + 10 mg/24h another 5d; 50% pulses of methylprednisolone 250 mg/24 h for 3d) + acetylcysteine 300 mg/6h/iv. 75% were associated with tocilizumab in a single dose (reasons for exclusion: age, contraindication, no indication). 33% received heparin at a therapeutic dose. All required continuous sedation for the first 72h and norepinephrine at low doses for 7.3d. 41.6% received broad spectrum antibiotic therapy for infectious complications.
Conclusions: Average age of 66 years, the majority men and with comorbidities, especially hypertension and DM. Admission to ICU between 9-10d of onset of symptoms, and with an intra-ICU mortality of 33%.

GENETIC LOCI ENRICHED IN AFRICAN ANCESTRY IN THE CANARY ISLANDERS CONTAIN ASTHMA RISK VARIANTS

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Introduction: Asthma is a chronic disease with a strong genetic component. However, a significant proportion of the genetic variation in asthma remains unknown and further studies in diverse populations are needed. The highest prevalence of asthma in Spain is found in the

Canary Islanders. These have also the largest proportion of African genetic ancestry observed among Southwestern Europeans, which has previously been linked to respiratory diseases, including asthma.
Objectives: Here we examined six genomic regions of the current Canary Island population enriched in African ancestry with the aim of identifying novel risk variants associated with asthma susceptibility.
Methods: We performed a two-stage study using genome-wide data from a total of 564 asthma patients from the GOA study and 923 population controls self-declaring having four grandparents with a Canary Islander origin. Genotyping was performed with an Axiom Genome-Wide CEU1 array (Affymetrix). After quality control steps and variant imputation, logistic regressions were performed for association testing and stages 1 and 2 were meta-analyzed for individual variants (Bonferroni-corrected $p = 1.20^{-6}$). Furthermore, imputation and association test of classic alleles from seven HLA genes were conducted with HIBAG (Bonferroni-corrected $p = 4.50^{-4}$).
Results: The study allowed to identify a variant within HLA-DQB1 associated with asthma susceptibility (meta-analysis $p = 1.30^{-7}$, OR [95%CI] = 1.74 [1.41-2.13]). The screening of classical HLA alleles revealed the novel allele HLA-DQA1*01:02 that significantly conferred asthma protection after meta-analysis ($p = 3.98^{-4}$, OR [95%CI] = 0.64 [0.50-0.82]). This allele had previously been related to autoimmune diseases, including type-1 diabetes, and peanut allergy.
Conclusions: The targeted analysis of six genomic regions enriched in African ancestry in the Canary Islands population revealed a novel classical HLA allele associated with asthma protection.
Funding: Instituto de Salud Carlos III (PI14/00844, PI17/00610, FI18/00230, CD19/00231), co-funded by ERD Funds, "A way of making Europe" from the EU; ITER agreement OA17/008.

HUMAN SURFACTANT PROTEIN A BINDS TO THE HUMAN ANTIMICROBIAL PEPTIDE CATHELICIDIN, DECREASING ITS CYTOTOXIC AND INFLAMMATORY EFFECTS ON ALVEOLAR EPITHELIAL CELLS

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Introduction: Human cathelicidin (LL-37) is a host defense peptide with direct antimicrobial activity against several pathogens. However, LL-37 also can cause tissue injury through binding to host membranes causing a cytotoxic effect. Secretion of LL-37 by alveolar epithelial and immune cells increases after infection or tissue injury. Because of SP-A abundance in the lung and its known role in immune defense, we asked whether SP-A was involved in the local regulation of LL-37 activity.
Objectives: To analyze whether SP-A binds to LL-37 and to evaluate whether SP-A is involved in modulating local LL-37 cytotoxic and pro-inflammatory activity.
Methods: SP-A binding to LL-37 was analyzed by tryptophan fluorescence and dynamic light scattering. Antimicrobial activity of LL-37 in the presence or absence of SP-A was studied through killing assays. Cytotoxic activity of LL-37 was measured by propidium iodide staining of target cell DNA, determined by flow cytometry and confocal microscopy. The proinflammatory effect of LL-37 on pneumocytes, with or without SP-A, was evaluated by IL-8 detection.
Results: SP-A bound to LL-37 with high affinity (Kd = 0.01 ± 0.006 pM) in physiological conditions. SP-A/LL-37 interaction results in reduction of LL-37 cytotoxicity on alveolar epithelial cells and U937 macrophages at high LL-37 concentrations, without affecting LL-37 antimicrobial activity against respiratory pathogens (Klebsiella pneumoniae K2, Pseudomonas aeruginosa O1, and nontypeable Haemophilus influenzae). In addition, SP-A decreased the secretion of IL-8 induced by LL-37 in alveolar epithelial cells, preventing LL-37 pro-inflammatory effects.
Conclusions: Our data indicate that SP-A protects against the cytotoxic and pro-inflammatory action of high concentrations of LL-37 by interaction with LL-37. This blocks LL-37 cytotoxicity without affecting LL-37 bactericidal effects.
Funding: Supported by Ministry of Science, Innovation and Universities [grant numbers SAF2015-65307-R and RTI2018-094355-B-I00] and Instituto de Salud Carlos III [grant number: CIBERES-CB06/06/0002], Spain.

IMPACT OF CYCLIC STRETCH ON LUNG MESENCHYMAL STEM CELLS CULTURED ON PULMONARY EXTRACELLULAR MATRIX HYDROGELS

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Introduction: Pulmonary cells are continuously subjected to a characteristic mechanical stimulus which is intrinsic to the pulmonary function. In fact, these cells interact biophysically with the extracellular matrix (ECM) triggering cellular responses through mechanotransduction. Furthermore, the ECM is highly involved in ensuring the regenerative capacity of tissues and, also, undergoes modifications upon diverse pathological conditions. Thus, it is crucial to replicate the real conditions in vitro to understand lung physiology processes.
Objectives: To develop a novel approach to subject pulmonary cells to physiological mechanical stimuli cultured on a new lung-ECM derived hydrogel.
Methods: Rat lung mesenchymal stem cells (MSCs) were cultured on 3D porcine lung ECM hydrogels with a stiffness of 0.7kPa. Hydrogels were attached to a lung-on-a-chip device allowing cyclic stretch. Cells were subjected to a stretch of 10% at breathing frequency of 0.2 Hz. After 5h, actin and paxillin were stained to measure the length of focal adhesions of the cells. Respective control groups without cyclic stretch and cultured on conventional 2D collagen I coating were performed.
Results: In 2D conditions, the application of stretch resulted in a drastic reduction on focal adhesions (2.5 ± 0.2 μm vs 1.8 ± 0.1 μm, $p < 0.01$). However, the level of focal adhesions in hydrogel were less compared to 2D conditions (1.4 ± 0.1 μm, $p < 0.001$). Interestingly, the application of stretch did not result in a further decrease of focal adhesion in cells cultured on 3D hydrogels (1.7 ± 0.1 μm).
Conclusions: The data indicate that the effect that has culturing lung MSCs under cyclic stretch is dependent on how the mechanical stimuli are transmitted to the cells. Thus, lung ECM-derived hydrogels could be a useful scaffold for the development of novel in vitro models based on lung-on-a-chip devices.
Funding: This work is partially funded by SEPAR and Spanish Ministry of Economy and Competitiveness.

IMPACT OF DIESEL EXHAUST PARTICLES ON INFECTED THP-1 MACROPHAGES WITH MYCOBACTERIUM BOVIS BCG

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Introduction: Diesel is one of the main contributors to pollution and it is reported to be able to modify susceptibility to lung infections,

which could include tuberculosis disease. Indeed, countries with high diesel consumption have a high incidence of tuberculosis.
Objectives: Evaluate the impact of diesel exhaust particles (DEP) exposure on mycobacterial infections in an in-vitro human cell model.
Methods: DEP were added to THP-1 cells to test the cytotoxicity, and to Mycobacterium bovis BCG to test their effect on bacterial growth. THP-1 cells were also infected with M. bovis BCG and exposed to a non-cytotoxic concentration of DEP. Extracellular and intracellular bacterial counts were assessed by plating onto Middlebrook, infection percentage and apoptosis were analyzed by flow cytometry, and levels of IL-1β, TNF-α, IFN-γ, IL-8 and GM-CSF were measured by Luminex.
Results: We found cytotoxicity correlated directly with the dose of DEP, being 12.5 μg/ml cytotoxic for THP-1 cells at day 6. We did not find any impairment on the growth of M. bovis BCG exposed to DEP. In infection assays, we observed that the percentage of infected cells exposed to the DEP tended to be lower than cells unexposed to DEP. However, the intracellular recovery of colony forming units (CFU's) was not affected by DEP exposure. Apoptosis increased in infected cells at day 6 in cells exposed to DEP comparing to the control, and extracellular recovery of CFU's tended to be higher on cells exposed to DEP. All cytokines tested except IL-8 increased with the infection, but no differences were observed by exposing cells to DEP.

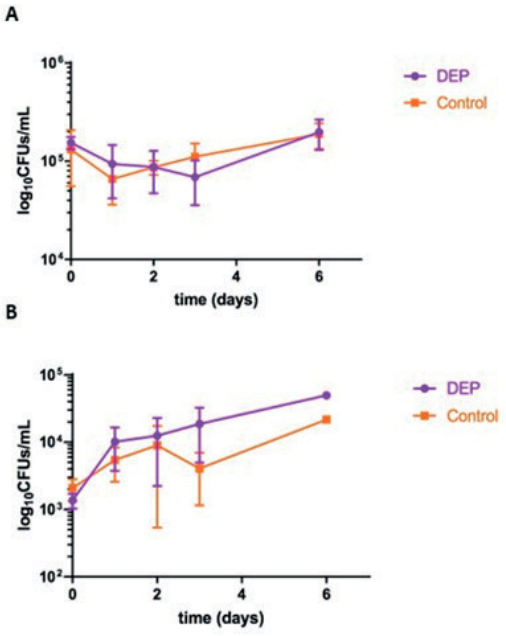

Figure 1. *M. bovis* BCG intra- and extra- cellular CFUs counts over 6 days. THP-1 macrophages were exposed to 6.25 μg/ml DEP. A. Intracellular and B. extracellular growth of bacilli was assessed. The results are expressed as the average and standard deviation of triplicates of at least three independent experiments.

Conclusions: Diesel exhaust particles are highly cytotoxic and could have some effects on the ability of macrophages to contain M. bovis BCG.
Funding: This work was supported by: SEPAR (Spanish Society for Pulmonology and Thoracic Surgery; Extraordinary Call for Research PII Smoking, Research Project nº: 16/024); European Respiratory Society Long-Term Research Fellowship (LTRF-2015-5934); Instituto de Salud Carlos III (PI16/1912 y PI17/01139 projects, integrated in the National Plan R+D+I and funded by the ISCIII and the European Regional Development Fund.) and the CERCA Programme/Generalitat de Catalunya. We also thank MJ. Cruz and X. Muñoz from Institut de Recerca Vall d'Hebron and Hospital Universitari Vall d'Hebron (Barcelona) for kindly providing us the diesel exhaust particles for this study.

IMPLEMENTATION OF AN ANTIBIOTIC STEWARD PROGRAM IN THE INTENSIVE CARE UNIT OF A SECOND LEVEL HOSPITAL

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Introduction: Antibiotic stewardship Programs (AMS) have been shown to improve how antibiotics (AB) are prescribed by clinicians. They represent a change in culture and a complex challenge in which dissemination and training are essential. A single Service does not have sufficient force to generate these changes, so ideally it should be an institutional project.
Objectives: Objective: Implementation of an AMS-ICU. Update ourselves on infectious, audit > 80% alerts and spread our activity. Expanded objectives: 1) Determination of vancomycin levels. 2) Annual epidemiological ma.p 3) Preparation of antibiotic guide. 4) Create an institutional project. 5) Training.
Methods: 6-bed polyvalent ICU. Team composed of intensivists, nurses, pharmacist and microbiologist. Target population: patients with prescription of ABs. Project design: Two-year descriptive prospective observational study. Communication via whatsapp alerting of bacteremia and MMR isolations. 3) Audits with the intervention of team AMS clinicians, discussed with the prescriber and offering recommendations. 6) Dissemination and feedback of relevant data.
Results: All the objectives are met with improvements at the hospital level. Income: 435. 68% are prescribed ABs. Interventions: 171. Most intervened processes: respiratory and urinary sepsis. Audit rate: 84% in bacteremia and 89% in isolates. Recommendation in 40% of interventions. 72% aimed at modifying the prescription, with a pressure reduction of 81%. Most intervened: carbapenems (36%), linezolid (26%) and piperacillin/tazobactam (20%). 87% acceptance. An Antimicrobial Guide for critics was developed. Recommended ABs prescription 85%. Item with less adherence: duration of the guidelines: 71%.
Conclusions: Most of the recommendations are aimed at reducing AB pressure. The degree of acceptance is high and without the perception that reduction negatively affects evolution. Our main limitation is not having previous data on the duration of ABs, mean hospital stays, or mortality, so we cannot provide objective comparative information. We are promoting the rational use of ABs and expanding our Service Portfolio.

INVESTIGATING NEW ANTIMICROBIAL THERAPIES AGAINST MULTIDRUG-RESISTANT RESPIRATORY PATHOGENS

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Introduction: In recent years, the appearance of multidrug-resistant respiratory pathogens such as Klebsiella pneumoniae or Pseudomonas aeruginosa has substantially increased the need to develop new antimicrobial therapies. To improve the efficacy of conventional antimicrobials, attention has been focused on combining clinically used antibiotics with human host defense peptides. Two promising host defense factors are pulmonary surfactant protein (SP-A) and the antimicrobial peptide SP-BN. We previously reported that both exert synergistic action against K. pneumoniae K2.

Objectives: To evaluate and characterize the potential synergistic antimicrobial activity of SP-A and SP-BN, employed with and without conventional antibiotics, against respiratory pathogens.
Methods: The effect of SP-A and SP-BN on bacterial membranes in vivo was studied by spectrophotometric techniques and transmission electron microscopy, while the effect in vitro on bacterial model membranes was analyzed by differential scanning calorimetry. Synergistic antimicrobial activity of SP-A and/or SP-BN with conventional antibiotics was studied through killing assay.
Results: The SP-A/SP-BN complex alters the bacterial ultrastructure of K. pneumoniae due to the ability to bind to lipopolysaccharide molecules present in the outer membrane, forming pores in the membrane that favor the translocation of both proteins to the periplasmic space. There they interact with the inner membrane by causing its permeabilization and depolarization, perhaps through the induction of toroidal pores. On the other hand, while SP-A only acts synergistically with polymyxin B, the peptide SP-BN could act synergistically with many conventional antibiotics.
Conclusions: The synergistic antimicrobial activity of the SP-A/SP-BN complex is based on the capability to alter the integrity of outer and inner bacterial membranes. SP-BN also improves the efficacy of conventional antibiotics against K. pneumoniae, expanding the possible antimicrobial use of this peptide. The characterization of mechanisms that govern synergistic activity with antibiotics may provide clues to improve current therapeutic treatments.
Funding: Supported by Ministry of Science, Innovation and Universities [grant numbers SAF2015-65307-R and RTI2018-094355-B-I00] and Instituto de Salud Carlos III [grant number: CIBERES-CB06/06/0002], Spain.

LPS TREATMENT ENHANCES THE REGENERATIVE CAPACITY OF MESENCHYMAL STROMAL CELLS-DERIVED EXOSOMES

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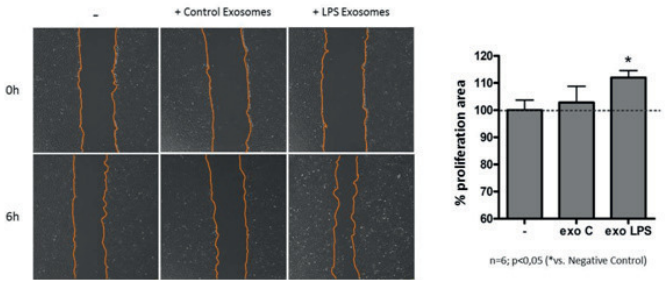
Introduction: Sepsis is a complex syndrome produced by a systemic infection that is accompanied by a dysregulated host immune response, affecting principally the lungs and causing, in many cases, an acute respiratory distress syndrome (ARDS) associated with a mortality rate of 40%. Despite decades of research, there is still a lack of a specific treatment targeting the regeneration of lung tissue injury. It is well established that the administration of mesenchymal stromal cells (MSC) has a remarkable therapeutic effectiveness and that its paracrine activity, mediated by the secretion of exosomes, has a crucial role on MSCs' action.
Objectives: In this study we aimed to determine the effect of the exosomes from MSC in vitro on cell proliferation, and to observe how pre-conditioning the MSCs to a septic environment, changes their exosomes' content and consequently, their regenerative capacity.
Methods: MSC were isolated from male Sprague-Dawley rats' femora and tibiae and cultured in non pre-stimulated and pre-stimulated (with lipopolysaccharide (LPS)) conditions. The secreted exosomes were obtained via standard ultracentrifugation protocol. Cell proliferation experiments were conducted by treating an epithelial cell line with exosomes from MSC and pre-stimulated MSC through an in vitro scratch assay. Exosomal protein profile was determined by a liquid chromatography-mass spectrometry analysis.
Results: The treatment of epithelial cells with exosomes derived from pre-stimulated MSC (LPS exosomes) increases a 15% its capacity to proliferate and to regenerate the wound healing in comparison with the epithelial cells treated with exosomes derived from non pre-stimulated MSC. The analysis of the protein content of both types of exosomes confirmed that pre-conditioning MSCs to a septic environment modifies the protein cargo of its exosomes, resulting in the appearance proteins related to cell cycle regulation and cell proliferation.



A. Representative scratch assay images over a 6 h period; B. Graphical representation of the scratch assay.

Conclusions: LPS treatment modifies Mesenchymal Stromal Cells-Derived Exosomes protein content and consequently, enhances their regenerative effects.
Funding: Fundación Ramon Areces.

METABOLOMICS IDENTIFIES BIOMARKERS OF EXACERBATIONS IN SMOKERS WITH AND WITHOUT COPD

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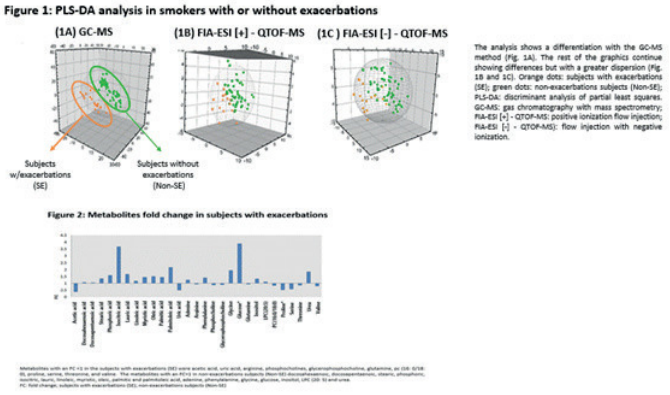
Introduction: The exacerbations are an important event in the natural history of COPD. These are associated with a worsening lung function, an increase of symptoms, more health resources use, and mortality. Biomarkers (BM) capable of predicting the risk of future exacerbations in COPD, is necessary.

Objectives: Determine the BM of clinical utility associated with the presence of respiratory exacerbations in serum sample the individuals of CHAIN cohort.
Methods: For this purpose, were selected serum samples of individuals, smokers, with or without COPD with ≥ 1 hospital admission or more than two episodes that require antibiotic treatment with/without systemic corticosteroids, in the previous year. The samples are classified in 21 subjects with exacerbations (SE) and 68 with non-exacerbations (Non-SE). First, we do an analysis of gas chromatography with mass spectrometry (GC-MS) and low injection (FIA) with positive and negative ionization. The results were processed to build the partial least squares analysis plots (PLS-DA) and compare profiles metabolic lost. The altered metabolites are selected according to the parameter "Variable importance in the projection" (VIP), a VIP > 1 is indicative of differences between the groups.
Results: The PLS-DA analysis with the three methods described, only GC-MS differentiates SE vs Non-SE group (Figure 1A, 1B, 1 C). However, the analysis identified 29 metabolites in group SE, although only 5 show significative differences in the ANOVA test (Table 1). In addition, we observed a decrease in most of the amino acids in the group SE (an exception of glycine and phenylalanine) in contrast with the increase of all fatty acids (Figure 2).



Conclusions: The decrease of amino acids and high levels of fatty acids in subjects' smokers with exacerbations profile was associated with the presence of respiratory exacerbations, founding 5 metabolites potentially involved (phosphoric acid, phenylalanine, glycine, glucose, and proline).
Funding: Scholarship not conditioned by Menarini laboratories. Scholarship not conditioned by AstraZeneca laboratories. Scholarship project IIS-AES PI16/01.

MICROBIOTA AS A MARKER OF MUCOID PSEUDOMONAS AERUGINOSA AND HAEMOPHYLUS INFLUENZAE IN NON-CYSTIC FIBROSIS BRONCHIECTASIS

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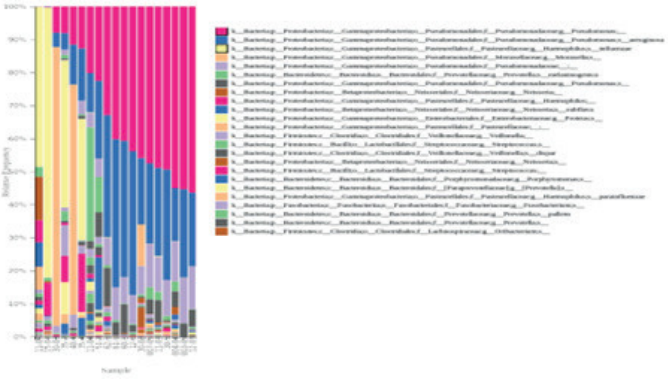
Introduction: Pseudomonas aeruginosa (PA) and Haemophylus influenzae (HI) are two of the main pathogens in non-CF bronchiectasis (BE) chronic colonization. However, scarce evidence exists on respiratory concomitant microbiota patterns associated to each of the two pathogens, in particular when mucoid PA is present.
Objectives: To determine if Pseudomonas aeruginosa (PA) non-mucoid (group 1) or mucoid (group 2), Haemophylus influenzae (HI) (group 3), and combined presence of mucoid PA and HI (group 4) is associated to any particular pattern of concomitant microbiota also clustering by BE alone or plus Chronic obstructive pulmonary disease (COPD).
Methods: Prospective collection of sputum in BE patients with at least 1 previous PA isolation. DNA of sputum samples were isolated. The preparation of the DNA libraries was based on the hypervariable regions for bacterial 16S rRNA. The pool of DNA libraries was introduced into Illumina Miseq platform. The bioinformatic analyses were performed by QIIME with GreenGenes microbiome database.
Results: Twenty-nine sputa were included in the analysis by groups 1 to 4 (n = 5, n = 9; n = 5 and n = 10, respectively). Twenty-seven taxonomic units were found. PA and HI were present in all groups having the highest abundance of PA or HI the group 4 (p = 0.022) or group 3 (0.007), respectively. HI was higher in the BE-COPD group compared to BE alone (520.5 [207.5-11,629.3] vs 49.5 [0.0-175.0], p = 0.007, respectively). Interestingly, the four study groups differed in terms of concomitant microbiota. Carnobacteriaceae were only found in group 1 Fusobacteriaceae, were only found in group 2, being the ones with higher abundance. Mycoplasmataceae, Gemellaceae and Peptostreptococcaceae were only found in group 4 being the Gemellaceae the ones with higher abundance.



Conclusions: The use of Fusobacteriaceae or Gemellaceae as markers of mucoid PA or mucoid PA with HI colonization needs further investigation but could impact in clinical management of BE patients.
Funding: FIS PI1800145.

MORBI-MORTALITY AND ECONOMIC IMPACT OF LOWER RESPIRATORY TRACT INFECTIONS IN THE HOSPITAL SETTING

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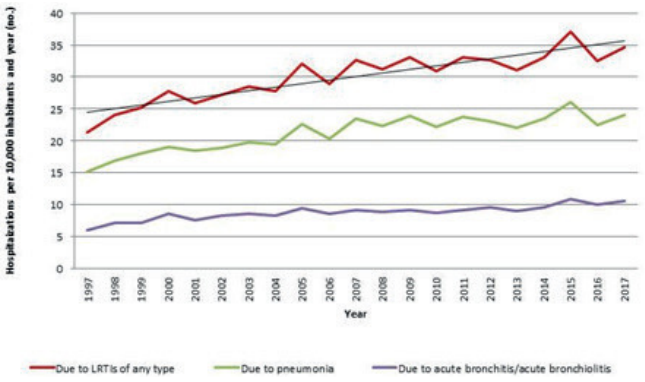
Introduction: Lower respiratory tract infections (LRTIs) are one of the leading causes of infectious disease mortality worldwide.
Objectives: The aim of the study was to analyze the health and economic impact of LRTIs from 1997 to 2017.
Methods: An observational study of hospitalizations due to LRTIs (pneumonia and acute bronchitis/bronchiolitis) in Spain from 1997 to 2017 was carried out. Data were extracted from the national information system for hospital data. Hospitalizations with LRTIs registered as primary diagnoses were included.
Results: Overall, 3.4% (range: 2.8-3.9%) of total hospitalizations were caused by LRTIs (n = 2,786,187), with a median incidence of 31.1 (range: 21.3-37.0) per 10,000 inhabitants/year. The incidence was higher for pneumonia cases than for acute bronchitis/bronchiolitis [median: 22.2 (range: 15.2-26.1) vs. 8.9 (range: 6.1-10.9) per 10,000

inhabitants/year; p < 0.001] and increased by 62.5% from 1997 to 2017 (Figure 1). A 47.9% of the hospitalizations due to LRTIs occurred in people over 74 years (Table 1).
In 89.2% of the cases, patients were discharged home. In-hospital mortality was 6.9%, and the median rate was 9,342 deaths (range: 5,257-11,991) per year. Mortality was significantly higher for pneumonia than for acute bronchitis/bronchiolitis [median: 9.0% (range: 8.2-10.1%) vs. 1.7% (range: 1.2-2.3%); p < 0.001] and showed a rising trend. A 75.1% of the deaths occurred in people over 74 years. In 2017, hospitalizations due to LRTIs had a median duration of 8.0 days (range: 4.9-10.0) and a cost of 4,439 € (range: 3,374-5,965 €) per hospitalization. Hospitalizations due to LRTIs amounted to 715.5 million € in 2017.

Age distribution of the patients hospitalized due to lower respiratory tract infections (LRTIs)

Age group	Main cause of the hospitalization		
	LRTIs of any type[a] (No. = 2,786,187)	Pneumonia[b] (No. = 1,968,672)	Acute bronchitis/ bronchiolitis[c] (No. = 817,515)
< 1 year	12.2%	1.2%	37.1%
1-14 years	9.9%	7.9%	14.3%
15-44 years	5.0%	6.1%	2.3%
45-64 years	11.8%	14.4%	6.1%
65-74 years	13.2%	15.7%	7.5%
> 74 years	47.9%	54.7%	32.6%
Total	100.0%	100.0%	100.0%

No.: number. [a]Percentage from total hospitalizations due to LRTIs of any type. [b]Percentage from total hospitalizations due to pneumonia. [c]Percentage from total hospitalizations due to acute bronchitis/bronchiolitis.



Temporal trend of the annual incidence rate of hospitalizations due to lower respiratory tract infections (LRTIs).

Conclusions: LRTIs are associated with a high morbi-mortality in Spain and have a relevant economic impact. There is an increasing trend in hospitalizations and deaths associated to LRTIs, which occurred mainly in older people. Therefore, effective measures that contribute to the prevention and treatment of LRTIs need to be adopted.
Funding: This research did not receive specific support from public agencies, the commercial sector or non-profit organizations.

OBESITY ATTENUATES THE EFFECT OF SLEEP APNEA ON ACTIVE TGF-β1 LEVELS AND TUMOR AGGRESSIVENESS IN PATIENTS WITH MELANOMA

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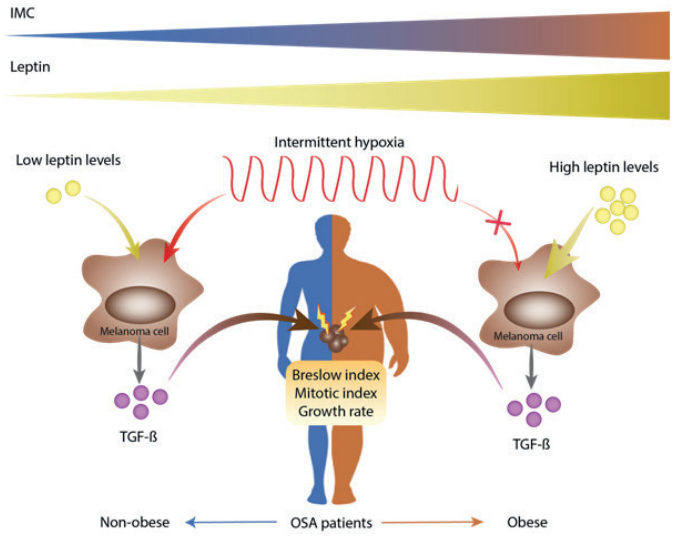
Introduction: Active transforming growth factor-β1 (TGF-β1), a cytokine partially regulated by hypoxia and obesity, has been related with poor prognosis in several tumors. OSA effects on tumor development and progression have been mainly attributed to the impact of intermittent hypoxia on the local tumor microenvironment, promoting melanoma cell proliferation, the release of pro-angiogenic factors, and alterations of the immune surveillance system. However, the impact of OSA on the intrinsic properties of tumor cells is less well understood.
Objectives: Our study objectives were to compare serum levels of active TGF-β1 in patients with CM according to the presence and severity of OSA, assess the correlation between active TGF-β1 levels and melanoma aggressiveness indices, and analyze their relationship with nocturnal hypoxia and serum levels of leptin in both obese and non-obese patients.
Methods: In a multicenter observational study, 290 patients with CM were underwent sleep studies. TGF-β1 and leptin plasma levels were measured by ELISA technique.
Results: TGF-β1 was increased in moderate-severe OSA patients vs. nonOSA or mild OSA patients with CM. In OSA patients, TGF-β1 levels correlated with mitotic index, Breslow index and melanoma growth rate, and were increased in presence of ulceration or higher Clark levels. In CM patients, OSA was associated with higher TGF-β1 levels and greater melanoma aggressiveness only in non-obese subjects. An in vitro model showed that IH-induced increases of TGF-β1 expression in melanoma cells is attenuated in the presence of high leptin levels.



Schematic representation of the proposed interaction between intermittent hypoxia, obesity, and circulating levels of TGF-β in patients with melanoma and OSA. In non-obese subjects, OSA-induced intermittent hypoxia could have a synergistic effect with leptin produced by adipocytes on the TGF-β expression by melanoma cells, promoting greater tumor aggressiveness. According to the data of the present study, this effect is lost in obese patients, since the basal overexpression of TGF-β caused by high levels of leptin is not enhanced by the additional presence of intermittent hypoxia.

Conclusions: In conclusion, TGF-β1 levels are associated with melanoma aggressiveness in CM patients and increased in moderate-severe OSA. Moreover, in non-obese patients with OSA, TGF-β1 levels correlate with OSA severity and leptin levels, whereas only associate with leptin levels in obese OSA patients.
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PARP EXPRESSION AND ACTIVITY IN LUNG TUMORS IN MICE AND PATIENTS: INFLUENCE OF COPD

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Introduction: Lung cancer (LC) is a major leading cause of death worldwide. Poly-ADP ribose polymerases (PARP)-1 and PARP-2 are key signaling pathways in cancer cells.
Objectives: To assess PARP-1 and PARP-2 expression and activity and DNA damage in tumors and non-tumor lungs of LC patients with/without underlying COPD and in an experimental model of lung cancer mice.
Methods: Patients: Lung tumor and non-tumor specimens were obtained through video-assisted thoracoscopic surgery (VATS) in LC patients with/without underlying COPD (two groups of patients, N = 15/group). Mice: Lung tumors (subcutaneous inoculation of 4×10^5 LP07 adenocarcinoma cells) were harvested from the lungs of wild-type BALB/C mice. Two groups of mice were established: non-tumor control mice and tumor-bearing mice (N = 9/group). Molecular biology analysis: PARP-1 and PARP-2 expression (ELISA in patients, immunoblotting in mice), activity (PARP colorimetric assay kit) and DNA damage (immunohistochemistry) levels were identified in all samples.
Results: 1) Patients: Both PARP-1 and PARP-2 expression levels were significantly lower in lung tumors (irrespective of COPD) of patients compared to non-tumor specimens, while DNA damage and PARP activity levels were significantly higher in lung tumors compared to non-tumor specimens only in LC-COPD patients; 2) Mice: PARP-1 expression and PARP activity levels were significantly lower, while DNA damage levels were significantly higher in lungs of LC mice compared to control mice. PARP-2 expression did not differ between the study groups.
Conclusions: In lung tumors of COPD patients, an overactivation of PARP enzyme was observed. A decline in PARP-1 and PARP-2 protein expression was seen in lung tumors irrespective of COPD. In lung tumors of wild type mice, PARP expression and activity also decreased, while greater DNA damage levels were seen in lung tumors of both patients and mice. Other phenotypic features beyond cancer may account for the rise in PARP activity seen in the tumors of patients with underlying COPD.
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POTENTIAL ROLE OF MIR-185-5P AS FEEDBACK MECHANISM FOR CONTROLLING AIRWAY REMODELING AND SMOOTH MUSCLE CONTRACTION

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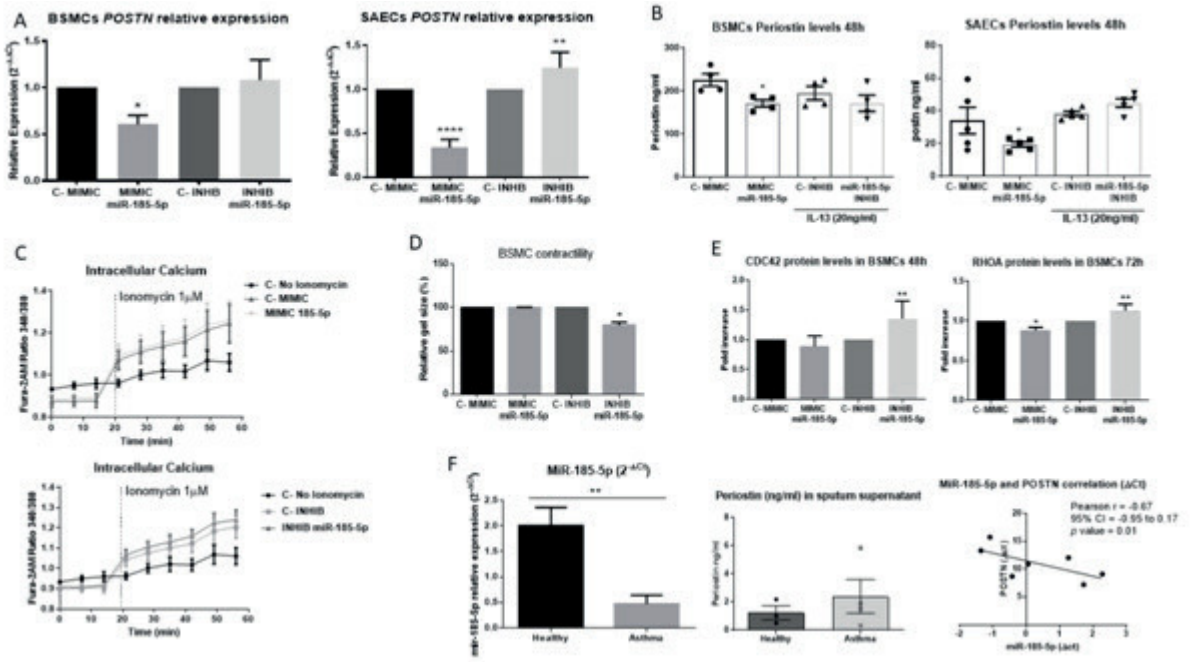
Introduction: Asthma is a chronic airway disease whose pathophysiologyis shaped by a melange of modulators including cytokines, exosomes and microRNAs. We previously described that microRNAs are differentially expressed in eosinophils and serum from asthmatics compared to healthy individuals, one of these is miR-185-5p.
Objectives: The aim of this study is to evaluate the role in airway remodeling and muscle contraction of miR-185-5p.
Methods: We used miR-185-5p mimic and inhibitors in Bronchial Smooth Muscle Cells (BSMCs) and Small Airway Epithelial Cells (SAEC) cultures to study gene expression and cellular functions. Gene expression and protein levels of periostin (POSTN), CDC42, and RHOA were analyzed by RT-PCR and ELISA/Western Blot respectively. BSMC contractility was analyzed using cell-embedded collagen gels and measurement of intracellular calcium was performed using Fura-2. Additionally, miR-185-5p and POSTN gene expression were evaluated in sputum cells from healthy (n = 3) and asthmatics (n = 4).
Results: MiR-185-5p overexpression downregulates periostin mRNA and protein in BSMCs and SAECs at 48 hours ($p < 0.05$; Figure 1A,B). MiR-185-5p modulation modifies contractile-related proteins CDC42 protein synthesis at 48 hours, and RHOA synthesis at 72 hours (Figure 1E). Inhibition of miR-185-5p produced higher BSMCs contraction induced by histamine (79% size gel size vs. 100% of scrambled control, $p < 0.05$; Figure 1D). Calcium mobilization was not modified by miR-

		Healthy (n = 3)	Asthmatics (n = 4)
Age (years)*		26.0 (25-27)	52.3 (36-70)
Sex (female) (%)		2 (66.7)	2 (50.0)
Atopy (%)		1 (33.3)	4 (100.0)
BMI		23.0 (22.9-23.2)	25.5 (23.4-28.3)
Smoking history	Current (%)	1 (33.3)	0 (0.0)
	Passive (%)	0 (0.00)	1 (25.0)
	Non-smoker (%)	2 (66.7)	3 (75.0)
FVC (%)†		105,8 (104.0-107.5)	110.3 (103.0-118.0)
FEV1 (%)†		103.3 (93.0-114.0)	100.8 (98.0-103.6)
FeNO		8.7 (5.4-12-0)	27.5 (18.0-37.0)
Peripheral blood eosinophils (cells/μl)*		200.00 (100-300)	250 (100-400)
Inhaled corticosteroids and LABA (%)		0 (100.0)	4 (100.0)
Squamous cell contamination* (%)		7.8 (3.0-14)	32.6 (7.7-65.0)
Cell viability* (%)		87.6 (78.0-94.8)	75.2 (69.2-83.1)

Results are shown as *Mean (range), †Median (range). FEV1: forced expiratory volume in first second. FVC: forced vital capacity, FeNO: exhaled nitric oxide test.



185-5p, showing that miR-185-5p role in BSMC contractility is performed by regulating CDC42 and RHOA instead (Figure 1C). Diminished miR-185-5p expression and a tendency for higher periostin levels was seen in sputum cells from asthmatics compared to healthy (clinically described in the Table). This miRNA-target relationship was confirmed, asmiR-185-5p expression inversely correlates with POSTN expression (Pearson r = -0.72 p < 0.05; Figure 1F).

Conclusions: We hypothesize that miR-185-5p is upregulated in asthmatic airways as a negative feedback loop to control airway remodeling through periostin secretion and smooth muscle contraction, evidencing its role as therapeutic target.

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PROANGIOGENIC FACTOR MIDKINE IS INCREASED IN MELANOMA PATIENTS WITH SLEEP APNEA AND INDUCES TUMOR CELL PROLIFERATION

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Introduction: Both sleep fragmentation and intermittent hypoxia (IH) that characterize OSA appear to contribute to some of the intermediate mechanisms that promote the oncogenic process. Among hypoxia-induced proangiogenic factors, the pleiotropic cytokine midkine (MDK) has recently acquired particular relevance in melanoma patients. Thereby we hypothesize that Midkine (MDK) might mediate the proangiogenic effect of intermittent hypoxia (IH) in patients with obstructive sleep apnea (OSA) and cutaneous melanoma (CM).

Objectives: Our objectives were to compare circulating MDK in CM patients with and without OSA, and their relationship with tumor aggressiveness, while exploring in vitro effects of soluble MDK on human lymphatic endothelial (HLEC) and melanoma cell proliferation.

Methods: In 360 CM patients, sleep studies and MDK serum level measurements were performed. The effect of MDK on cell proliferation was assessed using HLEC and melanoma cell lines with patient sera under both normoxia and IH.

Results: MDK levels were higher in severe OSA compared to mild OSA or non-OSA patients, whereas no differences in VEGF levels emerged. In OSA patients, MDK levels correlated with nocturnal hypoxemia and CM mitotic rate. In vitro, MDK promotes HLEC proliferation under IH conditions. Moreover, cultures of the human melanoma cell line C81-61 with sera from patients with the highest MDK levels promoted tumor cell proliferation, which was attenuated after addition of a MDK antibody. These responses were enhanced by IH exposures.

Conclusions: As conclusion, in CM patients, OSA severity is associated with higher MDK levels, which, appear to enhance both the lymphangiogenesis as the intrinsic aggressiveness of CM tumor cells.

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PULMONARY ARTERIAL FLOW MEASUREMENT WITH MAGNETIC RESONANCE IMAGING FOR PULMONARY HYPERTENSION DIAGNOSIS IN RODENTS

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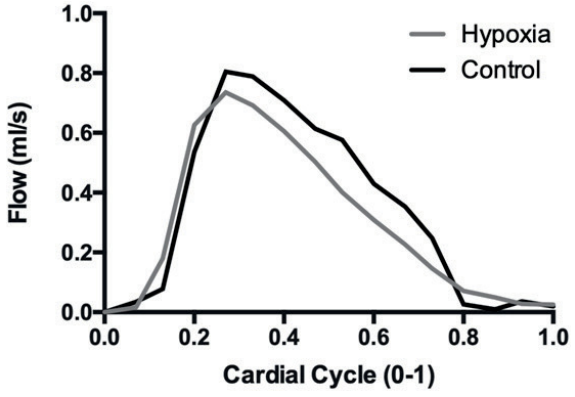
Introduction: Pulmonary hypertension (PH) is characterized by elevated mean pulmonary arterial pressure (mPAP). These altered pressure lead to changes in blood flow patterns that could be used as surrogate biomarkers of vascular remodelling. The pulmonary arterial flow parameters can be measured with magnetic resonance imaging and allow non-invasive diagnosis of PH[1].
Objectives: This study aims to determine the optimal magnetic resonance imaging protocol to quantify pulmonary arterial flow in mice.
Methods: PH was induced in 8-weeks old C57BL/6j male mice by chronic exposure to normobaric hypoxia (10% O2) during three weeks and received SUGEN 5416 IP injections weekly. Normoxic mice were treated with vehicle IP injections. Before the sacrifice, ventricular pressures through right heart catheterization via jugular vein was measured. Image acquisitions were performed at 7 Tesla Bruker MRI system with a 40 mm inner diameter volumetric coil. Imaging parameters included the following: TR: 5.49 ms; TE: 2.6 ms; slice thickness: 1.1 mm; matrix 128*128; VENC 120 cm/s; number of cardiac frames: 15. Data was acquired with dual cardiac and respiratory synchronization at the end of expiration and the pulmonary artery's main trunk.
Results: As expected from data in humans, as pulmonary arterial pressures and vascular resistance increase, blood velocity (min, max and mean) measurements show reduced values PH model comparing to the control group (Figure). A paired-t-test of flow values was performed throughout the cardiac cycle. Significant differences in total flow values between hypoxia and control ($p \leq 0.05$) were observed. Our results demonstrated strong correlations between ventricular pressures with PA flow velocities ($r^2 = 0.6339$).



Comparison of average total flow versus time between PH model and control group in pulmonary artery's main trunk.

Conclusions: Hereby, these preliminary results show that MRI-measured velocity analysis of PA flow can be an excellent technique to diagnose PH. With a non-invasive nature, this imaging modality can be relevant in understanding disease formation and therapeutic efficiency mechanisms, and, therefore, can be integrated into conventional imaging protocols.
Funding: This work was supported by grants from the Ministerio de Economía, Industria y Competitividad (MEIC) (SAF2017-84494-C2-R), and from the Gobierno Vasco, Dpto. Industria, Innovación, Comercio y Turismo under the ELKARTEK Program (Grant No. KK-2019/bmG19). JR-C received an Ayuda from the Fundación contra la Hipertensión Pulmonar (2018). CIC biomaGUNE is supported by the Maria de Maeztu Units of Excellence Program from the Spanish State Research Agency - Grant No. MDM-2017-0720.

PULMONARY FUNCTION AND RADIOLOGICAL FEATURES IN COVID-19 CRITICALLY ILL SURVIVORS AFTER HOSPITALIZATION DISCHARGE: A 3 MONTHS PROSPECTIVE COHORT

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Introduction: More than 20% of hospitalized patients with COVID-19 develop acute respiratory distress syndrome (ARDS) requiring UCI admission. The short-term respiratory sequelae of the surviving ICU patients remain speculative.
Objectives: To perform a detailed characterization of the short-term pulmonary sequelae in COVID-19 critically ill survivors.
Methods: Consecutive critical patients with COVID-19 requiring ICU admission were prospectively recruited and evaluated in a pulmonary consultation 3 months after hospitalization discharge. The systematic follow-up comprised: pulmonary function tests including lung volumes (TLC, VC, RV, FRC) and lung diffusing capacity for carbon monoxide (DLCO); exercise test; chest CT; and blood test.
Results: One hundred and twenty-five surviving ICU patients with ARDS secondary to COVID-19 were recruited between March and June 2020. Median ICU stay was 14.5 days and 39 (62.9%) patients were intubated. At 3 months follow-up, 62 patients were available for pulmonary consultation. Abnormal DLCO was reported in 84.3% of survivors. Reticular and fibrotic lesions were observed in 47.1% and 23.5% of the patients, respectively. Those survivors with more severe affectation in the chest CT showed worse pulmonary function and presented more degree of desaturation in the exercise test. Factors associated with the severity of lung damage in the chest CT were age and prone positioning.
Conclusions: Pulmonary structural abnormalities and functional impairment are persistent in surviving ICU patients with ARDS secondary to COVID-19 3 months after hospitalization discharge. Pulmonary evaluation should be considered for critical ill survivors at short-term.
Funding: Supported by ISCIII (CIBERESUCICOVID, COV20/00110), co-funded by ERDF, "Una manera de hacer Europa".

RELATIONSHIP BETWEEN EOSINOPHILS AND COVID-19 IN CHRONIC RESPIRATORY DISEASES

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Introduction: In December 2019, a new human coronavirus was identified in Wuhan for first time. World Health Organization named

it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), producing coronavirus disease (COVID-19). It has been responsible for the pandemic, producing more than 32 million of confirmed cases and almost one million of verified deaths.
Objectives: Our main objective is to explore the relation that eosinophils have in COVID-19 patients with chronic respiratory diseases (CRD).
Methods: We got a database of 3018 patients attended in two public hospitals in Madrid from January-April 2020, who had confirmed SARS-CoV-2 infection by polymerase chain reaction test. De-identified medical records were confidentially collected from Microsoft Sql Integration Services (SSIS) reporting database. Clinical and laboratory parameters were analyzed using Fisher's exact test for categorical variables and by two-tailed Mann-Whitney test for continuous variables. $p < 0.05$ was considered significant.
Results: From the 3,018 patients, we included to 2,539 who had respiratory disease information. Among them, 384 presented some CRD (15.12%): asthma (4.45%), chronic obstructive pulmonary disease (COPD) (3.51%) and obstructive sleep-apnea (OSA) (3.19%). Regarding CRD population, eosinophils showed a significant reduction at discharge in comparison to admission analytics ($p < 0.0001$). Indeed, patients with eosinopenia were greater in discharge than in admission (10.94% vs. 25.52%, $p < 0.0001$). This phenomenon can be also observed in each respiratory disease separately. Also, we found a higher percentage of mortality in individuals with eosinopenia respect to non-eosinopenia patients ($p < 0.05$), and eosinopenia percentage was further up in severe patients at discharge ($p < 0.0001$). Finally, patients with asthma had significant lower percentage of deaths than COPD ($P < 0.0001$) and OSA ($p < 0.05$).
Conclusions: In CRD population, lack of normal values of eosinophils have a worse prognosis in COVID-19 patients. Interestingly, asthmatic patients showed lower mortality than COPD and OSA patients.
Funding: This study was supported by Fondo de Investigación Sanitaria - FIS and FEDER (Fondo Europeo de Desarrollo Regional) [PI15/00803, PI18/00044 and FI16/00036], CIBERES, Merck Health Foundation funds and RTC-2017-6501-1 (Ministerio de Ciencia, Innovación y Universidades).

RESPIRATORY POLYGRAPHY PATTERNS AND RISK OF CARDIOVASCULAR EVENTS IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Introduction: Obstructive sleep apnea (OSA) severity is exclusively based on the apnea hypopnea index (AHI). Nevertheless, AHI is a simplistic measure that could result inadequate to capture the severity of the disease and its potential deleterious consequences in cardiovascular disease (CVD).
Objectives: To explore specific respiratory polygraphy patterns that could contribute to identify the risk of recurrent cardiovascular events in patients with acute coronary syndrome (ACS).
Methods: Post-hoc analysis of the ISAACC study, including 909 patients admitted for ACS (NCT01335087). To identify specific respiratory polygraphy patterns a principal component analysis (PCA) was performed using six respiratory polygraphy parameters: AHI, oxygen desaturation index, mean and minimum SaO2, average duration of events and percentage of time with SaO2 < 90%. All analyses for studying respiratory polygraphy patterns contribution on the risk of recurrent cardiovascular events was stratified by ACS phenotypes described as patients with no-previous CVD and patients with previous CVD.
Results: PCA showed that two respiratory polygraphy patterns accounted for 70% of variance. A first pattern was mainly characterized by a hypoxic component (hypoxic OSA pattern) and a second pattern that is characterized by longer events with less oxygen desaturation (non-hypoxic OSA pattern). The non-hypoxic OSA pattern was associated with an increased risk of recurrent cardiovascular events with an adjusted HR (95% CI) of 2.10 (1.16 to 3.78) (p-value = 0.01) for patients with no-previous-CVD phenotype, whereas a decreased risk of recurrent cardiovascular events with an adjusted HR (95% CI) of 0.41 (0.18 to 0.98) (p-value = 0.04) was found for patients with previous-CVD phenotype. For the hypoxic OSA pattern, no significant association was found for the risk of recurrent cardiovascular events in any of the ACS phenotypes.
Conclusions: A pattern of sleep apnea, mainly characterized by longer events with less oxygen desaturation, is associated with different degrees of risk of recurrent cardiovascular events in specific profiles of patients who have suffered from ACS.
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RISK FACTORS ASSOCIATED WITH PULMONARY HYPERTENSION IN OBESITY HYPOVENTILATION SYNDROME

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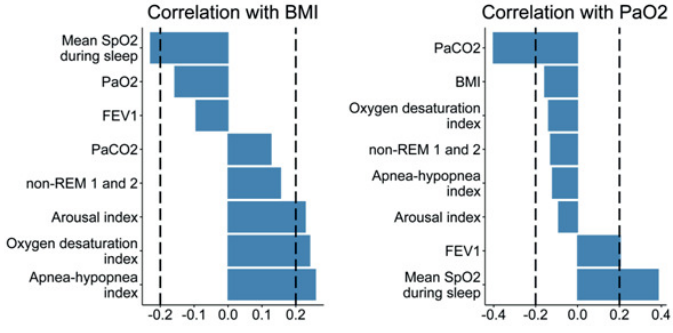
Introduction: Pulmonary hypertension (PH) is prevalent in obesity hypoventilation syndrome (OHS). However, there is a paucity of data assessing risk factors associated with the pathogenesis of PH.
Objectives: Analysis of risk factors of PH in OHS. In a post-hoc analysis of the Pickwick trial, we performed a bivariate analysis of baseline characteristics between patients with and without PH. Variables with a p value ≤ 0.10 were defined as potential risk factors for PH.
Methods: Risk factors were initially grouped by theoretical pathogenic mechanisms and adjusted models were evaluated. To assess the pathogenic groups together, a process of selection of potential risk factors using a Least Absolute Shrinkage and Selection Operator (LASSO) model was carried out. Finally, the results of the LASSO model served to develop a multivariate logistic model. Similar analysis was

carried out for the two OHS phenotypes, with and without severe concomitant obstructive sleep apnea (OSA).
Results: Of the 246 patients, 122 (49.6%) had echocardiographic evidence of PH. PaO2 and body mass index (BMI)were independent risk factors with a negative and positive adjusted linear correlation, respectively (adjusted odds ratio 0.96; 95%CI 0.93 to 0.98; p = 0.003 for PaO2 and 1.07; 95%CI 1.03 to 1.12; p = 0.001 for BMI). In the severe OSA phenotype, PaO2 and BMI were independent risk factors. In the non-severe OSA phenotype, E/A ratio and BMI were independent risk factors.

	Age-adjusted model*		Global model †	
	OR (95%CI)	p value	OR (95%CI)	p value
Obesity				
BMI, kg/m²	1.08 (1.04;1.13)	< 0.001	1.07 (1.03;1.12)	0.001
FEV1,% of predicted	0.98 (0.97;0.99)	0.013		
Sustained hypoxemia				
PaO2, mmHg	0.95 (0.92;0.98)	0.001	0.96 (0.93;0.98)	0.003
Mean SpO2 during sleep				
First tertile	Reference			
Second tertile	0.67 (0.35;1.26)	0.214		
Third tertile	0.7 (0.38;1.31)	0.269		
OSA				
Arousal index¥	1.18 (1.03;1.36)§	0.017		
Oxygen desaturation index¥	1.13 (1.01;1.27)§	0.049		
Apnea-hypopnea index¥	1.09 (0.98;1.21)§	0.1		
non-REM 1 and 2, %	1.02 (1.00;1.03)	0.079		
Hypoventilation				
PaCO2, mmHg				
First tertile	Reference			
Second tertile	1.23 (0.66;2.30)	0.512		
Third tertile	2.2 (1.18;4.12)	0.013		

Data presented coefficient (25;75 IQR). * The individual factors were adjusted only by age; ¥: log-transformed variable were used; §odds ratio for a 50% increase in variable; †: selection variables using Least Absolute Shrinkage and Selection Operator (LASSO). Note: Mean SpO2 during sleep and PaCO2were categorized by tertiles due to their non-linear relationship with the presence of pulmonary hypertension (see FigureS2). Abbreviations: OR: odd ratio; CI: confidential interval; BMI: body mass index; FEV1: forced expiratory volume in the first second; and SpO2: oxygen saturation by pulse oximetry.



Dashed line indicates limit above which the correlation value reaches statistical significance with p value equal to 0.05.Abbreviations: BMI = body mass index; FEV1 = forced expiratory volume in the first second; SpO2 = oxygen saturation by pulse oximetry.

Conclusions: Sustained hypoxemia and obesity are implicated in the origin of PH in OHS. The risk factors for PH vary based on OHS phenotypes. Although severe obesity is a shared risk factor, in those with severe OSA, sustained hypoxemia is an additional risk factor. Inpatients without severe OSA, a component of diastolic dysfunction (post-capillary mechanism) is an important risk factor.

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RUCAPARIB TREATMENT ATTENUATES PROTEOLYSIS AND AUTOPHAGY IN PERIPHERAL AND RESPIRATORY MUSCLES IN CANCER-CACHEXIA MICE

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Introduction: Overactivation of Poly(ADP-ribose) polymerases (PARP) lead to a deleterious effect on tissues, including muscles.
Objectives: We aimed to evaluate the effects of rucaparib treatment, a PARP inhibitor drug, on muscle fiber atrophy and other molecular and structural alterations in peripheral and respiratory muscles of mice with lung cancer.
Methods: PARP activity, muscle phenotype and morphometry (immunohistochemistry), muscle structural abnormalities (hematoxylin-eosin staining), and proteolytic and autophagic markers (immunoblotting, spectrofluorometry) were explored in diaphragm and gastrocnemius at day 30 of the study in the following groups of mice: 1) non-cachexia control, 2) non-cachexia control treated with rucaparib (150 mg/kg body weight/24h for 20 days, intragastric, gently provided by Clovis Oncology, San Francisco, CA, USA), 3) cancer-cachexia (4×10^5 LP07 cells inoculated subcutaneously in the left flank) and 4) cancer-cachexia treated with rucaparib. Furthermore, plasma troponin-I concentration (ELISA) and physical activity were analyzed in all the study animals.
Results: In gastrocnemius and diaphragms of cancer-cachexia mice muscle fibers size decreased compared to non-cachexia control. Moreover, PARP activity, muscle structural alterations and proteolytic (including troponin-I and tyrosine release) and autophagic markers increased in cachectic mice compared to non-cachectic controls. Rucaparib treatment on cancer-cachexia mice attenuated muscle structural alterations and reduced PARP activity on peripheral and respiratory muscles compared to cancer-cachexia mice. Besides, treatment of cachectic mice with rucaparib decreased significantly plasma troponin-I concentration, proteolytic (MuRF-1, atrogin-1, proteasome, ubiquitination) and autophagic (LC3B, beclin-1) markers (gastrocnemius particularly) on both study muscles. Furthermore, a decline in physical activity was observed in cancer-cachexia mice in comparison with non-cachexia controls while rucaparib treatment in cachectic mice showed an improvement in locomotor activity compared to untreated cachectic mice.
Conclusions: Conclusively, rucaparib treatment enhances the locomotor activity of mice with cancer cachexia and additionally attenuates muscle damage and reduces levels of proteolysis and autophagy markers. These findings may have future therapeutic implications in cancer-induced cachexia patients.
Funding: FIS 18/00075 (FEDER), CIBERES, and SEPAR 2018.

SENSITIVITY OF NASOPHARYNGEAL VERSUS OROPHARYNGEAL SAMPLES FOR SARS-COV-2 DETECTION

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Introduction: Obtaining biological samples from the upper respiratory tract is the most widely used method for SARS-CoV-2 detection.

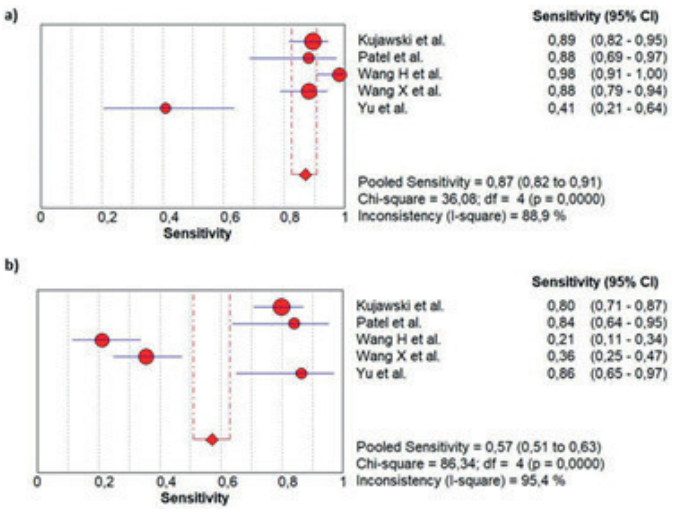
Characteristics and sensitivity results of the studies*

Author	Population	Sensitivity of NS	Sensitivity of OS	Absolute difference in sensitivity between NS vs OS
Wang et al.	353 patients with suspicion of COVID-19 (45.6% hospitalized). Age: 54 (range: 20-88) years.	67/76 (88.2%)	27/76 (35.5%)	52.7%
Patel et al.	146 patients. For each patient, the first pair of NS and OS obtained ≤ 7 days from symptoms onset was analyzed. Age: 40 (IQR: 24-56) years	22/25 (88.0%)	21/25 (84.0%)	4.0%
Wang et al.	120 hospitalized patients with confirmed COVID-19 (69.2% severe). For each patient, a pair of NS and OS was analyzed. Samples were obtained 27 days (IQR: 23.0-31.5) from the symptoms onset.	56/57 (98.2%)	12/57 (21.1%)	77.1%
Yu et al.	50 hospitalized patients with COVID-19. Age: 57 (range: 25-87) years.	9/22 (40.9%)	19/22 (86.4%)	-45.5%
Kujawski et al.	12 patients with SARS-CoV-2 in at least one NS (58.3% hospitalized). Age: 53 (range: 21-68) years. 117 pairs of NS and OS obtained from the 12 patients throughout the clinical course were analyzed.	93/104 (89.4%)	83/104 (79.8%)	9.6%
Wolfel et al.	9 patients. Age not provided. Samples were obtained throughout the entire clinical course.	NA	NA	NA

IQR: interquartile range; NA: not available; NS: nasopharyngeal sample; OS: oropharyngeal sample. *Any positive obtained from NS or OS was considered true positive.

Collecting nasopharyngeal samples (NS) can be uncomfortable and difficult in infants, and oropharyngeal samples (OS) may represent an option.

Objectives: To analyze the available evidence for determining the sensitivity of OS with respect to NS for SARS-CoV-2 identification.
Methods: A literature search was carried out in PubMed in August 20, 2020. Primary studies analyzing both NS and OS for SARS-CoV-2 detection were included. Results of reviews were described. Any positive obtained from NS or OS was considered true positive. Meta-disc software (http://www.hrc.es/investigacion/metadisc_en.htm) was used for meta-analyses.
Results: Six primary studies were identified (Table). Pooled sensitivity of NS was 87% (95%CI: 82-91%; I2 = 88.9%) and varied from 40.9% to 98.2% (Figure). Pooled sensitivity of OS was 57% (95%CI: 51-63%; I2 = 95.4%) and varied from 21.2% to 86.4% (Figure). Overall, sensitivity was 30% higher for NS than for OS, although a high heterogeneity was found. A sensitivity analysis excluding Yu et al. study, which had the lowest sample size and whose results differed substantially from the rest, showed a 36% higher sensitivity for NS than for OS [91% (95%CI: 87-94%; I2 = 54.1%) vs. 55% (95%CI: 48-61%; I2 = 96.1%)], remaining a high heterogeneity for OS. NS had a lower Ct (higher viral load) than OS. Viral RNA persistence in samples was similar for both samples (30-40 days). A review analyzing different samples of patients with confirmed COVID-19 found that the percentage of positive samples was highest for sputum [71% (95%CI: 61-80%; n = 11 studies)], followed by NS [54% (95%CI: 41-67%; n = 8 studies)] and OS [43% (95%CI 34-52%; n = 7 studies)]. The detection rate was higher at the time of symptoms onset and was reduced over time.



Pooled sensitivity for a) Nasopharyngeal samples; and b) Oropharyngeal samples.

Conclusions: Overall, NS had a higher sensitivity than OS for SARS-CoV-2 detection.
Funding: This research did not receive specific support from public agencies, the commercial sector or non-profit organizations.

SENSITIVITY OF SALIVA VERSUS ORO/NASOPHARYNGEAL SAMPLES FOR SARS-COV-2 DETECTION

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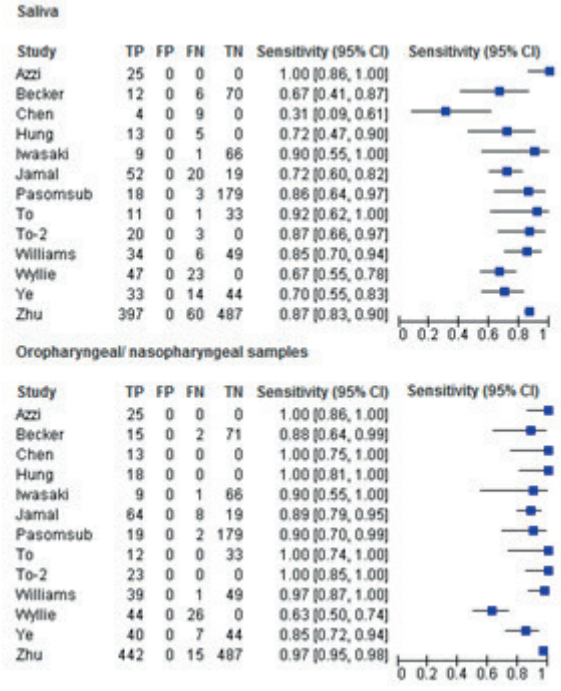
Introduction: Oropharyngeal and nasopharyngeal swab specimens (OS/NS) testing by reverse-transcriptase polymerase chain reaction (RT-PCR) is current standard to detect SARS-CoV-2, but this sampling method can be uncomfortable in some populations, and requires close physical contact with the health professional collecting the sample, which poses a certain risk of transmission. Saliva specimens may be a valid diagnostic sample alternative.



Sensitivity for saliva samples and Oropharyngeal/Nasopharyngeal samples.

Objectives: To analyze the available evidence on the sensitivity of saliva samples compared to OS/NS for the identification of SARS-CoV-2 by RT-PCR.
Methods: A literature search was carried out in PubMed in August 2020. Primary studies analyzing samples of both saliva and OS/NS for SARS-CoV-2 detection in suspected or confirmed cases were included. All positives obtained from any type of sample (OS/NS or saliva) were considered true positives (it was assumed that no false positives occurred). Therefore, specificity is not calculated. Meta-disc software (http://www.hrc.es/investigacion/metadisc_en.htm) was used for meta-analyses. In studies with serial samples, the first sample of each patient was used for the analysis whenever possible.
Results: Thirteen primary studies were identified, with a total of 1,773 participants (range 13-944). Five studies included only confirmed cases of COVID-19, while eight studies included suspected cases. Pooled sensitivity of saliva was 82% (95%CI 79-84%; I2 = 78.9%) and varied from 31% to 100% (Figure). Pooled sensitivity of OS/NS was 92% (95%CI 90-94%; I2 = 85.8%) and varied from 63% to 100% (Figure). The sensitivity of saliva samples for the study with the largest sample size (n = 944) was 86.9% (IC95% 83.4-89.8%), and for OS/NS it was 96.7% (IC95% 94.6-98.2%). Overall, sensitivity was 10% higher with OS/NS than with saliva samples, although a high heterogeneity was found.
Conclusions: Nasopharyngeal/pharyngeal samples had a higher sensitivity than saliva for SARS-CoV-2 detection by RT-PCR.
Funding: This research did not receive specific support from public agencies, the commercial sector or non-profit organizations.

SKELETAL MUSCLE DYSFUNCTION AND BODY COMPOSITION ALTERATIONS IN NON-CYSTIC FIBROSIS BRONCHIECTASIS PATIENTS: GENDER DIFFERENCES

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Introduction: Muscle dysfunction and nutritional abnormalities are common manifestations in chronic respiratory diseases. Whether patients with non-cystic fibrosis bronchiectasis (NCFB) may experience alterations in muscle mass and performance remains unanswered.
Objectives: To assess muscle mass, function, nutritional parameters, and exercise tolerance in NCFB patients.
Methods: Body weight and composition (muscle mass, bioimpedance), blood nutritional parameters, lung function (spirometry, lung volumes, and diffusion capacity), peripheral muscle function (dynamometry, upper and lower limb muscle groups, handgrip and QMVC, respectively), respiratory muscle function, and exercise capacity were evaluated in 131 NCFB patients that were prospectively and consecutively recruited in the Bronchiectasis Multidisciplinary Unit at Hospital del Mar (years 2018-2020, Barcelona).
Results: Ninety-eight female patients were recruited (33 males). All patients had mild-to-moderate airway obstruction and air trapping. Compared to male patients, female patients were older, muscle strength of both upper and lower limbs was significantly reduced, and exercise capacity was decreased. When patients were subdivided according to disease severity (FACED, females and males), patients with greater FACED scores exhibited a significant reduction in body weight, muscle strength (upper and lower extremities), exercise capacity, and lung function.
Conclusions: Nutritional abnormalities and reduced muscle function are very prominent in female NCFB patients as opposed to males. Moreover, these features were especially present in the more severe patients according to FACED scores. The study of the systemic manifestations in patients with bronchiectasis warrant further attention in the near-future.
Funding: CIBERES (ISCIII), unrestricted grant from Menarini SA, and SEPAR 2020.

SMAD4 OVEREXPRESSION IN PATIENTS WITH SLEEP APNOEA MAY BE ASSOCIATED WITH CARDIOMETABOLIC COMORBIDITIES

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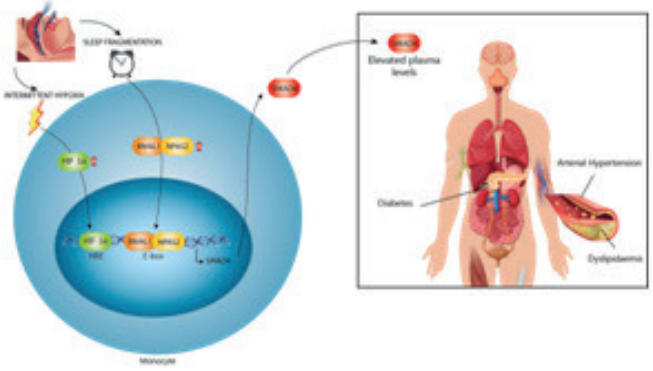
CIBERES, Madrid, Spain.

Introduction: Obstructive sleep apnoea (OSA) is associated with several diseases related to metabolic and cardiovascular risk. Although the mechanisms involved in the development of these disorders may vary, OSA patients frequently present an increase in transforming growth factor-beta (TGFβ), the activity of which is higher still in patients with hypertension, diabetes or cardiovascular morbidity. Smad4 is a member of the small mother against decapentaplegic homolog (Smad) family of signal transducers and acts as a central mediator of TGFβ signaling pathways.
Objectives: The aim of this study is to evaluate Smad4 as a possible link between OSA pathogenesis and the presence of common comorbidities.
Methods: In this study, we evaluate Smad4 plasma protein and mRNA expression in monocytes from 52 newly diagnosed OSA patients, with an apnoea-hypopnoea index (AHI) ≥ 30 and 26 healthy volunteers.
Results: These analyses reveal that OSA patients exhibit high levels of SMAD4 which correlates with variation in HIF1α, mTOR and circadian genes. Moreover, we associated high concentrations of Smad4 plasma protein with the presence of diabetes, dyslipidaemia and hypertension in these patients.



Conclusions: Our results suggest that increased levels of SMAD4, mediated by intermittent hypoxaemia and circadian rhythm deregulation, may be associated with cardiometabolic comorbidities in patients with sleep apnoea.
Funding: Fondo de Investigación Sanitaria (FIS) and Fondos FEDER PI13/01512 and PI16/00201 to F. García-Río and CP18/00028 Fondo de Investigación Sanitaria (FIS) PI19/01363 to C. Cubillos-Zapata.

SOLUBLE GUANYLATE CYCLASE STIMULATION WITH BAY 41-2272 ATTENUATES LIMB MUSCLE PROTEOLYSIS IN GUINEA PIGS EXPOSED TO CHRONIC CIGARETTE SMOKE

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Introduction: Cigarette smoke (CS) exposure induces skeletal muscle dysfunction and oxidative stress.
Objectives: We aimed to evaluate the effects of the sGC stimulator BAY 41-2272 on muscle proteolytic, apoptotic, and autophagic markers in the gastrocnemius of guinea pigs chronically exposed to CS.
Methods: Experimental groups (N = 7/group): 1) Control-sham (vehicle), 2) Control-sham treated with sGC stimulator BAY 41-2272 (3 mg/kg, oral gavage, 5 days a week) (sham+BAY), 3) CS-exposed for 3 months treated with vehicle (CS), and 4) CS-exposed for 3 months treated with BAY 41-2272 (CS+BAY). In the gastrocnemius of all guinea pigs, proteolytic, apoptotic and autophagic markers (immunoblotting), TUNEL positive nuclei (TUNEL assay, immunohistochemistry), muscle fiber typing and morphometry (immunohistochemistry), and muscle structural abnormalities (hematoxylin-eosin staining) were quantified.
Results: In gastrocnemius muscle of guinea pigs exposed to CS compared to non-exposed controls, levels of proteolytic markers (MuRF-1, atrogin-1, 20s proteasome C8 subunit, and total protein ubiquitination) and total muscle abnormal fraction increased, while the size of fast-twitch muscle fibers significantly decreased. No significant differences were observed in the levels of apoptotic (Bax, Bcl-2, or TUNEL positive nuclei) or autophagic markers (P62 and LC3B). Treatment of the animals with BAY 41-2272 elicited in the gastrocnemius a significant reduction in levels of all proteolytic markers, without inducing any further modifications in the levels of the apoptotic or autophagic markers or in muscle structure.
Conclusions: Limb muscles of guinea pigs chronically exposed to CS exhibited a rise in proteolysis that was significantly attenuated by treatment with the soluble guanylate cyclase BAY 41-2272 for 3 months. These findings have clinical implications for the management of smokers with/without respiratory conditions.
Funding: CIBERES, FIS 18/00075 (FEDER), SEPAR 2020

STABILITY OF ASTHMA BIOMARKERS IN TIME: A NEW STRATEGY FOR THE PREDICTION OF THE DISEASE

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Introduction: Asthma is a complex disease comprising various phenotypes, which need solid biomarkers for accurate classification. Previously, we defined specific genes and proteins which could be useful for diagnostic improvement. The MEGA cohort, is a prospective, multicenter study focused in the study of asthma patients with different severity diagnosis, from severe to mild.
Objectives: To examine the stability and utility as diagnosis tool of PI3 and CHI3L1, previously defined as asthma biomarkers, in blood and sputum samples.
Methods: Sputum and blood samples from 70 asthmatic patients (at time 0 (T0), 12 (T1) and 24 (T2) months) and 46 healthy controls were obtained from the MEGA biobank. RNA was extracted from sputum cells and peripheral blood mononuclear cells (PBMCs) using the TRIzol method. PI3 and CHI3L1 gene-expression was analyzed by qRT-PCR. Protein levels were determined by ELISA (in serum or sputum supernatant). Statistical analysis was performed by the GraphPad program.
Results: A multivariate study was done, analyzing PI3 and CHI3L1 gene and protein-expression in samples from allergic (AA) or nonallergic (NA) asthma patients with different severity, at different times. Overall, statistically significant correlation between gene and protein levels at different times was observed. Presence of both biomarkers was higher in sputum than in peripheral samples but there was good correlation in the samples analyzed. However, several statistically significant differences were found in PI3 serum protein levels comparing T0 to T1 and T2 in moderate-mild NA patients ($p = 0.019$, $p = 0.033$ respectively); as well as in severe AA patients ($p = 0.038$, $p = 0.0067$ respectively). An exhaustive analysis among groups and severity confirmed the relevance of these biomarkers to discriminate clinical phenotypes.
Conclusions: Our data confirm that PI3 and CHI3L1 are good candidates to differentiate asthma pathology and severity. Also support the idea that their stability study in blood can be a predictive/prognosis tool in clinical uses.
Funding: PI17/01682, SEAIC 2018.

SURFACTANT LIPIDS LIMIT ALVEOLAR MACROPHAGE ALTERNATIVE ACTIVATION INDUCED BY IL-4 OR LACTATE

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Introduction: Alternative activation of macrophages induced by IL-4 is necessary for tissue repair, although excessive activation causes pathology associated with fibrosis. Another factor that favors alternative activation of macrophages is lactate, whose levels increase in lung pathologies, such as fibrosis and COPD. We asked if surfactant lipids have any effect on alternative activation because they are continuously endocytosed by alveolar macrophages (aMΦs).
Objectives: To analyze the effect of surfactant lipids on alternative activation and proliferation of aMΦs induced by IL-4, lactate, and IL-4+lactate.
Methods: aMΦs were pre-incubated with surfactant lipids, to allow their endocytosis, and then were stimulated with IL-4, lactate, or IL-4+lactate. Analyses of alternative activation and proliferation of aMΦs, and signaling were performed by enzymatic and proliferation assays, and western blot.
Results: Surfactant lipids inhibited alternative activation and proliferation of aMΦs elicited by IL-4, lactate, and IL-4+lactate. Surfactant lipids inhibited IL-4-mediated activation of aMΦs by inhibiting the Akt-mTORC1 signaling axis.
Conclusions: Our results show that surfactant lipids limit alternative activation of macrophages induced by IL-4 or by the pro-fibrotic factor lactate. Levels of surfactant lipids decrease, whereas lactate levels

increase in idiopathic pulmonary fibrosis, which may contribute to the pathophysiology of the disease.
Funding: Supported by Ministry of Science, Innovation and Universities [grant number RTI2018-094355-B-I00] and Instituto de Salud Carlos III [grant number: CIBERES-CB06/06/0002], Spain.

SYSTEMIC INFLAMMATION IN PATIENTS WITH STABLE NON-CYSTIC FIBROSIS BRONCHIECTASIS

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Introduction: Non-cystic fibrosis bronchiectasis is a chronic airway disease with various etiologies and severities. Systemic inflammation is involved in the pathophysiology during acute exacerbations. Whether systemic inflammation is predominant in patients with stable non-CF bronchiectasis remains to be elucidated.
Objectives: We aimed to evaluate whether systemic inflammation takes place in patients with stable non-CF bronchiectasis with no acute exacerbation within at least three months prior to study entry.
Methods: This was a prospective investigation in which 30 patients with clinically stable non-CF bronchiectasis and 20 age- and sex-matched healthy controls were recruited. Systemic inflammatory parameters: CRP, GSV, fibrinogen, ceruloplasmin, alpha-1 antitrypsin, IgA, and IgG; blood nutritional parameters: albumin and prealbumin; and baseline lung function: spirometry, lung volumes, and diffusion capacity were assessed in both patients and healthy controls. Sputum samples were collected in patients with bronchiectasis for microorganism culture. Statistical differences of all the study variables between the two groups were explored using the Student´s t-test. Correlations between physiological/clinical and biological variables were examined using Pearson's parametric test.
Results: In patients with stable non-CF bronchiectasis compared to the healthy controls, patients with non-CF bronchiectasis, exhibited mild-to-moderate airway obstruction, systemic levels of CRP, GSV, fibrinogen, ceruloplasmin, IgA and IgG were significantly greater, while those of albumin and prealbumin were significantly lower in the patients. Levels of ceruloplasmin were shown to positively correlate with systemic markers such as CRP, GSV, and alpha-1 antitrypsin, and to negatively correlate with albumin. Prealbumin levels were positively correlated with lung function parameters, while IgA and IgG were negatively associated with FEV1. Pseudomonas aeruginosa colonization was identified in 36% of the patients in this series.
Conclusions: In patients with stable non-CF bronchiectasis, systemic inflammation takes place independently of previous acute exacerbations.
Funding: FIS18/00075(FEDER), CIBERES, unrestricted grant from Menarini SA, and SEPAR 2020.

THE IMPACT OF LONG-TERM POSITIVE AIRWAY PRESSURE THERAPY IN OBESITY HYPOVENTILATION SYNDROME BASED ON DISEASE SEVERITY

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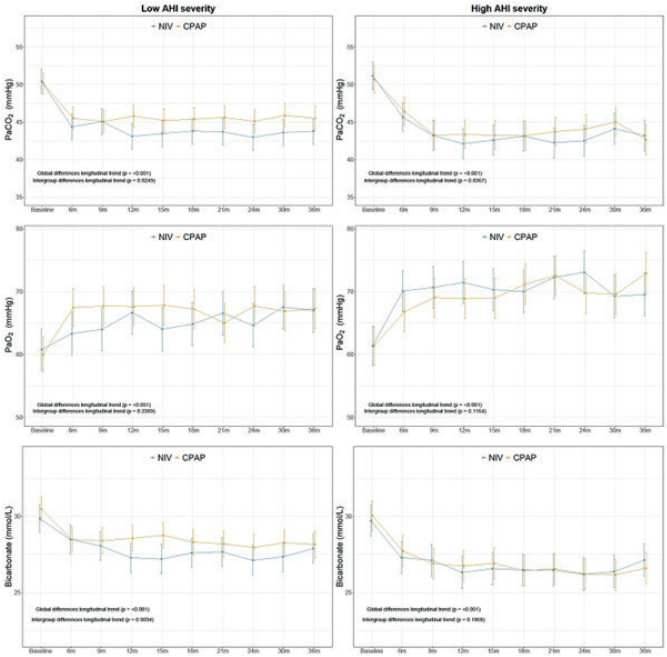
Introduction: Obesity hypoventilation syndrome (OHS) is treated with CPAP or noninvasive ventilation (NIV) during sleep. NIV is costlier, but may be advantageous because it provides ventilatory support. However, there are no long-term trials comparing these treatment modalities based on OHS severity.
Objectives: Does CPAP have similar effectiveness when compared to NIV according to OHS severity subgroups?
Methods: Post-hoc analysis of the Pickwick randomized clinical trial in which 215 ambulatory patients with untreated OHS and concomitant severe obstructive sleep apnea (OSA), defined as apnea-hypopnea index (AHI) ≥ 30 events/hour, were allocated to NIV or CPAP. In the present analysis, the Pickwick cohort was divided in severity subgroups based on the degree of hypercapnia (daytime median PaCO2 of 49.8 mmHg) and severity of OSA (median AHI of 68.4). Repeated measures of PaCO2, PaO2 and bicarbonate during the subsequent 3 years were compared between CPAP and NIV in the four severity subgroups. Statistical analysis was performed using linear mixed-effects model with treatment-time interaction.



Adjusted longitudinal changes of ABG parameters (mean and 95% CI) from linear mixed-effects models during the follow-up related to intervention treatment groups in low and high AHI severity subgroups. P values correspond to adjusted (age, sex and smoking status) longitudinal changes for ABG and for the inter-group CPAP and NIV comparison. ABG = arterial blood gases; AHI = apnea and hypopnea index; CPAP = continuous positive airway pressure; NIV = noninvasive ventilation; and CI = confidence interval.

	PaCO2 severity			AHI severity		
	Low-severity	High-severity	p value	Low-severity	High-severity	p value
	N = 102	N = 102		N = 102	N = 102	
Age, years	61.0 [51.2;69.0]	64.5 [53.8;72.0]	0.110	67.0 [57.0;72.0]	60.0 [47.2;67.0]	< 0.001
Sex, male	50 (49.0%)	39 (38.2%)	0.158	35 (34.3%)	54 (52.9%)	0.011
Smokers	29 (28.4%)	22 (21.6%)	0.525	21 (20.6%)	30 (29.4%)	0.309
Smoking, pack/year†	25.0 [15.0;39.4]	36.8 [15.1;50.6]	0.092	30.0 [10.0;40.0]	30.0 [15.0;47.8]	0.513
Drinkers‡	24 (23.5%)	15 (14.7%)	0.154	20 (19.6%)	19 (18.6%)	1.000
Alcohol, g†	30.0 [20.0;57.0]	30.0 [13.0;40.0]	0.265	30.0 [18.0;40.5]	31.0 [15.2;66.5]	0.543
BMI, kg/m^2	41.7 [37.9;47.1]	44.2 [38.4;49.6]	0.103	41.5 [37.0;46.4]	44.4 [39.0;50.0]	0.004
Neck circumference, cm	44.3 (4.44)	44.8 (4.82)	0.491	43.0 (4.26)	46.1 (4.50)	< 0.001
ESS¶	10.8 (4.88)	11.1 (5.29)	0.726	9.97 (4.94)	11.9 (5.05)	0.006
FOSQ	78.0 [62.0;93.0]	68.0 [53.0;86.0]	0.004	72.5 [55.8;88.2]	74.5 [59.8;95.2]	0.310
SF 36-Physical	38.3 [29.9;46.1]	33.0 [27.7;42.5]	0.062	33.6 [28.3;43.8]	37.9 [28.6;45.7]	0.431
SF 36-Mental	45.2 [30.3;52.9]	45.7 [33.5;53.2]	0.289	43.0 [30.1;53.0]	46.5 [33.7;53.2]	0.307
Dyspnea MRC scale ≥ 2						
Hypertension	63 (61.8%)	77 (75.5%)	0.050	76 (74.5%)	64 (62.7%)	0.097
Diabetes	39 (38.2%)	37 (36.3%)	0.885	43 (42.2%)	33 (32.4%)	0.192
Dyslipidemia	39 (38.2%)	51 (50.0%)	0.121	45 (44.1%)	45 (44.1%)	1.000
Heart failure	16 (15.7%)	14 (13.7%)	0.843	19 (18.6%)	11 (10.8%)	0.166
Stroke	9 (8.82%)	7 (6.86%)	0.795	10 (9.80%)	6 (5.88%)	0.435
Arrhythmia	10 (9.80%)	7 (6.86%)	0.612	11 (10.8%)	6 (5.88%)	0.311
Ischemic heart disease	9 (8.91%)	9 (8.82%)	1.000	9 (8.91%)	9 (8.82%)	1.000
Leg arteriopathy	8 (7.84%)	2 (1.96%)	0.105	6 (5.88%)	4 (3.92%)	0.746
Pulmonary hypertension	9 (8.91%)	8 (7.84%)	0.983	10 (9.90%)	7 (6.86%)	0.598
pH	7.41 [7.38;7.43]	7.39 [7.38;7.41]	0.009	7.40 [7.38;7.43]	7.40 [7.38;7.42]	0.299
PaO2, mmHg	63.2 [57.2;70.0]	58.5 [54.0;64.0]	< 0.001	61.0 [56.0;66.1]	60.0 [55.7;67.1]	0.890
PaCO2, mmHg	47.2 [46.3;48.2]	53.0 [51.0;55.9]	< 0.001	49.0 [47.0;52.0]	50.0 [48.0;53.8]	0.411
Bicarbonate, mmol/l	28.1 [27.0;30.2]	30.5 [28.6;33.0]	< 0.001	29.8 [28.0;31.8]	29.3 [27.8;31.9]	0.715
FEV1 in% of predicted	81.0 [71.0;92.0]	73.0 [61.2;84.0]	< 0.001	77.0 [66.0;89.0]	79.0 [66.0;88.8]	0.882
FVC, in% of predicted	84.0 [72.0;96.0]	75.5 [64.0;85.0]	0.001	79.0 [66.0;94.0]	80.0 [69.2;89.8]	0.913
6-MWD in meters	384 [303;475]	360 [246;431]	0.096	360 [240;420]	398 [312;480]	0.007
Polysomnographic parameters						
TST, hours	5.27 (1.30)	5.32 (1.31)	0.789	5.16 (1.30)	5.43 (1.30)	0.143
Non-REM stage 1 and 2,%	85.5 [74.7;91.4]	84.6 [73.0;92.0]	0.688	80.7 [65.1;88.7]	89.1 [81.3;94.0]	< 0.001
Non-REM stage 3,%	5.00 [0.00;11.6]	4.64 [0.00;16.1]	0.933	10.0 [2.65;18.9]	1.60 [0.00;9.85]	< 0.001
REM sleep%	7.00 [2.48;14.2]	8.20 [4.50;14.0]	0.573	9.80 [4.60;15.9]	7.00 [2.48;11.7]	0.046
Arousal index	53.0 [31.0;73.1]	59.7 [32.7;85.3]	0.178	36.0 [26.1;56.9]	80.1 [56.1;95.7]	< 0.001
AHI	68.3 [44.9;91.3]	72.5 [43.4;96.5]	0.721	44.2 [32.2;54.7]	96.4 [82.2;103]	< 0.001
ODI	69.0 [40.7;90.5]	73.4 [44.6;96.0]	0.569	46.6 [29.1;64.1]	93.2 [73.8;103]	< 0.001
Mean SpO2	86.8 [83.0;90.0]	84.0 [79.0;87.0]	0.002	86.2 [83.0;90.0]	84.0 [80.0;88.0]	0.011
TST with SpO2 < 90%,%	70.0 [45.0;92.5]	81.0 [57.0;96.6]	0.010	77.5 [49.8;95.3]	77.2 [53.6;94.1]	0.981
PAP adherence (> 4hours/day)	64 (62.7%)	69 (67.6%)	0.557	61 (59.8%)	72 (70.6%)	0.142
Oxygen therapy	20 (19.6%)	30 (29.4%)	0.143	32 (31.4%)	18 (17.6%)	0.034
Oxygen therapy flow, L/min†	1.50 [1.50;2.00]	1.75 [1.12;2.00]	0.806	1.50 [1.00;2.00]	2.00 [1.50;2.00]	0.123

*: Data presented%, median (25;75 IQR) or mean (SD); †: Includes only patients who reported to be active smokers or drinkers or with oxygen therapy. ‡: people who drink more than 30 g of alcohol/day in men and 20 g in women. Abbreviations: SD: standard deviation; IQR: interquartile range; BMI: body mass index; EES: Epworth sleepiness scale; MRC: Medical Research Council; FEV1: forced expiratory volume in the first second; FVC: forced vital capacity; 6-MWD: six-minute walk distance; TST: total sleep time; AHI: apnea-hypopnea index; ODI: 3% oxygen desaturation index; and SpO2: oxygen saturation by pulse oximetry.

Results: 204 patients, 97 in the NIV group and 107 in the CPAP group were analyzed. The longitudinal improvements of PaCO2, PaO2 and bicarbonate were similar between CPAP and NIV based on the PaCO2 severity subgroups. In patients with AHI below the median, PaCO2 improved more with NIV.
Conclusions: In ambulatory patients with stable OHS and concomitant severe OSA, long-term NIV therapy is superior to CPAP in improving hypercapnia in the subset of patients with less severe OSA.
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THERAPEUTIC APPROACHES AGAINST LUNG CANCER-INDUCED CACHEXIA IN A MOUSE MODEL: EFFECTS OF TREATMENT WITH CURCUMIN AND RESVERATROL

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Introduction: Cancer cachexia is characterized by body weight and muscle mass loss and is associated with cancer. Curcumin and resver-

atrol, two natural phenolic compounds, have shown protective effects against muscle dysfunction and wasting.
Objectives: We hypothesized that the treatment of cachectic mice with these compounds separately will attenuate the deleterious effects on muscles
Methods: A well-validated model of lung cancer (LC) cachexia in mice was used consisting of the inoculation of 4 x 10^5 LP07 lung adenocarcinoma cells on the left flank of female BALB/c mice and concomitant intraperitoneal treatment with each treatment for the last 15 days. The research was conducted for a period of 1 month. Mice were divided into 3 groups (N = 10/group); LC-cachexia (no treatment), LC-cachexia + curcumin (1 mg/kg/day of curcumin) and LC-cachexia + resveratrol (20 mg/kg/day of resveratrol). Body weight was evaluated every day, muscle and tumor weights were evaluated at the end of the study. In gastrocnemius, proteolytic markers (ubiquitin ligases and proteasome, immunoblotting), atrophy signaling pathways (FoXO, NFkB, SIRT1, immunoblotting), muscle structural abnormalities, and fiber type composition and morphometry (immunofluorescence) were identified. Plasma levels of troponin (muscle damage, ELISA) were also measured.
Results: Compared to LC-cachexia, mice treated with either curcumin or resveratrol had a lower decrease in body weight gain and in gastrocnemius muscle weight during the study. MURF-1 and proteasome levels (gastrocnemius) and systemic troponin I levels (blood) significantly decreased in LC-cachexia + curcumin and LC-cachexia + resveratrol groups compared to non-treated animals. Moreover, type I and II muscle fiber areas increased in LC-cachexia + curcumin and LC-cachexia + resveratrol in the gastrocnemius compared to non-treated cachectic mice.
Conclusions: Curcumin and resveratrol treatment induce a protective effect against cachexia, by reducing MURF-1 and proteasome protein levels and muscle wasting and damage. These results may have future therapeutic implications in patients with muscle wasting.
Funding: CIBERES, FIS 18/0075 (FEDER), SEPAR 2018, and unrestricted grant from Menarini SA 2014.

UNCOVERED CONTRIBUTION OF KV7 CHANNELS TO PULMONARY VASCULAR TONE IN PULMONARY ARTERIAL HYPERTENSION

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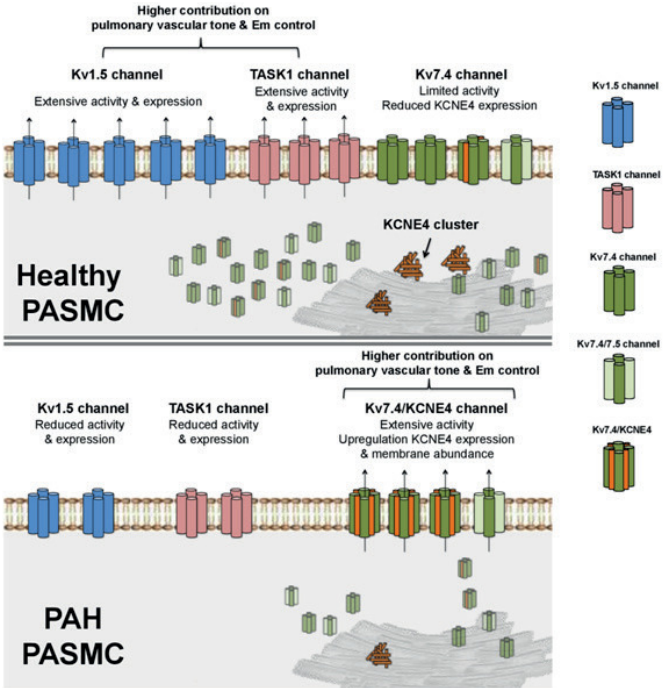
Introduction: K+ channels play a fundamental role regulating membrane potential of pulmonary artery (PA) smooth muscle cells (PASMCs) and their impairment is a common feature in pulmonary arterial hypertension (PAH). Kv7 channels (KCNQ1-5) and their KCNE regulatory subunits are known to regulate vascular tone, but whether Kv7 channel function is impaired in PAH and how this can affect the rationale for targeting Kv7 channels in PAH remains unknown.
Objectives: Herein we have studied the role of Kv7/KCNE subunits in rat PA and their possible alteration in PAH.
Methods: Kv7 channels activity was analyzed using patch-clamp technique and vascular reactivity studies. Kv7 channels expression was studied through qRT-PCR, Western blot and immunocytochemistry techniques.
Results: We found that the total K+ current is reduced in PASMC from pulmonary hypertension (PH) animals (Su/Hpx) and Kv7 currents made a higher contribution to the net K+ current. Likewise, enhanced vascular responses to Kv7 channel modulators were found in PH rats. Accordingly, KCNE4 subunit was highly upregulated in lungs from PH animals and patients. Additionally, Kv7 channel activity was enhanced in the presence of Kv1.5 and TASK-1 channel inhibitors and this was associated with an increased KCNE4 membrane abundance. Compared with systemic arteries, PA showed a poor response to Kv7 channel modulators which was associated with reduced expression and membrane abundance of Kv7.4 and KCNE4.



Conclusions: Our data indicate that Kv7 channel function is preserved and KCNE4 is upregulated in PAH. Therefore, compared to other downregulated channels, the contribution of Kv7 channels is increased in PAH resulting in an enhanced sensitivity to Kv7 channel modulators. This study provides insight into the potential usefulness of targeting Kv7 channels in PAH.
Funding: This work was funded by Ministerio de Economía y Competitividad grants (SAF2016-77222R to FPV and AC), the Cardiovascular Medical Research and Education Fund (AC), Fundación Contra la Hipertensión Pulmonar-EMPATHY, Comunidad de Madrid (B2017/BMD-3727 to AC and LM) and from Instituto de Salud Carlos III (PI15/01100 to LM). GM-P is supported by a predoctoral CIBERES grant and was beneficiary of CIBERES Training Program and Fellowships. MC, SE-R, MA.O and MM are supported Universidad Complutense, FPU, Fondo Social Europeo/Programa Operativo de Empleo Juvenil (YEI) and Comunidad de Madrid/Fondo Social Europeo co-financed grant, respectively.

VITAMIN D EFFECT ON CORTICOSTEROID SENSITIVITY IN OBESE ASTHMATIC PATIENTS BEFORE AND AFTER BARIATRIC SURGERY

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Introduction: Recent studies show the immunomodulatory effect of 1,25-dihydroxyvitamin D3, the active form of vitamin D. Its deficiency has been described in obesity and is related with less control of asthma symptomatology. We hypothesized that vitamin D treatment could enhance in vitro corticosteroid response by modifying anti-inflammatory pathways altered in obese asthmatic patients. Furthermore, bariatric surgery could increase corticosteroid sensitivity in these patients.
Objectives: Analyse the vitamin D influence in the corticosteroid response in obese asthmatic patients. Study the corticosteroid response of obese asthmatic subjects before and after bariatric surgery.
Methods: Severe obese (body mass index [BMI] ≥ 35 kg/m^2) patients with asthma (OA) (n = 25) and without asthma (O) (n = 15) were evaluated and compared with non-obese asthma patients (A) (BMI < 30 kg/m^2) (n = 15) and healthy subjects (H) (n = 19). Corticosteroid sensitivity was determined in vitro through peripheral blood mononuclear cells (PBMC) proliferation assay. PBMC were cultured with dexamethasone (from 10-11 to 10-5M) and/or with vitamin D 10-7M. Forced spirometry was performed in all groups. OA and O patients were reevaluated six months after bariatric surgery.
Results: OA group had a mean age of 56 ± [SD] 7 years, BMI 40 ± 5 kg/m^2 and FEV1 80 ± 18%; the O group a mean age of 51 ± 8 years, BMI 45 ± 8 kg/m^2 and FEV1 92 ± 12%; the A group a mean age of 49 ± 15 years, BMI 24 ± 2 kg/m^2 and FEV1 91 ± 8% and the H group 40 ± 11 years, BMI 24 ± 2 kg/m^2 and FEV1 99 ± 11%. Vitamin D showed an antiproliferative effect in PBMC. Dexamethasone IC50 value was reduced when vitamin D was added to the in vitro treatment ($p \leq 0{,}001$). PBMC from OA group increased corticosteroid sensitivity 6 months after they underwent bariatric surgery ($p \leq 0{,}001$).
Conclusions: Both vitamin D and obese asthmatic patients' bariatric surgery improve PBMC sensitivity to the in vitro corticosteroid treatment.
Funding: Supported by Sociedad Española de Neumología (SEPAR), Fundació Catalana de Pneumologia (FUCAP) and Menarini.

VTE PROPHYLAXIS AFTER DISCHARGE IN PATIENTS ADMITTED FOR COVID19. FOR EVERYONE?

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Introduction: Most recent studies seem to reduce the incidence of thromboembolic complications in the acute phase of Covid19, the concern caused by the preliminary studies led to reconsider, prophylaxis protocols, suggesting their extension after discharge. A recent consensus document 5 acknowledes the impossibility of issuing recommendations in this regard in the absence of post-discharge follow-up studies, but accepts that extended prophylaxis could provide benefits for Covid patients with low bleeding risk, if the risk of symptomatic VTE were above 1.8%, 35-42 days after discharge.
Objectives: Analyze the incidence of VTE in the 3-month follow-up after discharge from Covid.
Methods: Retrospective study of the follow-ups carried out systematically.
Results: Between 3-31/3/2020, 342 patients (53.8% male) with a mean age of 57.62 (SD: 12.73) were seen in our Pulmonology Service, of which 46 (13.45% [13.45% [IC95: 10.24-17.48%]) died during their stay. The rest (296) could be discharged after an average stay of 6.20 days (SD 9.19). 9 patients were discharged with standard anticoagulant treatment (2 for VTE diagnosis during their stay, and 7 for anticoagulation prior to admission due to cardiovascular indication), and only 8 (2.79% [2.79% [IC95: 1.42-5.40%) of 287 being eligible for prophylaxis heparinic disease, received it at the time of discharge. No case of VTE (0% [IC95: 0-0.01]) was recorded among the remaining 277 (in 2 patients there was no follow-up information), after an average follow-up of 100.02 (SD 12.02) days after discharge.
Conclusions: The incidence of symptomatic VTE after discharge without prophylaxis in Covid19 patients in our setting appears to be low. The figures that we have found in our study are below those that could justify the recommendation to systematically implement extended prophylaxis. The prescription of extended heparin prophylaxis at the time of discharge in Covid19 patients should probably be limited to cases with other risk factors.