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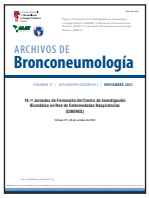

Comunicaciones

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

Virtual, 27 y 28 de octubre de 2021

3. A PREDICTION MODEL FOR FATAL OUTCOME IN COVID-19-RELATED CRITICAL ILLNESS BASED ON MICRORNA PROFILING OF BRONCHIAL ASPIRATES

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Background: The analysis of molecular patterns in respiratory specimens could provide valuable information for the clinical management of COVID-19.
Objectives: We analyzed the expression profile of microRNAs (miRNAs) in bronchial aspirate (BAS) samples from ICU COVID-19 survivors and nonsurvivors to identify miRNA signatures as prognostic biomarkers of fatal outcome in COVID-19-related critical illness and to define molecular pathways involved in the development of adverse events.
Methods: A prospective study cohort (n = 57) composed of COVID-19 survivors and nonsurvivors among patients assisted by invasive mechanical ventilation (IMV) (Table). BAS samples were collected by bronchoaspiration after IMV initiation. miRNA quantification was performed using RT-qPCR.
Results: Five miRNA ratios (miR-1-3p/miR-124-3p, miR-125b-5p/miR-34a-5p, miR-126-3p/miR-16-5p, miR-199a-5p/miR-9-5p and miR-221-3p/miR-491-5p) were deregulated when comparing ICU survivors and nonsurvivors (Figure 1A, 1B & 1C). A prediction model for ICU mortality based on three miRNA ratios (miR-125b-5p/miR-34a-5p, miR-199a-5p/miR-9-5p and miR-221-3p/miR-491-5p) was constructed using Random Forest (Figure 1E). The model (AUC 0.85) and the miR-199a-5p/miR-9-5p ratio (AUC 0.80) showed an optimal discrimination value and outperformed the best clinical predictor for ICU mortality (days from first symptoms to IMV initiation, AUC 0.73) (Figure 1D & 1F). Survival analysis confirmed the usefulness of miRNAs in risk stratification (Figure 1G). Pathological mechanisms implicated in fibrosis, coagulation, viral infections, immune responses and inflammation were identified by functional enrichment analyses.
Conclusions: miRNA profiling in BAS allows risk stratification in critically ill COVID-19 patients under IMV and provide molecular information about the factors that mediate the fatal outcomes.
Funding: MM is the recipient of a predoctoral fellowship (LT017/20/000142) from Generalitat de Catalunya. Financial support was provided by the Instituto de Salud Carlos III de Madrid (COV20/00110 to AT and CP20/00041 to DdGC) and CIBERES. Co-funded by the European Development Regional Fund (A Way to Make Europe).

4. MICRORNA PATTERNS ASSOCIATED WITH PULMONARY ABNORMALITIES IN SURVIVORS OF SARS-COV-2-INDUCED ARDS

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Background: Persistent pulmonary dysfunction has been described in around 80% of survivors who developed ARDS secondary to SARS-CoV-2. Despite the clinical relevance, there is a limited knowledge of the molecular pathways that determine the pulmonary sequelae.
Objectives: To identify the underlying molecular mechanisms involved in the functional and structural pulmonary outcomes from SARS-CoV-2-induced ARDS through microRNA (miRNA) profiling.
Methods: A prospective study including survivors who developed ARDS secondary to SARS-CoV-2 infection (n = 167) was performed 3 months after hospital discharge. The follow-up included a pulmonary function evaluation and a chest computed tomography (CT). Lung diffusing capacity for carbon monoxide (DLCO) and total severity score

(TSS) of CT were used to characterize diffusion impairment and radiologic abnormalities, respectively. Plasma miRNAs were quantified using RT-qPCR. miRNA signatures were constructed using the random forest algorithm (RF). KEGG pathway and GO enrichment analyses for experimentally validated miRNA:gene interactions were conducted.
Results: DLCO was altered (< 80%) in the 81.8% of the population and the TSS showed a median value of 5 [2; 8]. The prediction model based on RF identified a circulating miRNA profile (miR-17-5p, miR-27a-3p, miR-126-3p, miR-146a-5p and miR-495-3p) that independently correlated with DLCO levels. In relation with structural outcomes, a miRNA signature composed by miR-27a-3p, miR-214-3p and miR-495-3p was independently associated with TSS values. Bioinformatic analyses using the miRNA signatures informed on multifactorial molecular mechanisms involved in pulmonary sequelae, comprising fibrosis, cell death, inflammation, immune response and coagulation, among others.
Conclusions: The pulmonary function and radiological features in survivors of ARDS secondary to SARS-CoV-2 infection are linked to specific plasma miRNA patterns. The multifactorial mechanisms identified provide future insights to improve postacute management.
Funding: Financial support was provided by the Instituto de Salud Carlos III de Madrid (COV20/00110 to AT and CP20/00041 to DdGC) and CIBERES. Co-funded by the European Development Regional Fund (A Way to Make Europe). MCGH is the recipient of a predoctoral fellowship from "University of Lleida".

5. SERUM MICRORNAS CATALOG ASTHMATIC PATIENTS BY PHENOTYPE

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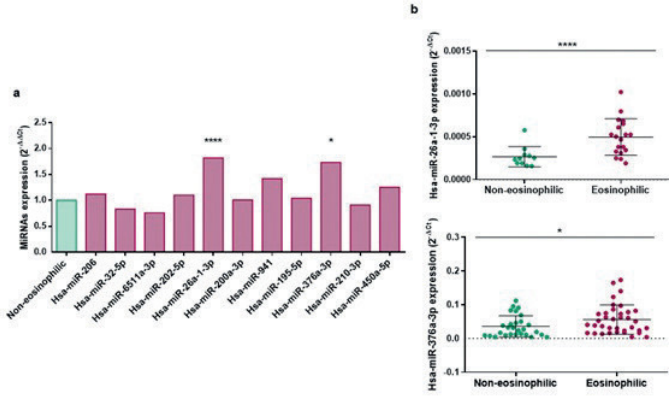
Background: Asthma is a chronic inflammatory condition of the airways with a complex pathophysiology. Stratification of asthma subtypes into phenotypes and endotypes should move the field forward, making treatment more effective and personalized. Eosinophils are the key inflammatory cells involved in severe eosinophilic asthma. Due to the health threat posed by eosinophilic asthma, there is a need for reliable biomarkers to identify patients and treat them properly with novel biologics. A promising tool for this matter are miRNAs.
Objectives: The main aim of this study was to find serum miRNAs that can phenotype asthmatic patients.
Methods: Serum miRNAs of eosinophilic (N = 40) and non-eosinophilic (N = 36) asthmatic individuals were evaluated by NGS, specifically miRNAs-seq, and selected miRNAs were validated by RT-qPCR. Statistical analysis (Table) and pathways enrichment analysis of deregulated miRNAs was performed.
Results: NGS analysis revealed 15 differentially expressed miRNAs between eosinophilic and non-eosinophilic asthmatic patients (Figure). Of the 15 differentially expressed miRNAs, hsa-miR-26a-1-3p and hsa-miR-376a-3p were validated by RT-qPCR. Expression levels of these 2 miRNAs were higher in eosinophilic than in non-eosinophilic asthmatics. Furthermore, expression values of hsa-miR-26a-1-3p inversely correlated with peripheral blood eosinophil count and hsa-miR-376a-3p expression values with fractional exhaled nitric oxide (FeNO) values and exacerbations number. Additionally, in silico pathway enrichment analysis revealed that these 2 miRNAs regulate signaling pathways related with asthma pathogenesis.

Demographic, inflammatory, functional and clinical characteristics eosinophilic and non-eosinophilic patients

		Eosinophilic (n = 40)	Non-Eosinophilic (n = 36)	P-value
Age (years)†		44.5 (35.3-58.8)	51.5 (38.3-59.8)	N.S.
Sex (%)	Female	27.0 (67.5%)	24.0/35.0 (66.7%)	N.S.
BMI†		25.2 (22.4-28.3)	25.8 (22.9-29.7)	N.S.
Smoking habit (%)	Smokers	4.0/39.0 (10.3%)	2.0/32.0 (6.3%)	N.S.
	Passive	4.0/39.0 (10.3%)	3.0/32.0 (9.4%)	N.S.
	Ex-smokers	11.0/39.0 (28.2%)	11.0/32.0 (34.4%)	N.S.
	Non-smokers	20.0/39.0 (51.3%)	16.0/32.0 (50.0%)	N.S.
Blood eosinophils (cells/#mL)†		700.0 (525.0-800.0)	100.0 (100.0-100.0)	****
Sputum eosinophils (%)†		2.3% (0.0-20.0)	1.0% (0.0-4.0)	N.S.
Atopy (%)		24.0 (60.0%)	28.0 (77.8%)	N.S.
IgE (IU)†		209.0 (87.4-497.0)	124.0 (62.3-391.0)	N.S.
FEV1/FVC (%)†		78.3% (69.2-84.3)	78.0% (71.9-84.1)	N.S.
FeNO (ppb)†		50.0 (31.5-78.0)	19.0 (11.3-28.1)	****
Exacerbations (%)		22.0 (55.0%)	14.0 (38.9%)	N.S.
Severity (%)	Severe	22.0/37.0 (59.5%)	11.0/30.0 (36.7%)	N.S.
	Moderate	9.0/37.0 (24.3%)	5.0/30.0 (16.7%)	N.S.
	Mild	5.0/37.0 (13.5%)	13.0/30.0 (43.3%)	*
	Intermittent	1.0/37.0 (2.7%)	1.0/30.0 (3.3%)	N.S.
ACT†		20.0 (13.3-23.0)	20.5 (18.0-24.8)	N.S.
ICS and LABA (%)		33.0 (82.5%)	32.0 (88.9%)	N.S.

†Results are expressed as median (IQR); N.S., Non-significant; ****p < 0.0001; **p < 0.01; *p < 0.05; BMI, Body Mass Index; FEV1, Forced Expiratory Volume measured during the first second; FVC, Forced Vital Capacity; FeNO, Fractional exhaled Nitric Oxide; Ppb, parts per billion; ICS and LABA, Inhaled Corticosteroids and Long-Acting β2-Agonists; ACT, Asthma Control Test.



Serum miRNA deregulation in eosinophilic and non-eosinophilic asthmatic patients (a). Subjects with eosinophilic asthma showed higher expression levels of hsa-miR-26a-1-3p and hsa-miR-376a-3p than non-eosinophilic asthmatics (b). ****p < 0.0001; *p < 0.05.

Conclusions: Hsa-miR-26a-1-3p and hsa-miR-376a-3p could be used to distinguish eosinophilic and non-eosinophilic asthmatic patients.
Funding: This study was supported by Fondo de Investigación Sanitaria and Fondo Europeo de Desarrollo Regional (FIS and FEDER) [PI18/00044]; for Biomedical Network of Respiratory Diseases (CIBERES), Instituto de Salud Carlos III (ISCIII). M.G.-M. was supported by a Contrato predoctoral de formación en investigación (PFIS) contract (FI19/00067) from the FIS (Ministerio de Sanidad y Consumo, Spain). P.M. has a Miguel Servet contract (CP16/00116) funded by the ISCIII.

6. THE ROLE OF NOTCH3 PATHWAY IN PULMONARY HYPERTENSION

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Background: Pulmonary arterial hypertension (PAH) is a disease characterized by excessive vascular smooth muscle cell proliferation leading to elevated pulmonary vascular resistance with consequent right ventricle failure. Notch receptor signaling is implicated in controlling pulmonary artery smooth muscle cell (PASMC) proliferation and maintaining them in an undifferentiated state. The communication between PASMC and pulmonary artery endothelial cells (PAEC) seems to have a critical role in PAH development and since Notch3ICD expression is upregulated in the lung of PAH patients, we hypothesize that Notch3 signaling mediates this intercellular communication and represent a relevant mechanism to investigate.
Objectives: To identify the source of the Notch3 signal. Thus, which is the Notch ligand provided by endothelial cells to PASMCs to activate Notch3.
Methods: Experiments were carried out with 6-8 weeks old male C57BL6/J. Hipoxia+Sugen and Bleo14 days post-induction(dpi) PAH mouse model were used to study the expression of Notch3 pathway related proteins.
Results: We first characterized the Notch3 pathway in two PH disease mouse models: a) Hipoxia + Sugen and b) Bleomycin 14 dpi. We observed an increase in NICD3-expressing cells in PH-induced lungs and in particular in PASMC. These cells exhibit higher proliferation as demonstrated by Ki67 immunostaining, and they present a lower expression of the markers of the contractile phenotype Myh11, transgrelin and calponin. In addition, we detect the Notch ligands Jag1, Jag2 and Dll1 in endothelial cells at homeostasis and Jag1 and Jag2 seems to be upregulated in PAECs of the PH lungs.
Conclusions: Changes in the expression of Notch ligands in PAECs upon PH damage suggest that this may trigger the pathogenesis of PH promoting Notch3 activation in neighboring PASMCs driving the PH phenotype and therefore, these proteins emerge as therapeutic targets to prevent PH.
Funding: Ministerio de Economía y Competitividad; Fundación contra la hipertensión pulmonar; Excelencia de MarÍa de Maeztu.

7. INTERMITTENT HYPOXIA MEDIATES PARASPECKLE PROTEIN-1 UPREGULA-TION IN SLEEP APNEA

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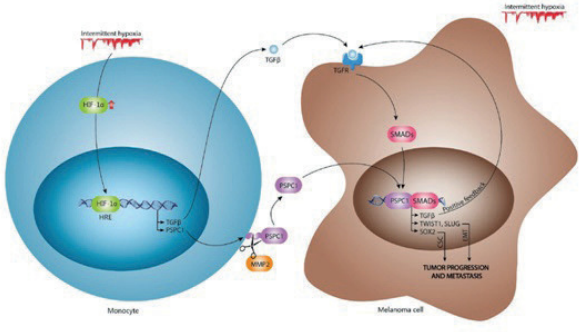
Background: Increasing information has recently emerged about the potential associations between obstructive sleep apnea (OSA), cancer aggressiveness, and mortality. However, the mechanisms by which cancer arises in OSA patients are not yet understood. Some evidence suggests that hypoxia might be an inducer of nuclear paraspeckle formation. PSPC1 is the main protein forming nuclear paraspeckles and has been described as the master modulator activating signature gene sets of EMT, CSC, and TGFβ signaling, processes that are upregulated in cancer.
Objectives: Our objectives were: (1) to analyze the expression of paraspeckle protein-1 (PSPC1) in patients with OSA, (2) to explore the role of intermittent hypoxia (IH) in PSPC1 expression in these patients, (3) to study the contribution of PSPC1 to the activation of tumor growth factor (TGF)β-SMAD pathway in patients with OSA (4) to determine PSPC1 role in promoting changes in intracellular signaling which justify the greater cancer aggressiveness reported in these patients.
Methods: Blood samples were obtained from newly diagnosed patients with OSA from the sleep unit of La Paz-Cantoblanco University Hospital. Plasma and monocytes were isolated for further analyses by ELISA, cytometry, WB and qPCR.
Results: We show that OSA patients exhibit elevated levels of PSPC1 both in plasma and in monocytes. Our data suggest that PSPC1 which

Comparisons between groups were performed by Student's t-test or the chi-squared test

	Severe OSA patients (n = 50)	Healthy volunteers (n = 20)	P-value
Male sex, n (%)	36 (72)	15 (75)	0.525
Age, years	59 ± 12	56 ± 8	0.418
Weight, Kg	92 ± 22	84 ± 8	0.332
Body mass index, Kg/m^2	32.6 ± 6.9	30.5 ± 1.9	0.370
Neck circumference, cm	42 ± 9	41 ± 8	0.413
Smoking habit, n (%)			0.231
Current smoker	16 (32)	6 (30)	
Former smoker	12 (24)	4 (20)	
Never smoker	22 (44)	10 (50)	
Epworth Sleepiness Scale	8.7 ± 4.2	2.0 ± 0.8	< 0.001
AHI, events/h	53.6 ± 16.9	2.7 ± 1.2	< 0.001
Oxygen desaturation index, events/h	51.0 ± 17.1	1.9 ± 1.1	< 0.001
Recording time with SpO_2 < 90%, %	31.7 ± 28.9	2.3 ± 2.1	< 0.001
Mean nocturnal SpO_2, %	90.6 ± 3.2	93.3 ± 1.5	0.003
Lowest nocturnal SpO_2, %	76.3 ± 8.9	90.2 ± 1.3	< 0.001

*Data are expressed as mean ± SD or number (percentage). Definition of abbreviations: AHI = apnea-hypopnea index; SpO_2 = oxyhemoglobin saturation.



PSPC1 expression in OSA patients increase the TGFβ expression effect on EMT. The intermittent hypoxia increases the activation of HIF1a, which could eventually, bound the promoter of genes such as PSPC1, MMP2 and TGFβ, leading to high levels of these proteins in severe OSA monocytes. The MMP2 plays a role in PSPC1 cleavage increasing the PSPC1 levels in plasma from patients. The combination of high levels of TGFβ and PSPC1 in plasma increases the EMT effect through TWIST and SLUG and CSC effect by SOX2 in melanoma cells.

is eventually delivered to the plasma through its cleavage from OSA monocytes by metalloproteases-2 (MMP2). Besides, IH promotes PSPC1, TGFβ, and MMP2 expression in monocytes through the hypoxia-inducible factor. Finally, both PSPC1 and TGFβ are capable to induce an increase in the expression of genes that drive the epithelial-to-mesenchymal transition and cancer stem cell.
Conclusions: Our study shows that hypoxemia up-modulates the extracellular release of PSPC1 by means of MMP2, in such a way that plasma PSPC1 together with TGFβ activation signaling promote tumor metastasis, supporting the high cancer aggressiveness in OSA patients.
Funding: FEDER and FIS.

8. PLASMA PROFILING REVEALS A BLOOD-BASED METABOLIC FINGERPRINT OF OBSTRUCTIVE SLEEP APNEA

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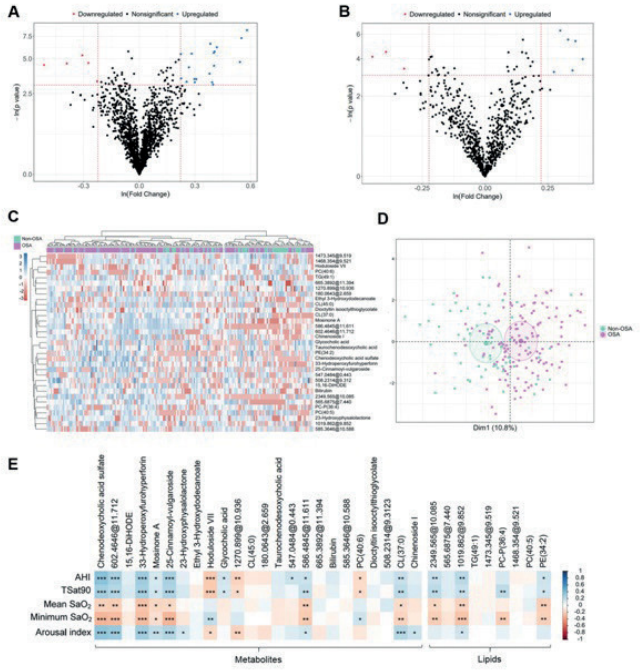
Background: Obstructive sleep apnea (OSA) is a chronic, heterogeneous and multicomponent disorder with associated cardiovascular and metabolic alterations. Despite being the most common sleep-disordered breathing, it remains a significantly undiagnosed condition. The study of metabolism at the molecular level has emerged as a tool with great potential not only in the identification of novel biomarkers but also can contribute to understand disease pathogenesis.
Objectives: We examined the complete plasma metabolome and lipidome of patients with suspected OSA, aiming to identify potential diagnosis biomarkers and to provide insights into the pathophysiological mechanisms underlying the disease. Additionally, we evaluated the impact of continuous positive airway pressure (CPAP) treatment on the circulating metabolomic/lipidomic profile affected by OSA.
Methods: Observational-prospective-longitudinal study including 206 consecutive subjects referred to the Sleep Unit. OSA was defined as apnea-hipoapnea index ≥ 15 events/h after full polysomnography (PSG) (Table). Patients treated with CPAP were followed-up for 6 months. Untargeted plasma metabolomic/lipidomic analysis was performed using liquid-chromatography coupled to mass-spectrometry.
Results: A plasma profile composed of 33 metabolites (mainly glycerophospholipids and bile acids) was identified in OSA vs. non-OSA patients (Figure A-D). This profile correlated with specific PSG measurements of OSA severity related to sleep fragmentation and hypoxemia (Figure E). Multivariate machine learning analysis disclosed a signature based on 4 metabolites which provided an accuracy (95%CI) of 0.98 (0.95-0.99) for OSA prediction. CPAP treatment was associated with changes in 5 plasma metabolites previously altered by OSA.
Conclusions: This analysis of the circulating metabolome and lipidome reveals a molecular fingerprint of OSA, which was modulated

Clinical and polysomnographic characteristics of the study population at baseline

	All (n = 206)	Non-OSA (n = 64) AHI< 15 events/h	OSA (n = 142) AHI≥ 15 events/h
Demographic variables			
Age, years	50.0 [44.0;55.0]	47.0 [40.0;54.0]	51.0 [45.0;56.0]
Sex, male	139 (67.5%)	39 (60.9%)	100 (70.4%)
Anthropometric measurements			
BMI, kg/m^2	29.3 [26.5;33.3]	28.0 [25.5;32.2]	29.8 [27.2;33.7]
Smoking status			
Never	80 (38.8%)	20 (31.2%)	60 (42.3%)
Former	61 (29.6%)	21 (32.8%)	40 (28.2%)
Current	65 (31.6%)	23 (35.9%)	42 (29.6%)
Polysomnography parameters			
AHI, events/h	27.6 [12.8;50.2]	9.26 [5.18;11.9]	41.0 [26.8;62.4]
TSat90, %	1.76 [0.18;8.86]	0.08 [0.00;0.67]	3.65 [0.74;14.6]
Mean SaO_2, %	94.0 [92.0;95.0]	94.0 [93.0;95.0]	93.0 [91.0;94.0]
Minimum SaO_2, %	83.5 [76.0;88.0]	88.5 [85.0;90.2]	81.5 [72.8;86.0]
Arousal index, events/h	33.7 [21.2;53.6]	20.7 [14.1;25.2]	43.3 [32.1;62.3]
Epworth Sleepiness Scale	11.0 [7.00;14.2]	10.0 [6.50;13.5]	11.0 [7.00;15.0]
Medical history			
Hypertension	72 (35.1%)	15 (23.8%)	57 (40.1%)
Dyslipidemia	50 (24.5%)	9 (14.3%)	41 (29.1%)
Diabetes	21 (10.2%)	3 (4.69%)	18 (12.7%)
Medications			
ACE inhibitors	50 (24.3%)	8 (12.5%)	42 (29.6%)
Beta-blockers	36 (17.5%)	7 (10.9%)	29 (20.4%)
Diuretic agents	28 (13.7%)	7 (11.1%)	21 (14.9%)
Calcium-channel blockers	17 (8.29%)	3 (4.69%)	14 (9.93%)
Angiotensin II receptor blockers	15 (7.35%)	4 (6.25%)	11 (7.86%)
Lipid-lowering agents	43 (21.0%)	8 (12.5%)	35 (24.8%)
Anticoagulants	5 (2.43%)	0 (0.00%)	5 (3.52%)
Insulin	10 (4.85%)	1 (1.56%)	9 (6.34%)

Data are presented as the median [p25;p75] for quantitative variables and n (%) for qualitative variables. Definition of abbreviations: ACE: angiotensin-converting enzyme; AHI: apnea-hypopnea index; BMI: body mass index; IQR: interquartile range; OSA: obstructive sleep apnea; SaO2: oxygen saturation; TSat90: time with SaO2 < 90%.



Untargeted metabolomic and lipidomic profiling in patients with suspected OSA. (A & B) Volcano plots of the fold change (FC) (x-axis) and p value (y-axis) for each detected metabolite (A) and lipid (B) in the comparison of OSA vs. non-OSA subjects. Red dots represent significantly downregulated (FC < 0.8) molecules, and blue dots represent significantly upregulated (FC > 1.25) molecules in OSA patients. The results are adjusted by confounding factors (age, sex and BMI). The p value threshold defining statistical significance was < 0.05. (C) Heat map representing the hierarchical clustering of dysregulated features in OSA. Each column represents a patient. Non-OSA patients appear in green, and OSA patients appear in pink. Each row represents a metabolite or lipid. Unknown features are presented as exact mass@retention time. The color scale illustrates the relative expression level of each molecule in each patient and ranges from red to blue, indicating relatively low to high expression, respectively. (D) PCA using the dysregulated features in OSA. Each dot represents a patient. Green dots represent the non-OSA subjects, and pink dots represent the OSA patients. (E) Correlations between polysomnographic parameters of OSA severity and the differentially expressed metabolites and lipids. Unknown features are presented as exact mass@retention time. The color scale illustrates the degree of correlation and ranges from red to blue, indicating negative and positive correlations, respectively. Significance is illustrated by *p < 0.05, **p < 0.01, ***p < 0.001. Definition of abbreviations: AHI: apnea-hypopnea index; BMI: body mass index; CL: cardiolipin; FC: fold change; OSA: obstructive sleep apnea; PC: phosphatidylcholine; PE: phosphatidylethanolamine; SaO2: oxygen saturation; TG: triglyceride; TSat90: time with SaO2 < 90%.

after CPAP treatment. Our results suggest blood-based biomarker candidates with potential application in the personalized management of OSA and suggest the activation of adaptive mechanisms in response to OSA-derived hypoxia.

Funding: ISCIII (PI14/01266, PI19/00907), co-funded by ERDF "A-way-to-make-Europe"; IRBLleida; CERCA-Programme / Generalitat-de-Catalunya. LP is a fellow from the Ministry of Universities of Spain (FPU19/01555).

9. NEURAMINIDASE INHIBITORS AND SINGLE-DOSE BALOXAVIR ARE EFFECTIVE AND SAFE IN UNCOMPLICATED INFLUENZA: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background: Scarce evidence verifying the clinical impact of baloxavir on influenza complications is found.
Objectives: To performed a systematic review and meta-analysis (SRMA) of randomized controlled trials (RCT) to assess the efficacy and safety of NAIs and baloxavir administration preventing complications when used to treat influenza infection.
Methods: PubMed, Cochrane Library, and Web of Science databases were searched through December 2020. Randomized-controlled trials (RCT) that enrolled patients with laboratory-confirmed influenza receiving neuraminidase inhibitors (NAI) or baloxavir comparing to placebo were assessed. PROSPERO Registration-number: CRD42021226854.
Results: Twenty-one RCTs (11,697 patients) were included. Antiviral administration significantly reduced time to clinical resolution (mean difference: -21.3 hours) and total influenza-related complications (OR: 0.55, 95%CI: 0.42-0.73). Specifically, antivirals significantly decreased bronchitis (OR: 0.54, 95%CI: 0.38-0.75), sinusitis (OR: 0.51, 95%CI: 0.33-0.78), acute otitis media (OR: 0.48, 95%CI: 0.30-0.77), and antibiotic prescription (OR: 0.62; 95%CI: 0.48-0.80). A positive trend favored antivirals administration to reduce pneumonia (OR: 0.47, 95%CI: 0.16-1.33), or hospitalization rates (OR: 0.65; 95%CI: 0.34-1.24) compared to placebo, but did not reach statistical significance. Adverse events (AE) were reported in 11%, 8.9%, and 5.1% of NAIs, placebo and baloxavir recipients, respectively. Compared with NAIs, administration of baloxavir showed non-significantly reduced AEs (OR: 0.74, 95%CI: 0.53-1.04).
Conclusions: Single-dose baloxavir and NAIs were superior to placebo to reduce complications in uncomplicated influenza, with 40% significant reduction in antibiotic prescription. Safety and efficacy of single-dose baloxavir were non-inferior to NAIs.
Funding: This work was funded by CIBERES, Instituto de Salud Carlos III, Madrid, Spain (Fondos FEDER) (CB06-06-036).

10. SEVERE BRONCHIAL ASTHMA ON BIOLOGICAL THERAPY AND COVID-19 INFECTION

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Background: It is not clear if there could be a relationship between asthma and asthma medication with COVID-19 infection incidence and severity.
Objectives: Describe clinical characteristics of patients with severe bronchial asthma receiving biological therapy who developed COVID-19 infection. Compare these features of inpatients versus outpatients.
Methods: Retrospective cohort study of patients with severe asthma on biological therapy during 2020 in Cáceres health area who had COVID-19 infection. Epidemiological, clinical, lung function, treatment and follow-up variables were analyzed.
Results: Out of a total of 150 patients with severe asthma on biologic therapy, 14 (9.3%) (six men, eight women, with a mean age: 52.7 years) had SARS-CoV-2 infection. Regarding cardiovascular risk factors: four patients had hypertension, three diabetes and six dyslipidaemia; six patients were ex-smokers and eight never-smoked. The mean BMI was 27.7. They presented mean values of FEV1: 82%, FVC: 93%, FEV1/FVC: 74%, FENO: 36 ppb. All were receiving biologic treat-

ment and a combination of ICS-LABA, 64.3% also LAMA and 14.3% took daily oral corticosteroid. Asthma control was good (mean ACT: 22), although five patients had at least an exacerbation in the previous year. Most had a correct perception of effectiveness with biological therapy evaluated by GETE. Half of patients required hospitalization for respiratory support with low-flow oxygen therapy and only one needed high-flow nasal cannula oxygen therapy. There was one death. Inpatients had a lower ACT (20.1 *vs.* 24.0, p 0.051) and five of them had exacerbations in the previous year versus none in outpatients (p 0.026).
Conclusions: 10% of patients with severe asthma receiving biologic agents in our area had SARS-CoV-2 infection. The profile of patient is a middle-aged woman, never-smoker, overweight, with normal lung function and good asthma control. Inpatients who had a severe COVID-19 infection had presented poorer asthma control and higher number of previous exacerbations last year.

11. DESCRIPTIVE STUDY OF THE PROFILE OF THE PATIENT WITH INDWELLING TUNNELED PLEURAL CATHETER (IPC) IN OUR AREA

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Background: The indwelling tunnelled pleural catheter (IPC) is an option in the treatment of symptomatic recurrent pleural effusion, especially in the case of a lung trapped or after a failed pleurodesis.
Objectives: The aim of our study is to describe the characteristics of patients in our area with IPC.
Methods: A retrospective descriptive study was carried out based on the reports of the Interventional Pulmonology Unit of our hospital. Epidemiological and clinical variables were collected from the 43 patients who had an IPC implanted during the period 2000-2021. Among the epidemiological variables we collected age and sex. Clinical variables we analyzed were type of effusion, its cause, the treatment received in the case of being malignant and the immediate complications related to the procedure, as well as the catheter-related delays. Furthermore, the time of catheter duration and the reason for removal. Statistical study is carried out using the PASW Statistics software.
Results: 43 patients with a mean age of 70 years were included. 63% were men and 37% women. The majority were exudative effusion (91%) and moderate (56%) (mild 14% and massive 30%). The vast majority were malignant of pulmonary origin (53%). Most patients received oncological treatment (65%). The mean duration with IPC is 72 days and the most frequent reason for withdrawal was the death of the patient (50%) or low debit (40%). Complications were not frequent, highlighting, in the moment of insertion, entry of air and pain (both in 9%) and in a late stage, cellulitis (5%), tumor spread through the catheter (5%) and pain (7%).
Conclusions: The profile of the patient with IPC in our area is that of a 70-year-old man with exudative effusion due to pulmonary neoplasia. In the majority of patients there are no complications related to the procedure.

12. LONG-TERM EFFECT OF OSA AND CPAP TREATMENT ON BLOOD PRESSURE IN PATIENTS WITH ACUTE CORONARY SYNDROME

A. Zapater[1,2], E. Gracia-Lavedan[2,3], I. David Benitez[2,3], G. Torres[2,3], A. Sánchez-de-la-Torre[2,3], J. de Batlle[2,3], A. Targa[2,3], O. Mínguez[3], L. Pascual[3], A. Cortijo[3], D. Martínez[3], M. Dalmases[2,3], M. Sánchez-de-la-Torre[1,2] and F. Barbé[2,3]

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Background: Obstructive sleep apnea (OSA) is prevalent in acute coronary syndrome (ACS) patients and is a cause of secondary hypertension.
Objectives: To evaluate the long-term effects of OSA and CPAP treatment on blood pressure (BP) patients with ACS.
Methods: *Post hoc* analysis of the ISAACC study included 1803 patients admitted for ACS (NCT01335087). Patients with OSA (apnea-hypopnea index≥ 15 events/h) were randomly assigned to receive either CPAP or/and usual care and followed up for one to 5 years. Office BP was determined at each visit.
Results: We included 596 patients without OSA, 605 patients in the CPAP group, and 602 patients in the usual care group. 52% of the patients had a diagnosed of hypertension at baseline. Median age and body mass index were 59 [52.0;67.0] years and 28.2 [25.6;31.2] kg/m^2, respectively. After a median [25th;75th percentile] follow-up of 41.2 [18.3;59.6] months, BP changes were similar between OSA and non-OSA groups. However, we observed an increase in BP in the third tertile of the AHI (AHI> 40 events/h) with a maximum difference in mean BP of +3.3 mmHg at 30 months (Figure). OSA patients with good CPAP adherence (≥ 4 hours/night) reduced mean BP after 18 months compared to non-OSA/poor CPAP adherence patients, maximum mean difference (95% CI) of -4.7 (-6.7,-2.7) mmHg. In patients with severe OSA we observed a maximum mean difference of -7.1 (-10.3,-3.8) mmHg.
Conclusions: In patients admitted with an ACS, severe OSA is associated with a long-term increase in BP, which is reduced by good CPAP adherence.
Funding: ISCIII (PI10/02763, PI10/02745, PI18/00449, PI19/00907), co-funded by FEDER "Una manera de hacer Europa", IRBLleida-Fundació Dr. Pifarré, CERCA Programme/Generalitat de Catalunya, SEPAR, Catalonian Cardiology Society, ResMed Ltd. (Australia), EsteveTeijin (Spain), Oxigen Salud (Spain), ALLER, CIBERES.

13. IMMUNOPATHOLOGICAL MECHANISMS OF BIRD-RELATED HYPERSENSITIVITY PNEUMONITIS IN AN ACUTE AND CHRONIC MURINE MODEL

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Background: Bird-related hypersensitivity pneumonitis (BRHP) is an interstitial lung disease induced by avian proteins, especially pigeon antigens. However, the immunopathological pathways involved in the disease are still unknown.
Objectives: This study assesses the cellular immune response and the cytokine pattern in a mouse model of BRHP.
Methods: C57BL/6JOlaHsd mice were randomly divided in four groups. On days -3 and -1 mice were intraperitoneally sensitized with pigeon serum (PS) or saline. Intranasal instillations with PS or saline were carried out during three consecutive days per week over either 3 weeks (acute model, AC) or 12 weeks (chronic model, CR). Leukocytes and cytokines patterns in lung tissue and pulmonary inflammation in bronchoalveolar lavage (BAL) were analysed.

Results: Both AC and CR HP groups exhibited increases in resident monocytes, interstitial macrophages and type 2 dendritic cells (CD-11b+Ly6C- DCs) but also a decrease in inflammatory monocytes, alveolar macrophages and tolerogenic DCs (CD11b-Ly6C-) compared to their saline groups. AC HP mice also had increased levels of eosinophils and T cells with a decrease in neutrophils and B cells while CR HP mice showed high levels of B cells. AC and CR HP groups exhibited an increase of Th1 cytokines (IFN-γ, IL-1β, IL-10 and IL-12) but also of Th2 (IL-5, IL-6) compared with their respective controls. AC HP mice also had high levels of TNF-α (Th1) and IL-13 (Th2) while CR HP mice showed increased levels of a Th17 cytokine (IL-23). Increased levels of neutrophils, eosinophils and lymphocytes were observed between HP and controls.
Conclusions: The present study suggests that in BRHP alveolar macrophages undergo subsequent apoptosis and interstitial macrophages proliferate to restore the depleted population. Our results also support that in acute BRHP there is a mixed Th1/Th2 immune response while in chronic BRHP a switch towards Th2/Th17 mixed response takes place.
Funding: ISCIII (PI18/00345), FEDER and FUCAP.

14. CHARACTERISATION OF PULMONARY SEQUELAE IN PATIENTS DISCHARGED AFTER COVID-19 PNEUMONIA

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Background: The respiratory sequelae of patients recovered from COVID-19 pneumonia are not well defined. However, it is predicted that the most severe patients may have organized pneumonia, other respiratory illnesses or continue with symptoms of the acute phase after discharge from hospital.
Objectives: The aim of the present study is to analyze possible biomarkers that can predict the severity and progression of the disease towards stages of respiratory sequels developed after recovery from severe COVID-19 hospitalization.
Methods: Patients with COVID-19 disease requiring hospital admission (n = 48) or ICU admission (n = 41) were included in the study. At the time of admission and after one and six months of negative results of COVID-19, an exhaustive clinical study, complete lung function, blood extraction and chest CT scan were performed. Blood samples were drawn to measure different cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17A, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1β and TNF-α) and lung fibrosis biomarkers (YKL-40 and KL-6).
Results: Increased levels of IFN-γ and TNF-α were observed during infection (Visit 1). Higher IL-4, IL-5 and IL-6 levels (p = 0.039, 0.011 and 0.045, respectively) were observed in patients who required ICU admission (Group 2) compared to those who were admitted to ward (Group1). IL-7 and MCP-1 levels increased in both groups one month after suffering the infection. During infection, higher YKL-40 levels were found in Group 2 (p = 0.0022). YKL-40 and KL-6 levels were higher in patients who died.
Conclusions: The pathogenesis of COVID-19 involves multiple cytokine pathways and differential biomarkers are present depending on the severity of the disease. Patients who required ICU admission had higher levels of Th2 cytokines profile. A relationship was observed between the markers of progression to pulmonary fibrosis and the mortality of the patients.
Funding: Air liquid, ISCIII (PI21 / 01046) and FEDER.

15. TREATMENT OF STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PNEUMONIAE MIXED BIOFILMS BY ANTIOXIDANTS.

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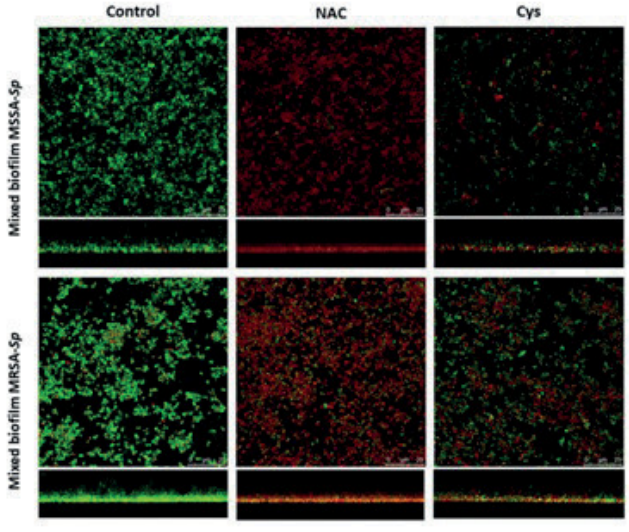
Background: Biofilm associate infections are a concern because of the increased antibiotic resistance and immune evasion. Co-colonization by Staphylococcus aureus and Streptococcus pneumoniae is possible and a threat in clinical practice.
Objectives: To investigate the interaction between S. aureus and S. pneumoniae in mixed biofilms and to test new antibiofilm therapies with antioxidants cysteamine (Cys) and N-acetyl-L-cysteine (NAC).
Methods: We developed two *in vitro S. aureus-S. pneumoniae* mixed biofilms in 96-well polystyrene microtiter plates. We treated in vitro biofilms with Cys and NAC and analyzed the effect of the antioxidants by CV staining, viable plate counting and confocal microscopy.
Results: S. pneumoniae needs a higher proportion of cells in the inoculum and planktonic culture to reach a similar population rate in the mixed biofilm. We demonstrated the effect of Cys in preventing *S. aureus* monospecific biofilms and *S. aureus-S. pneumoniae* mixed biofilms. Treatment with Cys had a low effect in both MSSA and MRSA strains. In the two mixed biofilms, Cys practically eradicated *S. pneumoniae* but only showed a 50% reduction in the S. aureus populations. NAC had a low effect preventing mixed biofilms but treatment with 5 mg/mL of NAC nearly eradicated the *S. pneumoniae* population and killed nearly 94% of MSSA cells and 99% of MRSA cells in the mixed biofilms. The methicillin resistance background did not change the antioxidants effect in *S. aureus.*
Conclusions: *S. aureus* and *S. pneumoniae* can interact and form stable biofilms. Cys and NAC are promising repurposed drugs to prevent and treat mixed biofilms.



CLSM of a MSSA-Sp and a MRSA-Sp mixed biofilm at 1:11 proportion after exposure to NAC and Cys. A MSSA-Sp and a MRSA-Sp mixed biofilm were untreated or treated with 2.5 mg/ml Cys or NAC for 1 h at 37 ºC. The cells in the biofilms were then stained with the BacLight LIVE/DEAD kit to reveal viable (green fluorescence) and non-viable (red fluorescence) bacteria. Projections were obtained in the x-y (individual scans at 0.5 μm intervals) and the x-z (individual scans at 5 μm intervals). Scale bars indicate 25 μm.

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16. SERUM MICRORNAS AS TOOL TO PREDICT EARLY RESPONSE TO BENRALIZUMAB IN SEVERE EOSINOPHILIC ASTHMA

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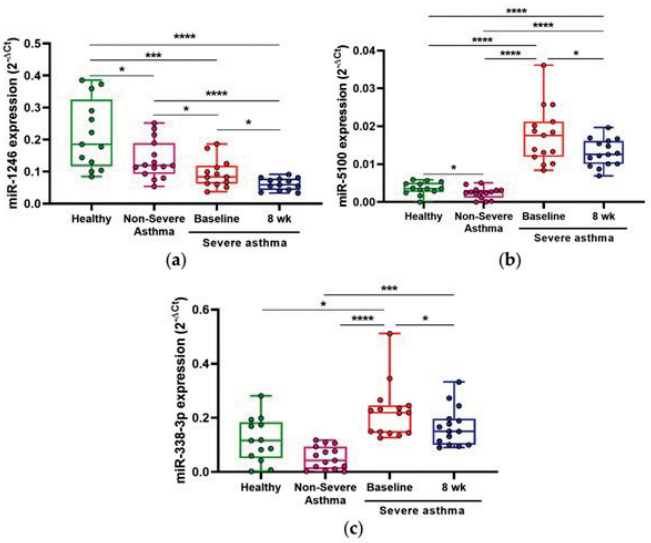
Background: Severe eosinophilic asthma implies a health and economic problem only controlled by biological biological drugs as benralizumab. This monoclonal antibody binds to IL-5 receptor alpha subunit depleting blood eosinophils, reducing asthma symptoms. Nevertheless, Benralizumab does not not benefit all patients g, and response-predictive biomarkers are needed. For this, serum microRNAs (miRNAs) can be useful.
Objectives: The aim of this study is finding possible serum miRNA biomarkers to detect an early response to benralizumab in severe eosinophilic patients.
Methods: This study was performed with fifteen severe eosinophilic asthmatic patients treated with benralizumab, fifteen moderate asthmatics and fifteen untreated healthy individuals. Serum miRNAs were evaluated by semi-quantitative PCR (qPCR) before and after treatment. Pathway analysis of deregulated miRNAs was performed using DIANA-miRPath v3.0 bioinformatic tool.
Results: Patients improved exacerbation rates and lowered eosinophil counts after benralizumab. miR-1246, miR-5100 and miR-338-3p were downregulated in severe asthmatic patients after eight weeks of therapy. Higher levels of miR-5100 and miR-338-3p were observed in severe asthmatic compared to patients with moderate asthma, while miR-1246 was lower in severe asthmatics at baseline than in non-severe patients. These three miRNAs were deregulated in severe asthmatics at baseline compared to healthy individuals. Inverse correlation of miR-1246 and exacerbation rates was observed at baseline, having otherwise a direct correlation with eosinophil counts and exacerbations after 8-week benralizumab. In silico, these three miRNAs control the MAPK signaling pathway, regulating genes as NFKB2, NFATC3 and DUSP family.
Conclusions: In this study, we observed a significant altered expression of miR-1246, miR-5100 and miR-338-3p after eight weeks of benralizumab administration and may serve as early response markers to benralizumab.
Funding: This manuscript was funded by Fondo de Investigación Sanitaria–FIS and FEDER [PI15/00803, PI18/00044, and FI16/00036], CIBER de Enfermedades Respiratorias (CIBERES), Merck Health Foundation, and Ministerio de Ciencia, Innovación y Universidades (RTC-2017-6501-1).

Clinical and demographic characteristics of studied patients

	Asthma (Mild-Moderate) (n = 15)	Asthma (Severe)		p-value
		Baseline (n = 15)	8 weeks (n = 15)	
Age (years)[a]	41.20 ± 8.75	46.86 ± 11.48		***
Male (%)	3 (20)	6 (40)		N.S.
Eosinophils (cell/μL)	300 (200-500)	217 (91-625)	0 (0-90)	N.S./****/‡
FEV_1 (%)[a]	97.99 ± 11.70	71.00 ± 16.56	74.08 ± 17.59	***/****/N.S.
Exacerbation per year[b]	1 (0-1.25)	3 (2-5)	0 (0-0)	***/*/†
ACT	23 (21-25)	11 (9.5-14)	13 (10.5-24.5)	***/N.S./N.S

[a]Results are expressed as mean ± SD; [b]Results are expressed as median (IQR) Comparisons were performed in the next order: Asthma *vs.* Baseline/Asthma vs. 8 weeks/Baseline vs. 8 weeks. Comparisons between Asthma and Severe Asthma are represented with asterisk: *p < 0.05, ***p < 0.001, ****p < 0.0001. Comparisons between Baseline and 8 weeks are represented with symbols: ‡p < 0.001, †p < 0.001. ACT, asthma control test; FEV1, forced expiratory volume measured during the first second; N.S. Non-significant.



Serum miRNA deregulation in severe patients treated with benralizumab. Severe eosinophilic asthmatic patients showed an altered expression of miR-1246 (a); miR-5100 (b); and miR-338-3p (c) after benralizumab administration. Also, miR-5100 and miR-338-3p expression levels were higher in severe asthmatics at baseline than in subjects with non-severe asthma. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

17. THE IMMUNOMODULATORY AND INFLAMMATORY EFFECTS OF THE INHALATION OF DEP IN A MURINE MODEL OF HEALTHY MICE

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Background: Exposure to environmental pollutants such as diesel exhaust particles (DEP) increases the risk of respiratory diseases exacerbation. However, the effects that DEP can produce by itself in healthy people remain poorly understood.
Objectives: The present study aimed to assess the immunomodulatory and inflammatory effects of the inhalation of DEP in a murine model of healthy mice.
Methods: BALB/c ByJ mice were randomly divided into five experimental groups. Control group received nasal instillations of saline during 1 week, 3 days per week, (Group 1) while the other four groups received nasal instillations of 150 μg of DEP 3 days per week during 1, 2, 3 and 6 weeks, Groups 2, 3, 4 and 5 respectively. Lung function assessment and flow citometry were performed.

Results: Inhalation of DEP decreased total monocytes during the first 3 weeks but basal levels are recovered after 6 weeks. Furthermore inflammatory monocytes were reduced after 3 weeks; however, resident monocytes increased at week 6. Regarding dendritic cells (DC) the inhalation of DEP increased the total population after 3 weeks. As regards macrophages, inhalation of DEP decreased total and alveolar populations, while interstitial macrophages populations increased during the whole experiment.
Conclusions: This mouse model provides evidence of the capacity of DEPs to increase DCs, interstitial macrophages and resident monocytes. Continuous exposure to DEP has an immunomodulatory and inflammatory effect.
Funding: This project received funding from the Fundació Catalana de Pneumologia (FUCAP), FIS PI18/00344 and FEDER.

18. NOVEL DNA METHYLATION MARKS ASSOCIATED WITH LUNG FUNCTION IN LATINO CHILDREN WITH ASTHMA

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Background: In the United States, ethnic disparities on lung function have been recognized by epidemiologic and genetic studies. Among Mexican American children, Native American ancestry is associated with higher lung function, whereas African ancestry is associated with lower lung function among Mexican American and Puerto Rican children. However, the role of the DNA methylome on lung function has not been specifically investigated in Latino populations.
Objectives: We sought to reveal DNA methylation (DNAm) signals for lung function in Latino youth with asthma, assess their transferability to European populations, and validate previous DNAm associations
Methods: We assessed the whole-blood DNA methylome for forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and their pre- and post- albuterol administration ratio in 247 Puerto Rican and 148 Mexican American youth with asthma. For that, we investigated differentially methylated CpG sites and regions. For the single CpG analyses, genome-wide significance was defined based on the total number of analyzed CpG markers (p-value = 0.05/427079 = 1.17×10^{-7}).
Results: We identified three CpG sites with DNAm levels genome-wide associated with lung function in Mexican Americans and Puerto Ricans: cg14441538 (PRKG1-AS1, p-value pre-FVC = 7.69×10^{-10}), cg00914963 (TBC1D16, p-value post-FVC = 5.14×10^{-8}), and cg20515679 (KCNJ6, p-value post-FEV1/FVC = 7.21×10^{-8}). Interestingly, these CpGs were not validated on publicly available data from 2,043 European adults. Moreover, two differentially methylated regions at LY6G5C and AURKC were associated with pre-bronchodilator lung function (adjusted p-value < 0.05) in both subethnic groups. We also replicated previously reported epigenetic marks for lung function.
Conclusions: We validated previous epigenetic associations and identified several novel epigenetic signals for lung function in Latino children with asthma that may exert population-specific effects.
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19. RNA-SEQ ANALYSIS ON LUNGS OF HOUSE DUST MITE-EXPOSED IGF1R-DEFICIENT MICE PROVIDES NEW INSIGHTS IN ALLERGIC AIRWAY INFLAMMATION

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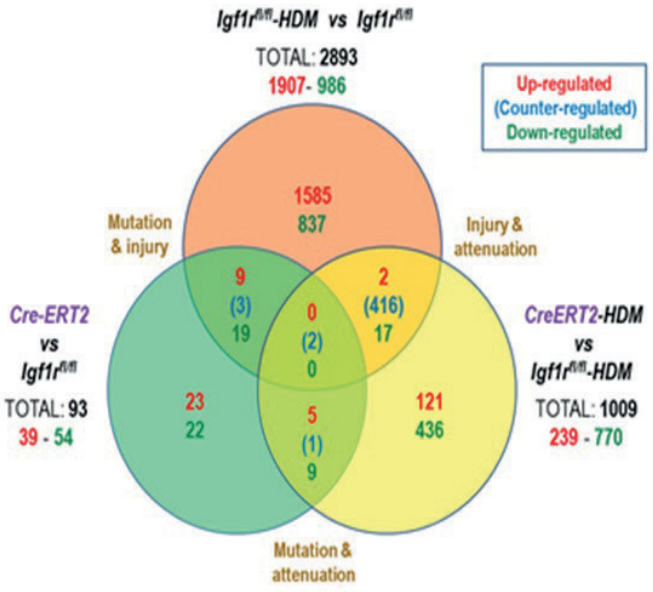
Background: Insulin-like Growth Factor 1 Receptor (IGF1R) is a trans-membrane tyrosine kinase that belongs to the IGF signaling system. IGF activity maintains lung homeostasis and it is involved in pulmonary diseases such as cancer, ARDS, COPD, asthma and fibrosis. IGF1R deficiency attenuates house dust mice (HDM)-mediated allergic lung inflammation (PLoS One 2016, 12: e0190159).
Objectives: To further explore the transcriptomic profile and regulation of acute asthma pathogenesis and their modulation by IGF signaling.
Methods: We performed RNA-seq in lungs of IGF1R-deficient and control mice after seven days of HDM or PBS intranasal administration. Raw reads were assessed for quality, trimmed and filtered. Clean reads were aligned, counted on gene features and normalized. Analysis of differential gene expression was then carried out and the resulting dataset was subjected to functional enrichment.
Results: Transcriptomic analysis identified a large number of differentially expressed genes between HDM-treated and untreated control mice which clustered in several biological processes and signaling pathways implicated in acute asthma biopathological fea-



Venn diagram of shared and independent significantly expressed genes between comparisons (FDR < 0.05, FC > 1,5/< 0.67).

tures. Analysis of differential gene expression due to IGF1R depletion in HDM challenged mice revealed reversal of a great part of the transcriptional regulation changes triggered by HDM challenge within those functional groups. Interestingly, data mining identified significant expression changes of gene clusters with key roles in mitochondrial homeostasis, metabolism and epigenetics, providing new insights into the molecular mechanisms underlying allergic airway inflammation and the implication of IGF1R signaling in this process.

Conclusions: These findings allow a more comprehensive view of acute asthma pathogenesis at the regulatory level and reinforce IGF1R as a potential therapeutic target in allergic asthma.

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20. GENETIC VARIANTS ASSOCIATED WITH 28-DAY SEPSIS SURVIVAL: A GENOME-WIDE ASSOCIATION STUDY

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Background: Sepsis is a severe systemic inflammatory response to infections that is accompanied by organ dysfunction. Sepsis has a high mortality rate in adult intensive care units (ICUs). To date, most genetic studies have focused on particular biological candidates.

Objectives: Here, we performed the first genome-wide association study (GWAS) of 28-day survival in sepsis.

Methods: We performed a two-stage GWAS on 687 European individuals from the GEN-SEP cohort (506 survivors and 181 deceased) and 7.5 million imputed genetic variants. Association analyses were conducted using Cox regression models, adjusting by gender, age and the two main principal components of genetic variation. The two stages were meta-analyzed and significance was established at $p < 5.0^{-8}$. Whole-blood transcriptomics from septic patients and *in silico* functional analysis were also assessed by focusing on the genes linked to the significant variants.

Results: The GWAS identified 3 independent common variants associated with reduced 28-day sepsis survival: intergenic to SLC5A12 and FIBIN (HR = 5.14, 95%CI = 3.06-8.65, $p = 7.04^{-10}$), intergenic to LINC00378 and MIR3169 (HR = 8.33, 95%CI = 4.10-16.91, $p = 4.56^{-9}$), and exonic to SAMD9 (HR = 4.75, 95%CI = 2.86-7.89, $p = 1.77^{-9}$). SAMD9, which may be related with the inflammatory response to tissue injury, was upregulated in non-surviving septic patients ($p = 1.67^{-3}$).

Conclusions: We completed the first GWAS of 28-day survival in sepsis patients and identified novel variants associated with reduced survival. These results will potentially allow to identify novel targets for sepsis treatment and patient risk stratification.

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21. PRIORITIZATION OF GERMLINE CAUSAL VARIANTS OF DISEASE: SOFTWARE ASSESSMENT ON WHOLE-EXOME SEQUENCING DATA

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Background: Whole-Exome Sequencing (WES) experiments analyze DNA sequences from protein-coding regions of the genome, where more than 80% of pathogenic and causal variants of Mendelian diseases are located. Frequently, WES provides a large number of variants per sample (15,000-25,000 variants). Despite causal variant prioritization is necessary for clinical diagnosis, bioinformatic standards are lacking.

Objectives: Here we benchmarked free software tools for variant prioritization of causal variants from empirical WES data.

Methods: A total of 62 WES data from patients with a known genetic diagnosis were obtained using a HiSeq 4000 Illumina® system, using 75 bp paired-end reads and a 100 bp padding to capture the exon-flanking regions. Consistent quality controls and BWA-GATK v3.8 Best Practices were followed for germline variant identification using the GRCh37/hg19 human genome as reference. Whenever necessary, the Human Phenotype Ontology terms were manually inspected according to the declared phenotypes. Different parameters were considered for the performance assessment of nine software tools.

Results: Five of the tools were fully evaluated, because of technical limitations and installation issues in the others. Based on conservative first-gene rankings, the highest diagnostic yield was found for Exomiser (69.3%), followed by AMELIE (46.7%), and Tapes (19.3%).

Conclusions: The tools for variant prioritization provided largely different diagnostic yields, with Exomiser being the best performing tool.

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22. IGF1R ACTS IN THE TUMOR MICROENVIRONMENT AS A CANCER-PROMOTING FACTOR FACILITATING THE IMPLANTATION AND PROGRESSION OF LUNG METASTASIS

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Background: Lung cancer is the leading cause of cancer death worldwide. The tumor microenvironment (TME) comprising blood vessels, immune cells, fibroblasts, signaling molecules and the extracellular matrix modulates tumor implantation and progression, thus its blockade is of therapeuthical interest. IGF1R (Insulin-like Growth Factor type 1 Receptor) is a ubiquitous membrane-bound tyrosine kinase receptor with recognized protumoral activity in the lung.
Objectives: To determine the impact of IGF1R deficiency on key components of the lung TME using murine models of tumor heterotopic transplantation and pulmonary metastasis.
Methods: In order to understand the role of IGF1R in the lung TME we generated Lewis and melanoma (LLC1/B16F10) lung cancer models using Igf1r-deficient mice and their controls (Transgenic Res24:279, 2015): i) LLC1 cells were intratracheally administered, awaiting for lung tumors to be developed after 21 days; ii) LLC1 cells were subcutaneously inoculated, followed by primary tumor resection on day (D)14 to allow pulmonary metastasis to be triggered until D35; and iii) LLC1 and B16F10 cells were administered via tail vein injection, awaiting for lung tumors to be developed after 14 days.
Results: IGF1R deficiency diminished BALF and bone marrow inflammatory total cell counts, and reduced lung tumor burden. Additionally, lack of IGF1R lowered mRNA expression of metastasis, hypoxia, tumor associated macrophage, tumor infiltrating lymphocyte, neutrophil and dendritic cell markers. Tumors of Igf1r-depleted mice also presented reduced proliferation, vascularization and presence of activated fibroblasts, sustained by decreased expression of epithelial to mesenchymal transition markers.
Conclusions: Our results support that IGF1R acts as a cancer-promoting factor in the TME contributing to implantation and progression of lung metastases.
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23. CARBOHYDRATE RECOGNITION BY COMPLEMENT FACTOR H AND RELATED PROTEINS VIEWED BY NMR

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Background: Human pathogenic microbes have several strategies to evade our immune system. These include interactions with the host complement system that may facilitate pathogen entry into cells and tissues. The Factor H (FH), a complement inhibitor glycoprotein, has a major role in protecting host cells from complement-mediated damage and elimination. Cell surface carbohydrates play an essential role in self/non-self recognition by FH distinguishing host cells from pathogens. The C-ter domain works as a cell-surface sensor by recognizing sialylated glycans. Factor H-related proteins (FHRs) are a group of complement proteins proposed to promote complement activation by competing with FH. Variants in C-ter homologous domains in FH and FHR-1, have been associated with various diseases. Interestingly, the carbohydrate recognition capabilities of FHR-1 are poorly characterized. Unraveling the molecular mechanism for surface recognition and complement de-regulation activity of FHR-1 will help to elucidate its role in health and disease.
Objectives: To study the molecular recognition processes between carbohydrates and FH/FHR-1 and mutants by means of NMR spectroscopy.
Methods: NMR Saturation transfer difference (STD) experiments are applied to characterize the recognition of sialylated glycans by complement proteins.
Results: The STD-NMR confirms that FH binds 2-3 linked sialic acid, while native FHR-1 does not. Such binding capacity can be restored by mutations (L290S, A296V) on the C-ter domain of FHR-1 that reproduce FH sequence. The FHR-1 mutant, (L290V) changes the binding preference towards the 2-6 isomer that is not bound by any other FH and FHR-1 variants tested.
Conclusions: FH, FHR1 and variants in their C-ter domain show differences in their carbohydrate recognition properties than can be related to their complement regulating or de-regulating activities.
Funding: Financial support from Comunidad de Madrid (project: S2017/BMD-3673) and Spanish Ministry of Science (2018_RTI2018-094751-B-C22) is acknowledged.

24. TRACKING OF A LONG-TERM SARS-COV-2 INFECTION IN A PATIENT WITH X-LINKED AGAMMAGLOBULINEMIA

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Background: Patients with impaired immune systems are characterised by persistent SARS-CoV-2 infections. Very few studies have reported intra-host viral evolution throughout infections in these patients.
Objectives: We describe the case of a 23-year-old immunocompromised male patient with clinically diagnosed X-linked agammaglobulinemia who was persistently infected with SARS-CoV-2 for a period of almost five months.
Methods: At the moment of hospital admission, the patient was confirmed with SARS-CoV-2 infection using RT-qPCR on a nasopharyngeal swab sample. Despite COVID-19 test negativizations, the patient was hospitalized most of the time and finally admitted to the intensive care unit where he died from multiorgan failure and shock. Over 149 days, 26 nasopharyngeal swab and bronchoalveolar lavage samples were collected, and 16 of them were sequenced using either Illumina or Oxford Nanopore Technologies sequencing. Consensus sequences were assigned to a PANGO lineage and mutations were called using Nextclade.
Results: All viral sequences were assigned to the same lineage supporting a single viral infection event. The accumulation of mutations throughout the course of the infection was accelerated and suggested the presence of compartmentalized viral subpopulations that evolved independently in the upper and lower respiratory airways.

Conclusions: These results support that long-term viral shedding in immunocompromised patients is one possible mechanism for the emergence of variants of concern and provide evidence towards infection control guidelines in these patients.
Funding: This work was supported by Cabildo Insular de Tenerife; the agreement with Instituto Tecnológico y de Energías Renovables (ITER); Instituto de Salud Carlos III and Ministerio de Ciencia e Innovación; the European Regional Development Fund (ERDF); Lab P2+ facility grant, by the ERDF and "Fundación CajaCanarias"; and the Spanish HIV/AIDS Research Network, by ISCIII and the ERDF; and by RIS-3 Canarias Strategy – "María del Carmen Betancourt y Molina" Program, "Consejería de Economía, Conocimiento y Empleo, Gobierno de Canarias".

25. ANALYSIS OF INSULIN LIKE GROWTH FACTORS AND IGF-BINDING PROTEIN SERUM LEVELS OF PATIENTS WITH DIFFERENT GRADES OF COPD, COPD AND LUNG CANCER AND EXACERBATED COPD

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Background: Members of the insulin-like growth factor (IGF) system, including IGF1 and 2 and IGF binding proteins (IGFBPs), are involved in lung homeostasis and respiratory diseases, including COPD.
Objectives: To assess IGF system components as serum biomarkers between groups of patients with different COPD status, smoking habit and controls.
Methods: Serum levels of IGF1 and IGF2, and IGFBP2 to 5, measured by ELISA and immunoblotting, were compared between seven groups of patients (n = 200): healthy controls (n = 30), smokers (n = 30), mild-COPD (n = 30), moderate-COPD (n = 30), severe-COPD (n = 23), COPD + lung cancer (n = 21) and exacerbated-COPD (n = 36).
Results: While IGF1 concentration was increased in smokers respect to controls and exacerbated-COPD patients, IGF2 was reduced in exacerbated-COPD respect to all groups, in COPD + lung cancer group respect to controls and smokers, and in severe-COPD respect to controls. IGFBP2 levels were elevated in both exacerbated-COPD, respect the rest of groups except COPD + lung cancer, and in COPD + lung cancer respect to controls, smokers and severe COPD. In contrast, IGFBP3 and IGFBP5 levels were reduced in exacerbated COPD with respect the rest of groups. The ratio fragmented/intact IGFBP3 was increased in smokers and exacerbated-COPD. Levels of IGFBP4 were significantly increased in COPD+lung cancer respect the rest of groups except smokers. Serum levels of IGF1R and IGFALS were found decreased in exacerbated-COPD respect to controls.
Conclusions: IGF components changed with COPD status. IGFBP2 and 3 showed opposed serum concentration profiles in COPD. While IGFBP4 was upregulated in COPD + lung cancer, variations in IGFs, IGF1R, IGFALS and IGBP2 and 3 levels were more marked in exacerbated-COPD. IGFs could be candidates serum biomarkers in exacerbated-COPD.
Funding: Spanish MICINN Project PGC2018-097397-B-I00 and Fundación Rioja Salud (Spain) Projects 6.FRS-ABC.019, all co-funded by the European Regional Development and European Social Funds (ERDF/ESF). E.A-A and A.U. PhD fellowships are granted by the Spanish Association Against Cancer (AECC) and Gobierno de La Rioja (Spain), respectively.

26. DEVELOPMENT OF NEW IMAGING BIOMARKERS FOR PULMONARY HYPERTENSION DIAGNOSIS BY FLOW MAGNETIC RESONANCE IMAGING

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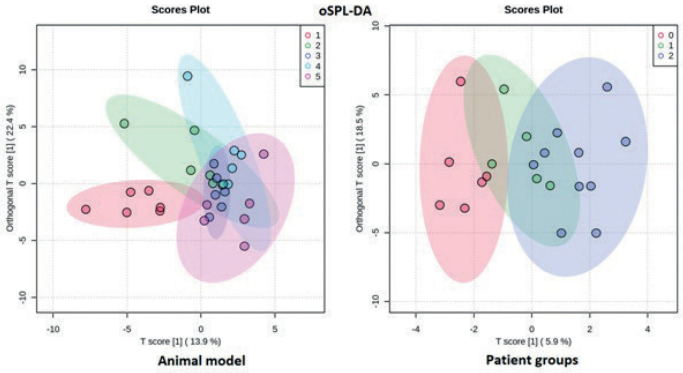
Background: Pulmonary hypertension (PH) is a chronic disease characterized by an increase in pulmonary vascular impedance and pulmonary vascular dysfunction and it eventually leads to right ventricle failure. Diagnosis must be confirmed in many cases by a right heart catheterization to measure a mean pulmonary artery pressure (mPAP) greater than 25 mmHg.
Objectives: We seek non-invasive diagnostic methods to characterize this disease. Cardiac Magnetic Resonance Imaging (MRI) and 4D Flow MRI (4DMRI) appear as non-invasive tools for right ventricle function and pulmonary vasculature evaluation.
Methods: An animal model has been studied in which four pigs were subjected to lung saline lavages followed by 2 hours of injurious mechanical ventilation. MRI datasets were acquired in baseline conditions, after the lung injury (ARDS) and in other three controlled ventilation conditions. Furthermore, more than 40 patients with PH associated with left heart disease (WHO group 2) are participating in a study from H.U. 12 de Octubre together with CNIC. From 4DMRI, several flow parameters have been calculated at different points of the pulmonary artery using a semi-automatic segmentation method. Unsupervised (PCA, K-means) and supervised (sPL-DA, oSPL-DA) analysis have been performed to reduce the 67 variables calculated to the most significant ones and to classify these subjects and patients into groups.
Results: The figure shows the oSPL-DA classification for animals in the five conditions (left) and a patient classification by hemodynamic conditions (right).



Conclusions: Helicity appears as a promising biomarker which correlates with mean pulmonary artery for patients. oSPL-DA success in classifying animal groups, hence we expect a similar result with patients groups avoiding right heart catheterization.
Funding: Proyecto FIS 2010, Evaluación de un Programa de Intervención Transversal en Insuficiencia Cardiaca. SAF2017-84494-C2-R, Elkartek KK-2019-bbmG19. Fundación contra la hipertensión pulmonar.

27. HIGH DOSES OF N-ACETYLCYSTEINE (NAC) INCREASE CELL SURVIVAL OF FIBROBLASTS CONTAMINATED WITH TOBACCO SMOKE EXTRACT

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Background: N-acetylcysteine (NAC) is an antioxidant molecule used in the treatment of respiratory diseases. There is a direct relationship between the dose of NAC and the protective proliferative effect on cells that are being affected by tobacco smoke contaminants.
Objectives: The objective of this study was to verify whether high doses of NAC can have a greater therapeutic effect on cells exposed to tobacco smoke extract (ET).
Methods: Lung fibroblasts were cultured in the presence of ET. For this, colorimetric studies of cell viability were carried out using the CCK-8 kit. The effect of different concentrations of NAC, added to the culture medium contaminated with ET, at the level of cell proliferation and the release of inflammation mediators by the cells present was studied in the culture medium.
Results: We found that all doses of NAC used in the assay have a proliferative protective effect against cell death caused by ET. At the lowest dose of NAC of 1.6 mM (equivalent to 600 mg in patients), a cell killing effect is still observed with the higher doses of ET. The 5 mM dose of NAC (equivalent to 1,200 mg) improves the effect of cell viability over that of 600 mg. Higher doses of 12 mM and 15 mM do not appear to improve the effect on cell viability over that of 5 mM.
Conclusions: A protective proliferative effect of NAC has been found in fibroblasts exposed to ET. This effect is dose dependent, with a significant increase in the protective therapeutic effect being found with the 5 mM (1,200 mg) dose compared to the 1.6 mM (600 mg) dose. No significant increase in the proliferative effect was found with doses higher than 12 or 15 mM.
Funding: This work has been funded by ZAMBON S.A.U. (Project ZB-NAC-01).

28. STANDARDIZATION OF VISCOELASTICITY OF SPUTUM FROM BRONCHIECTASIS PATIENTS

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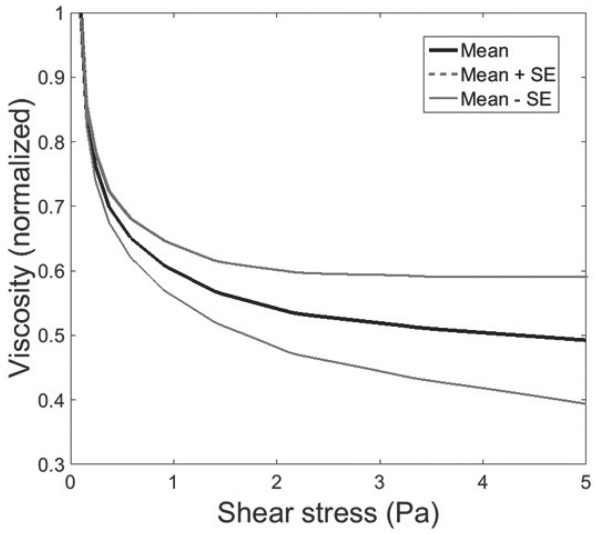
Background: It has been shown that rheological characterization of sputum in patients with non-cystic fibrosis bronchiectasis could be a valuable clinical tool for personalized management of the disease (Alcaraz-Serrano et al. Resp Med. 2019;154:46). Nevertheless, there is a lack of knowledge on the specific mechanical properties of the mucus secretions, making difficult the comparison between results obtained in trials conducted in different centers using different conditions for measuring sputum viscoelasticity.
Objectives: The aim of the present work was to assess the sputum rheological properties at different ranges of deformation to determine its fluid behavior.
Methods: The sputum from 48 patients with bronchiectasis were collected and frozen at -80 ºC for further rheological analysis. The sputum samples were thawed to 37 ºC, and rheological properties were measured by using a 35 mm serrated parallel-plate rheometer (Haake Rheostress1, ThermoFisher). Samples were subjected to shear stress from 0.1 Pa to 5 Pa at an angular frequency of 1 rad/s while measuring the shear force to determine the storage (G', elastic component) and loss (G'', plastic component) modulus. From deformation, G' and G'' data, sample viscosity was computed as a function of the shear stress.



Viscosity decrease with the shear stress applied to the sample.

Results: Bronchiectasis patient's sputum showed a clear non-Newtonian fluid behavior, as documented by a viscosity which markedly decreased with the shear stress applied to the sample (Figure). The fluid properties observed in these sputum samples were the classical ones for pseudoplastic or shear thinning materials.
Conclusions: As bronchiectasis patient's sputum showed a non-Newtonian fluid behavior, the measurements to be done in clinical studies must be carried out under well-defined conditions (shear stress, diameter of the plates, speed, etc.). Such characterization is required for clinical applications since it will allow to compare the sputum rheological properties measured at different centers.
Funding: Spanish Ministry of Science.

29. SERUM MICRORNAS AS BIOMARKERS OF SEPSIS AND RESUSCITATION

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Background: There is a lack of biomarkers of sepsis and the resuscitation status.
Objectives: To prove that the serum expression of certain miRNAs is differentially regulated in sepsis and is sensitive to different resuscitation regimes.
Methods: Anesthetized pigs (Sus scrofa domesticus) received no treatment (n = 15) or an intravenous live E. coli. Septic animals received saline 0.9% at 4 mL/kg/h (n = 8) (low resuscitation group, LoR), or 10-17 mL/kg/h (high resuscitation group, HiR) (n = 8 each group). Blood samples were obtained at the end of the experiment for measurement of 7 different miRNAs (RT-qPCR, Qiagen, Hilden, Germany).
Results: Serum expression of miR-146a-5p and miR-34a-5p increased significantly in the septic group, and miR-146a-5p was significantly lower in the HiR group than in the LoR group. Toll-like receptor signaling pathway involving 22 target proteins was significantly (adjusted $p = 3.87^{-4}$) regulated by these two microRNAs (KEGG). Highly significant (p value = 2.22^{-16}) protein-protein interactions (STRING) were revealed for these 22 hits.

Conclusions: miR-146a-5p and miR-34a-5p were identified as biomarkers of sepsis, and miRNA146a-5p seems to be a biomarker of the intensity of the resuscitation.
Funding: This study was supported by grants from Instituto de Salud Carlos III (PI19/01091) and Comunidad de Madrid (B2017/BMD-3727-EXOHEP-CM).

30. EPITHELIAL MESENCHYMAL TRANSITION IN LUNG CANCER PATIENTS: INFLUENCE OF COPD

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Background: Lung cancer (LC) is a major cause of morbidity and mortality worldwide. Chronic Obstructive Pulmonary Disease (COPD) is a risk factor for LC. The epithelial Mesenchymal Transition (EMT) pathway plays a key role in cancer progression and metastasis. Whether EMT expression may be influenced by underlying COPD remains to be fully explored.
Objectives: We hypothesized that EMT expression biomarkers will differ in LC patients with COPD from those without.
Methods: Lung tumor and non-tumor specimens were obtained through video-assisted thoracoscopic surgery (VATS) in LC patients with/without underlying COPD (N = 30 & 20 in each group, respectively). EMT markers: Snail1, Twist1, Vimentin, ICAM-1, E-cadherin, N-cadherin, Smad3/4, Zeb-2, and MMP-1/9 were analyzed in tumor and non-tumor samples of all the study patients using (qRT-PCR and immunoblotting, gene and protein expression levels, respectively). All the patients were clinically evaluated.
Results: The number of vimentin-positively expressed lung tumor samples was significantly greater in the LC-COPD group than in the LC patients. Nonetheless, no significant differences were observed in lung tumor gene expression of the markers Snail1, Twist1, Vimentin, ICAM-1, N-cadherin, Smad3/4, Zeb-2, and MMP-1/9 between the two study groups.
Conclusions: Among the different analyzed markers of EMT phenotype, vimentin appears as a potential surrogate of a differential expression in lung tumors of patients with underlying COPD. Increased expression of vimentin may favor LC cell adhesion, thus favoring a pro-tumoral microenvironment in patients with chronic airways diseases. These results offer a potential therapeutic avenue of research as vimentin can be targeted pharmacologically.
Funding: Funded by: FIS 18/00075 (FEDER) and CIBERES.

31. LONG-TERM EFFECT OF ANTITHROMBIN OR ARGATROBAN FOR THE TREATMENT OF ACUTE LUNG INJURY

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Background: Coagulation and inflammation play a critical role in Acute Respiratory Distress Syndrome (ARDS). We previously demonstrated that nebulized Antithrombin (AT) decreased pulmonary coagulation and inflammation in a model of Acute Lung Injury (ALI) at 48 h without altering systemic coagulation and no bleeding.
Objectives: To evaluate the potential therapeutic effects of nebulized AT or Argatroban in an ALI model at 72h.
Methods: The ALI model was induced in Sprague-Dawley rats (~250 g) by the intratracheal administration of 300 µl HCl (0.1 M) and 2 h later of 500 µl Lipopolysaccharide (LPS 30 µg/g body weight). Two or 3 doses of AT (500 IU/Kg body weight) or 3 doses of Argatroban (1 mg/Kg) were nebulized through a mesh AeronebPro nebulizer system (Aerogen Limited). Control animals received saline (0.9%) instead. Animals were sacrificed 72h after injury. Procoagulant, fibrinolytic, proinflammatory and chemoattractant molecules were evaluated in bronchoalveolar lavage (BAL) at protein level and in lung tissue at protein and mRNA levels. Statistics: One-Way-ANOVA and Newman Keuls post-hoc test was used. When data failed the normality test in the one-way ANOVA, the Kruskal-Wallis was used (Statistical significance: $p \leq 0.05$).
Results: All anticoagulant treatments decreased significantly mRNA proinflammatory mediators (IL1β and TNFα) and chemoattractant molecules (Gro-kc α and MCP1) in lung tissue at 72h, maintaining the effects found at 48 h (data not shown), although only 3 doses of AT decreased significantly the protein levels of these mediators at 72h (Figure). Prolonged anticoagulant treatments also reduced antifibrinolytic PAI-1 protein in lung tissue.

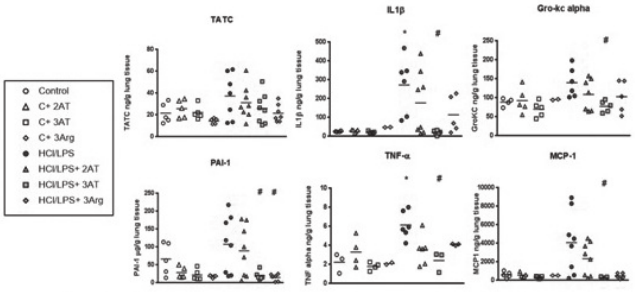

Figure 1: Protein concentration in lung tissue at 72 h: Procoagulant (TATC), antifibrinolytic (PAI-1), proinflammatory mediators (IL1β, TNFα) and chemoattractant mediators of neutrophils (Gro-kc alpha) and monocytes (MCP-1). Data represent mean ± SEM. *vs Control, # vs HCL/LPS. ps0.05

Conclusions: Nebulized anticoagulants reduce inflammation, neutrophils and monocytes recruitment in an ALI model at 72h, but only prolonged AT/Argatroban promote the fibrinolytic activation at this timepoint.
Funding: Grifols, S.A.: Investor-Sponsored Research Program grant and Grifols Antithrombin Research Awards (GATRA).

32. *IN VIVO* GENOME-WIDE DIFFERENTIAL GENE EXPRESSION PROFILING AND FITNESS ANALYSES HIGHLIGHT *HAEMOPHILUS INFLUENZAE* METABOLIC REQUIREMENTS DURING LUNG INFECTION

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Background: Nontypeable *Haemophilus influenzae* (NTHi) is a human-adapted pathogen causing lower airway infections in COPD patients. Bacteria and host elements dictating the complexity and fitness of NTHi within the host lung are not well understood. In vivo system multi-omics analyses are powerful approaches to explore the host–microbe complex, particularly when technologies offer distinct but complementary insights.
Objectives: To gather a comprehensive in vivo overview of host and bacterial factors that influence lower airway infections by NTHi.
Methods: We used a previously characterized murine model and two genomic technologies, RNA sequencing and transposon insertion mutagenesis coupled to next-generation sequencing, to screen differentially expressed genes, or genes required for bacterial fitness.
Results: Differential gene expression profiling of bacteria grown in vitro and recovered from BALF samples showed up-regulation of genes involved in purines and non-aromatic amino acid biosynthesis, and of part of the natural competence machinery. Bacterial gene inactivation allowed establishing a correlation between gene up-regulation and its involvement in infection. Profiling of Tn-mutant fitness by screening bacterial genes whose mutants were underrepresented in lung samples compared to in vitro growth identified dam methyltransferase and genes required for tryptophan biosynthesis. Tryptophan auxotrophy dampened in vivo bacterial fitness upon impaired transport, in turn modulated by strain-dependent distribution of the accessory tnaCAB locus (links tryptophan synthesis and indole excretion). Expression of these genes in vivo did not correlate with their involvement in the bacterial fitness.
Conclusions: Our results provide novel insights into *H. influenzae* metabolic requirements for lung infection, and demonstrate the power of using both gene expression and fitness profiling in vivo for probing bacterial virulence.
Funding: N.L.-L. and C.G.-C. are funded by PhD studentships 0011-1408-2017-000000 and PRE2019-088382. This work was funded by grants RTI2018-096369-B-I00 (MICIU), 875/2019 (SEPAR), PI003 Micro-EPOC and PC150-151-152 (Regional Navarra Govern) to J.G. CIBER is an initiative from Instituto de Salud Carlos III.

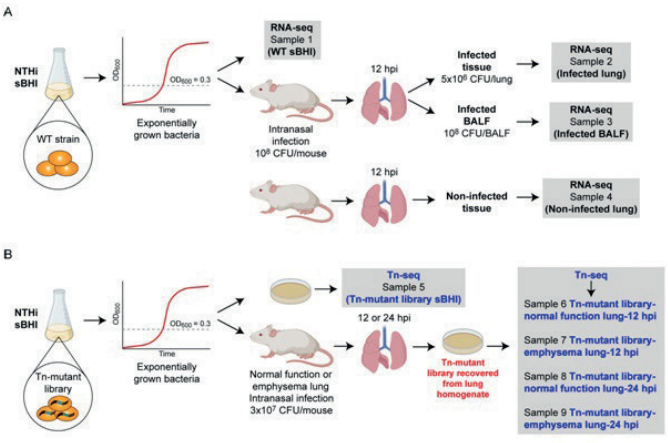

Figure 1. Schematic representation of the *in vivo* multi-omics analyses used to explore the host–microbe crosstalk during *H. influenzae* lung infection in a suitable and previously characterized mouse model. For *in vivo* bacteria gene expression profiling, we used RNA-seq to compare bacteria grown *in vitro* to those recovered from BALF samples (samples 1 and 3). For host gene expression profiling, we used RNA-seq to compare uninfected and infected lung samples (samples 2 and 4). For *in vivo* assessing bacterial fitness related genes, we used Tn-seq to compare lung-derived samples to *in vitro* growth and screen for bacterial genes whose mutants were under-represented in samples 6, 7, 8 and 9 compared to sample 5.

33. DIVERSITY AND COMPOSITION OF THE SALIVARY MICROBIOME ASSOCIATE WITH ASTHMA EXACERBATIONS

J. Pérez-García[1], A. Espuela-Ortiz[1], J.M. Hernández-Pérez[2], R. González-Pérez[3], O. Sardón[4], E. Martín-González[1], E. Mederos-Luis[5], P. Corcuera[6], I. Sánchez-Machín[5], P. Poza-Guedes[3], A. Callero[7], M. González[8], J. Korta-Murua[4], J. Villar[9], M. Pino-Yanes[10] and F. Lorenzo-Díaz[8]

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Background: Asthma exacerbations are the major contributor to the asthma burden. The nasal and lung microbiome has been associated with asthma exacerbations. However, to date, no study has investigated the role of the salivary microbiome in asthma exacerbations.
Objectives: To assess whether the diversity and composition of the salivary microbiome are associated with asthma exacerbations.
Methods: Saliva samples were collected from adults and young patients with asthma from the GEMAS study. Cases and controls were defined by the presence or absence of asthma exacerbations in the past 6 months (based on oral corticosteroids use to treat asthma symptoms). Microbial communities were profiled by targeted sequencing of the V3-V4 region of the 16S ribosomal ribonucleic acid gene. Bioinformatic analyses were conducted using QIIME2 and R (phyloseq). Differences between groups were assessed through logistic regression models or PERMANOVA tests, adjusting for age, sex, and antibiotics use.
Results: A total of 239 patients were retained after quality control (82 cases and 157 controls). Controls showed higher salivary microbiome richness (observed Operational Taxonomic Units) and diversity (Shannon and Faith indexes) than cases ($p < 0.04$). Dissimilarities in the average composition of bacterial communities (unweighted UniFrac distance) were observed between groups (pPERMANOVA = 0.002). The relative abundance of the Rothia genus was increased in the saliva from exacerbators compared to the non-exacerbator group (6.0% *vs.* 4.9%, $p = 0.007$).

Conclusions: Richness, diversity, and composition of the salivary microbiome associate with asthma exacerbations.
Funding: Funded by SAF2017-83417R MINECO/AEI/FEDER, UE.

34. A GENOME-WIDE ASSOCIATION STUDY OF VEGFR1 SERUM LEVELS IN PATIENTS WITH SEPSIS

E. Suárez-Pajes[1], T. Hernández-Beeftink[1,2], B. Guillén-Guio[1], M I. García-Laorden[2,3], A. Corrales[1,3], M. Prieto-González[4], A. Rodríguez-Pérez[5,6], D. Carriedo[7], J. Blanco[3,8], A. Ambrós[9], E. González-Higueras[10], E. Espinosa[11], A. Muriel[8], D. Domínguez[11], A. García de Lorenzo[12], J.M. Añón[3,12], M. Soro[13], J. Villar[2,3] and C. Flores[1,3,14]

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Background: The acute respiratory distress syndrome (ARDS) is a severe lung inflammatory process that is mainly caused by sepsis and that continues to have a disproportionate death rate in the Intensive Care Units. The FLT1 gene, encoding the VEGF receptor 1 (VEGFR1) implicated in vascular permeability and immunity, have been involved in predisposition to sepsis-associated-ARDS. Functional evaluations of FLT1 revealed higher expression in ARDS patients compared to other critical patients.
Objectives: Here we performed the first genome-wide association study (GWAS) of the serum VEGFR1 levels in sepsis patients to identify variants associated with the protein levels.
Methods: Protein levels were measured with the VEGFR1/Flt-1 DuoSet ELISA kit (R&D Systems) in serum samples from 231 patients collected within 24 h from sepsis diagnosis. Genotyping was performed with the Axiom Genome-Wide CEU1 array (Affymetrix) and imputation was conducted on the HRC panel. A linear association test was performed on 7.7 million variants.
Results: The most significant variant (rs59679804, Odds Ratio = 0.85, 95%CI = 0.81-0.91, p = 3.3^{-7}) was intergenic to PARL and ABCC5 genes. This variant was previously associated with the blood gene expression of both genes and with blood cells count.
Conclusions: We completed the first GWAS of VEGFR1 serum levels and identified a potential variant associated with its levels in sepsis patients. Further studies will be needed to explore if PARL/ABCC5 gene variants could be associated with sepsis outcomes.
Funding: Instituto de Salud Carlos III (PI17/00610, PI20/00876, FI17/00177), co-financed by the European Regional Development Funds, "A way of making Europe" from the European Union; by the agreement with Instituto Tecnológico y de Energías Renovables (OA17/008); by Ministerio de Ciencia e Innovación (RTC-2017-6471-1; AEI/FEDER, UE); and by Cabildo Insular de Tenerife (CGIEU0000 219140).

35. MICRORNAS AS BIOMARKERS OF DISEASE SEVERITY IN PATIENTS WITH COVID-19

A. Ferruelo[1,2], Y. Douhal[2], R. Herrero[2,1], R. Murillo[2,1], A. Silva[2], P. González-Rodríguez[2], D. Carriedo[2], C. Jaramillo[2], Ó. Peñuelas-Rodríguez[2,3] and J.Á. Lorente[2,1,4]

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Background: The time course of patients with COVID-19 is not predictable based on clinical grounds, and biomarkers of disease severity are lacking.
Objectives: To identify miRNAs differentially expressed in patients with COVID-19 of different severity.
Methods: We conducted a case control study of miRNAseq sequencing in samples obtained on the day of admission from patients discharged from the Emergency Department (mild group), admitted to the hospital ward (moderate group), and admitted to the intensive care unit (severe group) (n = 10 each group). Differential expression analysis of the miRNA sequencing data was conducted using DESeq2 in R. To reduce false discovery, we selected miRNAs with base mean expression > 50 cpm, ≥ 2-fold expression change, and an adjusted p value (FDR) of < 0.05 in all three pairwise comparisons. Target prediction and enrichment analysis was conducted using web-based tools (mirNet 2.0). RT-qPCR was used to validate the differential expression of miRNAs identified in the discovery cohort and in an additional validation cohort of 60 patients (n = 20 each group).
Results: We identified 6 miRNAs differentially expressed in the severe group as compared to the mild and moderate groups. Differential expression of all 6 miRNAs was validated by RT-qPCR. These miRNAs are involved in cellular response to stress, cellular senescence, intrinsic pathway for apoptosis, signaling by PDGF and innate immune system (Reactome) (adjusted p value < 0.001). In addition, two of the miRNA correlated (r > 0.40 or r < -0.40, p < 0.001) with albumin serum concentration, serum LDH, blood neutrophil/lymphocyte ratio and respiratory SOFA score.
Conclusions: miRNAs involved in apoptosis, inflammatory response and immune system are characteristic of severe disease in COVID-19. Our findings may have implications for biomarker and innovative therapeutic target discovery in COVID-19.
Funding: CIBERESUCICOVID Project (COV20/00110, ISCIII), Instituto de Salud Carlos III (PI19/01091) and Comunidad de Madrid (B2017/BMD-3727-EXOHEP-CM).

36. IMPACT OF UNIVERSAL PAEDIATRIC VACCINATION ON THE INCIDENCE OF ADULT INVASIVE PNEUMOCOCCAL DISEASE IN TIME OF SARS-COV-2 PANDEMIC

A. González-Díaz[1,2,3], S. Calvo-Silveria[1,2], J. Camara[1,2,3], E. Cercenado[4,3], N. Larrosa[5], M.D. Quesada[6], I. Sanfeliu[7], J.M. Marimon[8], J. Yuste[3,9] and C. Ardanuy[1,2,3,10]

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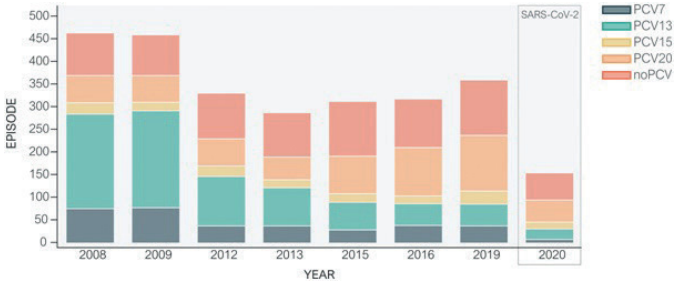
Background: Streptococcus pneumoniae is a major human pathogen primarily transmitted through respiratory droplets. In Spain, high vaccine coverage was obtained after the national approval of the PCV13 in the paediatric schedule in 2015-2016.

Objectives: To analyse the impact of universal paediatric vaccination with PCV13 in the adult invasive pneumococcal disease (IPD) in Spain as well the global SARS-CoV-2 pandemic.
Methods: All adult IPD episodes collected in six Spanish hospitals in 2019-2020 (uni-PCV13) were collected and serotyped. Antibiotic susceptibility was tested following EUCAST recommendations. The incidence was calculated as cases per 100,000 inhabitants. Results were compared with previous periods: pre-PCV13 (2008-2009), early-PCV13 (2012-2013) and late-PCV13 (2015-2016).
Results: In the uni-PCV13 period, a total of 542 episodes were collected: 374 in 2019 and 168 in 2020. The incidence increased from 8.5/100,000 habitants in 2016 to 9.3/100,000 in 2019, but a significant decrease was observed in 2020 (4.3/100,000 habitants). IPD episodes caused by non-PCV13 serotypes increased from 36.3% in 2008 to 71.7% in 2019. PCV13 serotypes increased from 19.5% in 2008 to 21.4% in 2013 with a further decrease to 10.4% in 2019. Serotypes 8 (16.8%), 3 (5.6%), 12F (5.6%) and 22F (5.6%) were the most frequent in 2019 whereas in 2020 were: 8 (19.7%), 3 (7.7%), 6C (5.4%) and 19A (4.8%). A decrease in the β-lactam non-susceptibility was observed between the late-PCV13 and uni-PCV13 period; penicillin (22.1% to 15.3%), amoxicillin (8.9% to 5.5%) and cefotaxime (7.0% to 4.1%). Rates of erythromycin and clindamycin resistance increased from 16.3% to 21.2% and from 15.0% to 15.5%, respectively.

Antibiotic non-susceptibility in the uni-PCV13 period

	mg/L	pre-PCV13	early-PCV13	late-PCV13	uni-PCV13
Penicillin	> 0.06	22.8	27.1	22.1	15.3
Amoxicillin	> 0.5	11.2	12.0	8.9	5.5
Cefotaxime	> 0.5	10.1	12.5	7.0	4.1
Erythromycin	> 0.5	21.0	23.8	16.3	21.2
Clindamycin	> 0.5	18.8	20.2	15.0	15.5
Chloramphenicol	> 8	6.1	5.3	5.2	3.7
Tetracycline	> 1	27.7	24.1	20.3	18.6
Cotrimoxazol	> 1	24.9	19.2	23.8	11.4



Conclusions: Despite universal children vaccination, the overall adult IPD increased in 2019. A great impact was observed during the SARS-CoV-2 pandemic, halving the number of episodes. Despite being included in PCV13, serotype 3 is a major cause of adult IPD.
Funding: Projects from the Fondo de Investigaciones Sanitarias "PI1800339" and CIBERES (CIBERES–CB06/06/0037).

37. ASSOCIATION OF ALPHA-1 ANTITRYPSIN GENETIC POLYMORPHISMS AND PROTEIN LEVELS WITH ASTHMA EXACERBATIONS

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Background: Asthma is a chronic inflammatory disease of the airways. Patients with asthma may experience episodic flare-ups, known as exacerbations. Different polymorphisms in the serpin family A member 1 gene (SERPINA1) can cause alpha-1 antitrypsin deficiency (AATD), especially the rs17580 (S variant) and rs28929474 (Z variant) polymorphisms, also related to different respiratory diseases, including asthma. However, the role of this genetic variants on asthma exacerbations has not been examined.
Objectives: This work aimed to analyze whether the genetic variants that characterize AATD and reduced alpha-1 antitrypsin protein levels are associated with asthma exacerbations.
Methods: Genetic data and alpha-1 antitrypsin (AAT) protein levels measured in serum samples from Canary Islanders from La Palma (n = 452) were analyzed as discovery. Genomic data from additional Canary Islanders (n = 217) and mainland Spaniards (n = 151) were analyzed for replication. Logistic regression analyzes were performed for S and Z variants and the classic SERPINA1 alleles derived from them, as well as for AATD, which was defined as serum AAT levels < 100 mg/dl. All analyzes were adjusted by age, sex, inhaled corticosteroid use, and smoking history. Multiple comparison adjustment was performed with Bonferroni correction.
Results: A statistically significant association was found for the polymorphisms rs17580 (*odds ratio* (OR) = 1.28, 95% confidence interval: 1.15-1.42, p-value = 4.12×10^{-6}) and rs28929474 (OR = 1.39, 95% confidence interval: 1.19-1.63, p-value = 4.47×10^{-5}) in individuals from La Palma. Likewise, AATD was associated with a higher risk for asthma exacerbations (p-value = 1.39×10^{-5}). At classic allele level, Pi*MS and Pi*SZ were also significantly associated with a higher risk for asthma exacerbations. However, these results were not validated in independent populations (p-value > 0.05).
Conclusions: Our results suggest a potential role of SERPINA1 genetic variants and AATD on asthma exacerbations that should be further explored in larger populations.
Funding: Funded by SAF2017-83417R MINECO/AEI/FEDER, UE.

38. VITAMIN D DEFICIENCY, A POTENTIAL CAUSE FOR INSUFFICIENT RESPONSE TO SILDENAFIL IN PULMONARY ARTERIAL HYPERTENSION

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Background: Phosphodiesterase 5 inhibitors (PDE5i), such as sildenafil is frequently used to treat pulmonary arterial hypertension (PAH). However, a sizeable proportion of PAH patients fail to maintain treatment goals with PDE5i. The results from REPLACE study show that patients remaining at intermediate risk, switching to riociguat is beneficial in terms of clinical improvement as compared to PDE5i maintenance therapy. Moreover, several reports have shown that vitamin D (vitD) regulates the NO signaling pathway in systemic arteries.
Objectives: The aim of this study is to analyze if vitD deficiency in PAH may account for the limited efficacy of sildenafil in some patients.
Methods: The vasodilator response to sildenafil and riociguat were studied in isolated pulmonary arteries (PA) from rats with PAH that had been exposed to vitD-free diet for 8 weeks with those after restoring vitD status for the last 3 weeks. We retrospectively compared the 25(OH)vitD plasma levels of PAH patients that responded (n = 17) *vs.* those who did not respond (n = 13) to PDE5i. Plasma samples were obtained from the Spanish National Pulmonary Hypertension Biobank and clinical data from the REHAP.
Results: VitD deficiency led to a poor vasodilator response to sildenafil in isolated PA which can be reverted by restoring vitD levels. The response to riociguat was unaffected by the vitD status. The responders to sildenafil had significantly higher 25(OH)vitD levels than non-responders.
Conclusions: VitD deficiency causes a poor vasodilator response to PDE5i, but preserved response to riociguat in rats with PAH, indicating that vitD deficiency reduces the apparent basal NO-dependent cGMP production. Lower levels of 25(OH)vitD in non-responders to PDE5i compared to responders suggest that vitD deficiency may cause insufficient response to PDE5i in some patients with PAH.
Funding: This study was supported by grants from Ministerio de Economía y Competitividad (SAF2016-77222-R; PID2019-107363RB-I00), Fundación Contra la Hipertensión Pulmonar and an unrestricted grant from MSD.

39. EVALUATION OF SAK PRODUCTION IN *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATES FROM THE LOWER RESPIRATORY TRACT

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Background: Mechanically ventilated patients are susceptible to develop lower respiratory tract infections. Staphylokinase (SAK) is a fibrinolytic agent that has been suggested to facilitate bacterial colonization and dissemination.
Objectives: To assess the expression of SAK in a collection of S. aureus clinical isolates and correlate its expression with clinical and microbiological features.
Methods: Forty-eight clinical strains isolated from patients under mechanical ventilation were selected (Lacoma A et al., 2021). Patients were classified according to clinical criteria into pneumonia, tracheobronchitis and bronchial colonization. A single colony was grown for 20 hours in tryptic soy broth and supernatant was collected. The assay was performed according to Kwiecinski J et al., 2013. Strains were categorized as high, moderate, low, very low or non-producers of SAK.
Results: The distribution of strains according to SAK expression is shown in the Figure. According to the three study groups, no differences were found regarding the production of SAK. Nevertheless, pneumonia strains show a trend towards a higher proportion of low producers and a lower proportion of negative strains in comparison to the other groups. Regarding its impact on clinical outcome, isolates from cases with respiratory complications and/or mortality related displayed a higher proportion of low and very low producers, accounting for 50% and 25%, respectively. None of the cases defined as persistent were considered high producers, while this category represents the 17% of the non-persistent isolates. Moderate producers are more frequent in the persistent group, accounting for 37% of the strains, while low producers are the most common isolates in the non-persistent group, accounting for a 38%.

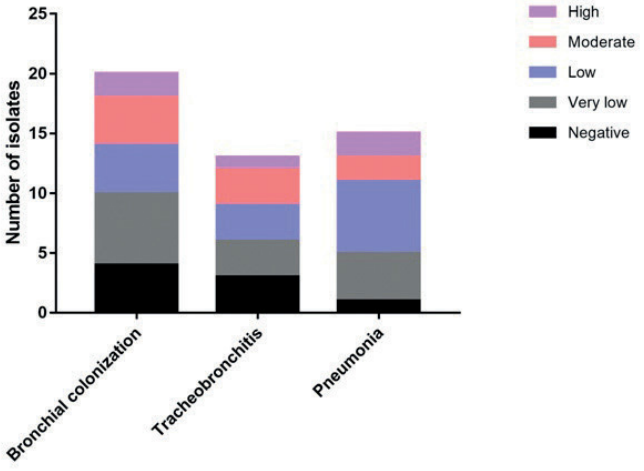

Figure 1. SAK production according to the study group

Conclusions: The expression of SAK is variable among respiratory clinical isolates. The expression of SAK is not related to study group nor clinical outcome. However, persistent and non-persistent strains show a different SAK expression pattern.
Funding: This work was supported by PI17/01139.

40. SOLUBLE GUANYLATE CYCLASE STIMULATORS REVERT IN VITRO CIGARETTE SMOKE EFFECTS THROUGH JNK PATHWAY NORMALIZATION

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Background: In previous studies we identified in a guinea pig model of COPD that emphysema and pulmonary hypertension (PH) induced by cigarette smoke (CS) exposure were related to alteration of genes of functional pathways such as oxidative stress, inflammation, apoptosis and cell proliferation (AJP-LCMP-2019;317:L222-34). Furthermore, treatment with the soluble guanylate cyclase (sGC) stimulator, BAY41-2272, induced the normalization of ≈50% of altered genes, especially those related to the MAPK pathway. Even more, an *in silico* analysis showed that genes in the JNK pathway were also altered in smokers.
Objectives: The aim of the present study was to evaluate in vitro the effects of CS on the JNK pathway in both endothelial (HPAEC) and smooth muscle cells (PASMC) from human pulmonary arteries, as well as the effects of the sGC stimulator BAY63-2521.
Methods: Commercially available human lung cells (HPAEC, PASMC) were cultured with CS extract (CSE) (1/5) and BAY63-2521 (EC50 100uM). Cell proliferation was calculated and gene expression analysed by qRT-PCR.
Results: The in vitro results show that HPAEC proliferation was more sensitive to CSE than in PASMC. Besides, CSE increased the expression of JUN and FOS in both cell types. JUN was 5 times more expressed in HPAEC and 3 times in PASMC (both $p < 0.05$). Moreover, FOS was expressed 170 times more in PASMC and 4 times in HPAEC as compared to respective controls (both $p < 0.01$). In both cell types, higher expression of these genes was associated with increased expression of the apoptotic genes CASP3 and P21 ($p < 0.05$). Interestingly, in PASMC treatment with BAY63-2521 normalized the expression of JUN and prevented the increase of P21 and CASP3 ($p< 0.05$).
Conclusions: In conclusion, these results highlight the importance of JUN and FOS as effector targets of the NO-cGMP pathway and suggest the use of sGC stimulators as a treatment for emphysema and PH in COPD.
Funding: FIS PI16/01147, SEPAR-888/2019.

41. ATROPHY SIGNALING PATHWAYS IN RESPIRATORY AND LIMB MUSCLES OF GUINEA PIGS EXPOSED TO CHRONIC CIGARETTE SMOKE: ROLE OF SOLUBLE GUANYLATE CYCLASE STIMULATION WITH BAY 41-2272

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Background: Cigarette smoke (CS) exposure induces skeletal muscle atrophy through the activation of the proteolytic, apoptotic, and autophagy systems. Soluble guanylate cyclase (sGC, nitric oxide receptor) plays key roles in muscle metabolism and function.
Objectives: We aimed to evaluate the activation levels of upstream signaling pathways, and the effects of the sGC stimulator BAY 41-2272 on the expression of the signaling pathways in the gastrocnemius and diaphragm of guinea pigs chronically exposed to CS.
Methods: Experimental groups (N = 7/group): 1) Control-sham (vehicle), 2) Control-sham treated with BAY 41-2272 (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 and diaphragm of all guinea pigs, levels of the following signaling pathways: phosphorylated NF-κβ subunits p50 and p65, phosphorylated p38 mitogen-activated protein kinases (MAPK), and phosphorylated Forkhead Box O3 (FOXO3) transcription factor (immunoblotting) were quantified.
Results: In the gastrocnemius of guinea pigs exposed to CS compared to non-exposed controls, levels of phosphorylated p50 increased, while no significant differences were observed in the levels of phosphorylated p65, p38, or FOXO3. In the diaphragm of guinea pigs exposed to CS compared to non-exposed controls, levels of phosphorylated p50 and FOXO3 increased, levels of phosphorylated p38 decreased, and no differences were observed in the levels of phosphorylated p65. Treatment of the animals with BAY 41-2272 elicited a reduction in levels of phosphorylated p50 and FOXO3 and increased levels of phosphorylated p38 in the diaphragm. No significant differences were detected in the limb muscle.
Conclusions: Limb and respiratory muscles of guinea pigs chronically exposed to CS exhibited differences in the activation levels of the upstream markers of the pathways leading to muscle atrophy and the treatment with the sGC stimulator BAY 41-2272 significantly attenuated the activation of those markers in the diaphragm muscle.
Funding: CIBERES, FIS-18/00075 (FEDER), SEPAR-2020.

42. IRON DYSREGULATION AND OXIDATIVE STRESS IN COPD PATIENTS WITH NON-ANEMIC IRON DEFICIENCY

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Background: Non-anemic iron deficiency (NAID) is a worldwide health problem that affects a large part of the population. Up to 40% of COPD patients present NAID, being a common systemic manifestation. We hypothesized that dysregulation of iron metabolism may take place in patients with NAID and that it may be associated with redox imbalance in the muscle and systemic compartments.
Objectives: In the vastus lateralis (VL) and in serum samples of COPD patients with NAID: 1) to explore iron metabolism, 2) to determine levels of oxidative stress, and 3) to examine muscle phenotype.
Methods: Forty patients with stable severe COPD (non-iron deficiency, ID and NAID, n = 20/group) were recruited. In the VL of all patients, ferritin, myoglobin, ferroportin-1, transferrin receptor, IRP-1, and IRP-2 were identified using immunoblotting. Muscle fiber type and morphometry were evaluated using immunohistochemistry. In serum samples of the same patients, 3-nitrotyrosine, malondialdehyde-protein adducts, reduced glutathione, trolox equivalent antioxidant capacity (TEAC), catalase, superoxide dismutase, and iron metabolism biomarkers were assessed using ELISA procedures.
Results: Compared to non-ID patients, in the VL of NAID patients, ferritin levels significantly decreased, while IRP-1 levels significantly

increased. In serum samples of NAID patients, levels of hepcidin and hemojuvelin were significantly lower than in non-ID patients. In the systemic compartment, 3-nitrotyrosine levels were significantly greater in NAID patients than in non-ID patients. Levels of TEAC significantly decreased in NAID patients compared to non-ID patients. Muscle fiber typing or morphometry did not differ between the two study groups.
Conclusions: Systemic iron deficiency in COPD patients has an effect on the regulation of iron metabolism in limb skeletal muscles probably through IRP1-dependent mechanism. ID in non-anemic COPD patients increases systemic oxidative stress and reduces antioxidant capacity. These findings add insight into the pathophysiology of the systemic manifestations in COPD.
Funding: FIS-17/00649, FIS-18/00075 (FEDER), BA-17/00025, CIBERES, SEPAR-409/2017, FUCAP-2017, Vifor Pharma-2018.

43. ENDOTHELIAL PROGENITOR CELLS IN PAEDIATRIC PULMONARY ARTERIAL HYPERTENSION: ROLE OF HYPOXIA AND INFLAMMATION

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Background: Pulmonary arterial hypertension (PAH) is a group of severe and rare diseases which are characterized by a sustained elevation in pulmonary arterial pressure. Paediatric PAH is less studied than the adult forms of PAH but it is estimated that up to 10% of children with congenital heart diseases (CHD) will develop PAH, which can be reversible during the early phases. Endothelial progenitor cells (EPCs) play a major role in vasculogenesis. However, their potential role in the progression and reversibility of pulmonary vascular remodeling in these patients is currently unknown.
Objectives: The aim of this study is to characterize the proliferation and secretion of inflammatory cytokines in EPCs isolated from paediatric patients with or without PH and to evaluate the potential role of hypoxia and innate immune receptors in the dysregulation of these cells.
Methods: EPCs were isolated from blood samples from four patients with PH (PH-EPCs) and four patients without PH (non PH-EPCs). EPCs were exposed to normoxia or hypoxia (3% O2) and treated with TLR agonists (Poly [I:C]-TLR3, LPS-TLR4, ssRNA40-TLR8 and ODN-TLR9) for 72 hours. The proliferative potential of EPCs was determined using the MTT test and IL-6 production was quantified by ELISA.
Results: PH-EPCs displayed a higher IL-6 secretory capacity compared to non PH-EPCs. Under normoxic conditions, PH-EPCs exhibited a higher IL-6 production following stimulation with TLR3, TLR4 and TLR8 agonist as compared to non PH-EPCs. Stimulation with the TLR9 agonist had no significant effects in any tested group. Notably, exposure to hypoxia increased IL-6 production following the stimulation with these agonists in EPCs from normotensive patients whereas reduced IL-6 secretion in PH-EPCs.
Conclusions: Our results demonstrate that EPCs from PH present a higher sensitivity to TLR stimulation and may actively contribute to the proinflammatory milieu intrinsic to the progression of PH.
Funding: PI19/01616; Youth Guarantee of Madrid.

44. STUDY OF A NOVEL HYPOXIA SYSTEM TO EVALUATE ENDOTHELIAL CELL DYSFUNCTION IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH)

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Background: Oxygen (O2) plays a key role in respiratory diseases and hypoxia can be an essential part of the progression of diseases such as CTEPH. The endurance of hypoxic conditions can contribute to a metabolic shift characterized by an abnormal cell proliferation, tissue hypertrophy and remodelling.
Objectives: The aim of the study is to evaluate the effect of chronic hypoxia on endothelial cells (ECs) derived from patients with CTEPH (EC-CTEPH) compared to healthy controls and to identify potential metabolic or angiogenic differences when EC are subjected to different conditions of O2.
Methods: To assess the role of hypoxia in EC dysfunction both patient and control cells were submitted to different O2 conditions (1%, 4%, 13% and 21% O2) for 48h. RT-PCR for angiogenic and metabolic genes and supernatant (SN) analysis were performed using EC-CTEPH (n = 5). HPAE cells were used as control group (n = 5).
Results: Lactate levels in control endothelial cells grown under hypoxia were significantly higher compared to cells grown in normoxia conditions. Likewise, control cells under 1% O2 showed a gene upregulation of HK2 and NIP3 metabolic and hypoxia signaling pathway, respectively. EC-CTEPH cultured under hypoxia did not show a significant increase in lactate levels. However, hypoxia inducible genes such as metabolic genes (HK2, PDK1 and LDHA); hypoxia signaling pathway (HIF-1α, VEGF, NIP3, AKT1 and ENO-α) and related to oxidative stress (NOX4 and SOD2) were upregulated compared to EC-CTEPH grown in normoxia.
Conclusions: Control cells seem to be affected by hypoxia in a different way than EC-CTEPH. At transcriptomic level, EC-CTEPH grown under hypoxia showed a more drastic upregulation of genes related to metabolism, response to hypoxia and oxidative stress, especially when cells were grown at 1% O2. Higher lactate levels in control cells showed that hypoxia also affects the metabolism of these cells.
Funding: Supported by SOCAP, SEPAR, ISCIII (CP17/00114, PI18/00960), FCHP, CIBERES.

45. TIGHT JUNCTIONS AS TARGETS OF SEPSIS AND MECHANICAL VENTILATION IN RAT LUNGS IN VIVO.

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Background: The tight junction (TJ) complexes of the alveolar septal walls regulate lung permeability.

Objectives: To determine whether sepsis and mechanical ventilation (MV) alter the TJ proteins in rat lungs.
Methods: Sepsis by cecal ligation and puncture (CLP) was performed in adult rats (300-350 g b.w.), followed 24 h later by MV for 4 h. Two MV strategies were used: a) low tidal volume (Vt = 9 ml/kg) + PEEP 5 cmH2O or b) high tidal volume (Vt = 25 mk/kg, no PEEP) in rats with or without CLP. A group of sham (laparotomy only) and CLP without MV were used as controls. We measured the levels of occludin and ZO1 (ELISA, inmunofluorescence), cytokines (ELISA) and apoptosis (caspase 3) in lung tissue, bronchoalveolar lavage fluid (BALF) and plasma. Statistical analysis: One-way ANOVA.
Results: Compared with sham, CLP and CLP combined with MV increased IL6 levels in plasma and caspase 3 activity in the lung ($p< 0.05$). Compared with control rats, occludin increased in the lungs of rats with CLP alone or combined with MV-Low Vt but decreased in all rats ventilated with high Vt ($p < 0.05$). MV with high Vt (alone or combined with CLP) caused the major elevation of occludin in BALF and plasma ($p < 0.05$). In contrast, ZO1 levels decreased in rat lungs after CLP, MV or CLP+MV ($p < 0.01$). CLP alone raised the levels of ZO1 in plasma ($p < 0.01$), and in BALF when associated with MV-high Vt ($p = 0.03$).
Conclusions: Sepsis and MV activate pro-apoptotic pathways and alter the expression of TJ proteins in the lung. Compared with sepsis, MV with high Vt is more deleterious on the TJ proteins and raises their levels in the alveolar airspaces and plasma. The release of TJ proteins in BALF and plasma might be used as biomarkers of severity in ARDS patients.
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46. IMPACT OF TIME TO INTUBATION ON MORTALITY AND PULMONARY SEQUELAE IN CRITICALL ILL COVID-19 PATIENTS: A PROSPECTIVE COHORT

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Background: Due to the high rate of admissions to the Intensive Care Unit of COVID-19 critically ill patients, it has been necessary to increase the use of noninvasive intensive respiratory support devices. However, it is unclear whether the delayed initiation of invasive ventilation in these patients may have affected their prognosis.
Objectives: Evaluate if the time between first respiratory support and intubation was associated with mortality or pulmonary sequelae.
Methods: Prospective cohort of critical COVID-19 patients on IMV. Patients were classified as early intubation if they were intubated within the first 48 hours from the first respiratory support, or delayed group if they were intubated later (> 48 hours). Survivors patients were evaluated between the 3rd and 6th months of hospital discharge.
Results: We included 205 patients, (140 as early IMV and 65 as delay IMV). The median [p25;p75] age was 63 [56.0;70.0] years old and 74.1% were male. Survival analysis showed a significant increase in the risk of mortality in the delay group with an adjusted Hazard Ratio (HR) of 2.45 (95%CI 1.29-4.65). Time to IMV as continuous predictor showed a non-linear association with risk of in-hospital mortality. A multivariate mortality model showed delay of IMV as the most important factor for mortality (HR of 2.40; 95%CI 1.42-4.1). In the follow-up, patients in the delay group showed worse DLCO (mean difference of -10.77 (95CI%, -18.40 to -3.15), with a greater number of affected lobes (+ 1.51 [95%CI, 0.89 to 2.13]) and a greater total severity score (+4.35 [95%CI, 2.41 to 6.27]) in the chest CT scan.
Conclusions: Among critically ill patients with COVID-19 who required IMV, the delay in intubation from the first respiratory support is associated with an increase in-hospital mortality and worse pulmonary sequelae.
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47. CIRRHOSIS CHANGES THE PULMONARY RESPONSES TO MECHANICAL VENTILATION IN RATS.

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Background: Liver dysfunction is the major determinant of survival in patients with Acute Respiratory Distress Syndrome (ARDS). The liver-lung axis plays a role in the development of lung injury.
Objectives: To characterize the effects of mechanical ventilation (MV) on the lung pathophysiology and expression of extracellular vesicles (EVs) in rats with cirrhosis.
Methods: Cirrhosis was induced in adult rats by oral gavage with carbon tetrachloride (CCl4) for 12 weeks. Then, the rats were mechanically ventilated for 2.5 h with low or high tidal volume (Vt: 6 ml/kg or 16 ml/kg b.w.) + PEEP 2 cmH_2O and FiO_2 0.3%. Respiratory rate was adjusted for arterial blood pH and $PaCO_2$. Arterial blood pressure, oxygen saturation and peak inspiratory pressure (PIP) were continuously monitored. After euthanasia, we assessed inflammation and apoptosis in the lung. Statistical analysis: one-way ANOVA.
Results: The initial decrease of mean arterial pressure caused by MV with high Vt was not observed in rats with cirrhosis. Compared with normal rats, rats with cirrhosis receiving MV with low Vt exhibited lower PIP ($p< 0.05$), higher PaCO2 ($p = 0.002$), and lower PaO2 ($p = 0.018$) associated with increased alveolar-arterial O2 gradient at the end of the experiment. Compared with normal rats, MV in rats with cirrhosis significantly increased CXCL1 levels in the lung ($p = 0.04$), tended to increase lung permeability, mainly with high Vt ($p = 0.14$),

and raised the levels of EVs in BALF (p = 0.007). Cirrhosis, but not MV, was associated with a higher degree of apoptosis in the lung compared with non-cirrhotic rats.
Conclusions: Cirrhosis influences physiological and molecular responses to MV in rats. MV with a protective strategy could require optimization in the presence of cirrhosis. The apparent increase in EVs in the alveolar airspace of rats with cirrhosis receiving MV supports their potential role in liver-lung communication and in the pathogenesis of ventilator-induced lung injury.
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48. A NOVEL INTELLIGENT RADIOMIC ANALYSIS OF PERFUSION SPECT/CT IMAGES TO OPTIMIZE PULMONARY EMBOLISM DIAGNOSIS IN COVID-19 PATIENTS

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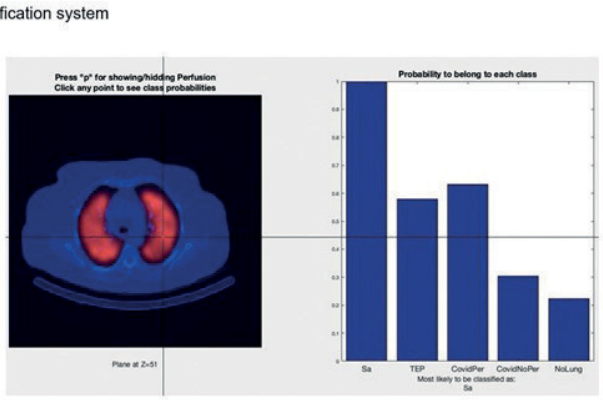
Background: COVID-19 pneumonia is associated with a high rate of pulmonary embolism (PE). In patients with contraindications for CT-pulmonary angiography (CTPA) or non-diagnostic, perfusion single-photon emission computed tomography/computed tomography (Q-SPECT/CT) is the diagnostic alternative.
Objectives: To develop a radiomic diagnostic system to detect PE based on the analysis of Q-SPECT/CT.
Methods: This system is based on a local analysis of SPECT-CT volumes. SPECT-CT is normalized to account for contrast agent concentration using the 0.99 quantile and the minimum of the intensity as, respectively, maximum and minimum values for normalization. We present a hybrid approach that uses radiomics features extracted from each scan as input to a siamese classification network, a fully connected neural network that takes as input the concatenation of the radiomic features and it optimizes a weighted cross-entropy loss trained to discriminate among different types of image patterns: healthy (control group), PE and pneumonia.
Results: The proposed radiomic diagnostic system has been trained on 20 patients (4.518 set of samples of 4 types of image patterns) and validated in a group of 39 patients (4.410 set of samples of 4 types of image patterns), of which 29 were COVID-19 patients. Four types of models have been used and the best of them presented a sensitivity, specificity, positive predictive value, and negative predictive value 75.1%, 98.2%, 88.9% and 95.4%, respectively for PE and 94.1%, 93.6%, 85.2% and 97.6% respectively for pneumonia. The AUC was 0.92 for PE and 0.91 for pneumonia.

Sensitivity, specificity, positive predictive value, and negative predictive value

	Pulmonary embolism	Pneumonia
	M1 3 × 3*	M2 5 × 5*
Sensitivity	75.1%	94.1%
Specificity	98.2%	93.6%
Positive predictive value	88.9%	85.2%
Negative predictive value	95.4%	97.6%

*M1 and M2 correspond to different normalization models. 3 × 3 and 5 × 5 indicate the size of the window used to compute radiomic texture descriptors.



Conclusions: This radiomic diagnostic system has been able to identify different lung imaging patterns and represents a first step towards a comprehensive intelligent radiomic system to optimize diagnosis of PE by Q-SPECT/CT.
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49. STUDY OF CD27, HLA-DR, CD38, AND KI-67 MARKERS ON CD4+ T-CELLS FOR TUBERCULOSIS MANAGEMENT

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Background: Diagnostic tools for tuberculosis (TB) management are insufficient. Tuberculin skin test (TST) has low specificity in patients with Bacille Calmette-Guérin vaccine and/or nontuberculous mycobacteria infection. The interferon-gamma release assays (IGRAs), based on Mycobacterium tuberculosis (Mtb) T-cell responses detection, overcome some of these limitations. However, there is still no standard method to discriminate active TB (aTB) from latent TB infection (LTBI). New biomarkers are therefore needed to improve diagnosis.
Objectives: To investigate CD27, HLA-DR, CD38, and Ki-67 markers on specific T-cells for TB immune diagnosis.
Methods: Blood was collected from 35 patients classified as: (i) 18 pulmonary aTB patients microbiologically confirmed with less than 1 month of treatment; and (ii) 17 LTBI individuals with positive TST and IGRAs. Peripheral blood mononuclear cells were fixed, permeabilized, and stimulated with ESAT-6/CFP-10 or PPD antigens. Samples were acquired in a LSRFortessa cytometer and labelled with: CD3-PerCP, CD4-BV786, CD8-BV510, IFN-γ-APC, TNF-α-PECy7, CD27-BV605, CD38-PE, HLA-DR-BV421, and Ki-67-FITC.

Results: The expression of CD27, CD38, HLA-DR, and Ki-67 markers was analyzed within Mtb-specific CD4+ T-cells, defined as CD4+ lymphocytes producing IFN-γ and/or TNF-α cytokines after ESAT-6/CFP-10 or PPD stimulation. The percentage of HLA-DR+ and Ki-67+ populations within specific CD4+ T-cells was significantly increased in aTB compared to LTBI after both ESAT-6/CFP-10 ($p < 0.001$) and PPD ($p < 0.01$ for HLA-DR+; $p < 0.001$ for Ki-67+) stimulation. Data also points to a significant increase in aTB of CD27- and CD38+ phenotype on Mtb-specific T-cells in response to ESAT-6/CFP-10 ($p< 0.05$). Furthermore, ratio based on the CD27 median fluorescence intensity (MFI) in CD4+ T-cells over CD27 MFI in Mtb-specific CD4+ T-cells was also measured, with a significant increase towards aTB after ESAT-6/CFP-10 stimulation ($p < 0.05$).
Conclusions: CD27, HLA-DR, CD38, and Ki-67 markers on CD4+ specific T-cells are differentially expressed on aTB versus LTBI. Their detection might serve for immune diagnosis and characterization of the disease.
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50. NOVEL METHOD FOR FAST DECELLULARIZATION OF LUNG SLICES

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Background: The perfusion of detergents and/or enzymes through the trachea and the pulmonary artery is the standard protocol to decellularize whole lungs. However, decellularization of the whole lung is not always the desired endpoint: some research questions require the analysis of native as well as decellularized lung samples. An optimal protocol has not yet been established for the decellularization of thin lung slices without detachment from a glass slide.
Objectives: To develop a fast and efficient decellularization method for lung slices that preserve their composition, without detachment from a glass slide.
Methods: Different decellularizing agents were compared and tested for their effectiveness in removing cellular content. The intensity of Hoechst staining was taken as an indicator of the remaining cells. The presence of collagen I, elastin and laminin were also quantified using immunostaining. Scaffolds resulting from the optimized protocol were mechanically characterized using AFM and recellularized with mesenchymal stem cells to assess their biocompatibility.
Results: SDC and SDS were the most effective agents for decellularization (93% and 99% decrease in DNA signal when compared to native tissue). Other decellularization agents (CHAPS, triton and ammonia hydroxide) did not achieve full cellular removal. Even though SDS was effective in cellular removal, its negative effects on matrix proteins were already widely reported, so SDC was chosen as the optimal treatment. SDC treatment was successful in the maintenance of ECM composition. This protocol was successful for porcine, mice and rat lungs. No significant mechanical differences were found before and after decellularization and the resulting lung scaffolds were shown to be biocompatible.
Conclusions: This novel method was successful and improved upon current decellularization options as it has the advantage of not requiring adherence treatments, is faster and is especially suitable for scarce samples, like biopsies.
Funding: Funded by the H2020European Research and Innovation Programme under the MarieSkłodowska-Curie grant agreement"-Phys2BioMed"contract no.812772.

51. INCIPIENT INTERSTITIAL LUNG DISEASE IN THE LUNG CANCER SCREENING COHORT: PREVALENCE, CHARACTERISATION AND PROGRESSION FACTORS (INCIEPID STUDY)

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Background: Interstitial lung abnormalities (ILA) are radiological findings representing a subclinical form of interstitial lung disease.
Objectives: To study the prevalence, characteristics and related progression factors of low-dose computed tomography (LDCT) of the lung cancer screening (LSC) cohort.
Methods: Subjects with radiological findings compatible with ILA and at least 3 years of consecutive follow-up between 2014-2020 in the LCS cohort of the IIS-Fundación Jimenez Diaz.
Results: 32 subjects met established criteria, mainly were male, current smokers, mean age of 64.86 ± 5.3 and a pack-year index of 45. (Table 1). The prevalence of ILA was 2.7%. The main radiological findings were reticulation (82.8%), ground glass (79.3%) and traction bronchiectasis (31%) (Figure and Table 2). 50% of the CT scans showed radiological features of incipient interstitial lung disease/desquamative interstitial pneumonia, followed by unclassifiable idiopathic interstitial pneumonia (18.7%). Functional progression was observed throughout the four years of follow-up with the year-on-year decrease in FVC ≥ 10%, 14.3% of patients and a mild decrease in the CO diffusion test. No statistically significant association was found between functional progression and clinical variables (dyspnea, acropachy or crackles) or body mass index. Patients with functional progression were mostly young females (Table 3). Radiological pro-

Table 1. General Characteristics

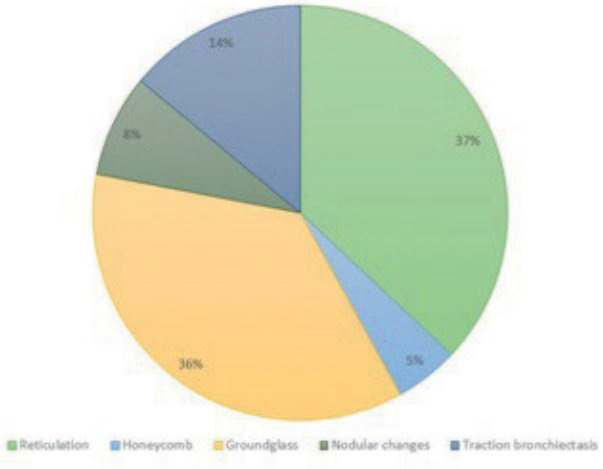
Variables	N = 32
Age	64.86±5.3
Males, n (%)	22 (68.8)
Body mass index, kg/m²	26.36±4.4
Smokers, n (%)	23 (71.9)
Pack-year index*	45 (40-66.5)
COPD, n (%)	14 (43.8)
Pulmonary function test, %	
FEV_1 posbroncodilatation	82.1±15
FVC posbroncodilatation	95.8±12.2
FEV_1/FVC posbroncodilatation*	74 (65.5-79.8)
DLCOsb	78.5±18.8
GOLD, n (%)	
1-2	12 (37.6)
3-4	2 (6.3)
Hospitalizations due to COPD exacerbation, n (%)	3 (9.3)
BODEx Index, n (%)	
0-2 points (mild)	11 (34.4)
3-4 points (moderate)	3 (9.4)
≥ 5 points (severe)	0 (0.0)
Comorbidities associated with PDID, n (%)	
Sleep apnea syndrome	11 (34.4)
Acute coronary syndrome	7 (21.9)
Gastroesophageal reflux disease	7 (21.9)
Arrhythmias	3 (9.4)
Pulmonary hypertension	2 (6.3)

COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in the first second; FVC: forced vital capacity; DLCOsb: diffusing capacity for carbon monoxide with single breathing technique; GOLD: Global Initiative for Chronic Obstructive Lung Disease; BODEx: body-mass, airflow obstruction, dyspnea and exacerbations index; ILD: interstitial lung disease. *Median (interquartile range).

Table 2. Characteristics of pulmonary interstitial abnormalities

Variables	N = 29*
Reticulation degree, (%) †	
Minor	21 (72.4)
Moderate	3 (10.3)
Severe	0 (0.0)
Reticulation lobar distribution, n (%) ‡	
Lower predominance	16 (55.2)
Upper predominance	4 (13.8)
Diffuse	4 (13.8)
Ground glass grade, n (%) †	
Minor	18 (62.0)
Moderate	5 (17.2)
Severe	0 (0.0)
Ground glass lobar distribution, n (%) ‡	
Lower predominance	12 (41.4)
Diffuse	8 (18.8)
Upper predominance	3 (10.3)
Ground glass axial distribution, n (%) ‡	
Peripheral	16 (55.1)
Diffuse	6 (20.6)
Central	1 (3.4)
Traction grade bronchiectasis, n (%) †	
Minor	8 (27.6)
Moderate	1 (3.4)
Severe	0 (0.0)
Bronchiectasis of traction lobar distribution, n (%) ‡	
Lower predominance	9 (31.0)
Upper predominance/diffuse	0 (0.0)
Traction bronchiectasis axial distribution, n (%) ‡	
Peripheral	7 (24.1)
Central	2 (6.9)
Diffuse	0 (0.0)
Honeycomb grade, n (%) †	
Moderate	2 (6.9)
Minor	1 (3.4)
Severe	0 (0.0)
Honeycomb lobar distribution, n (%) ‡	
Lower predominance	3 (10.3)
Upper predominance /diffuse	0 (0.0)
Honeycomb axial distribution, n (%) ‡	
Peripheral	3 (10.3)
Central/ Diffuse	0 (0.0)

Data presented as n (%). *Data from 29 CT scans of 32 (the three remaining could not be analysed as we were unable to access the image of the test performed. †For these variables the category "none" has not been represented. ‡For these variables the category "not applicable" has not been represented.



Distribution of pulmonary interstitial abnormalities

gression was only targeted in three subjects (data limited by data loss during follow-up). The presence of pulmonary hypertension was significantly associated with the presence of honeycombing and traction bronchiectasis in LDCT.

Conclusions: The presence of ILA was found in almost 3% of the LSC cohort. These findings were mainly associated with smoking and emphysema. Functional progression resulting from ILA was more frequent during the second year of follow-up and mainly in young women. Further identification of radiological patterns of subclinical ILD is required to validate them with functional prognostic parameters and early detection.

Table 3

		Year 1			Year 2	
Variables	No PF (N = 9) †	PF (N = 3) †	P ‡	No PF (N = 2) †	PF (N = 4) †	P ‡
Age	63.6 ± 2.34	66.0 ± 1.76	0.435	64.0 ± 6	63.1 ± 3.07	0.888
Sex, n (%)			0.127			1.000
Male	8 (88.9)	1 (11.1)		2 (40.0)	3 (60.0)	
Female	1 (33.3)	2 (66.7)		0 (0.0)	1 (100)	
Smokers, n (%)§	7 (77.8)	2 (22.2)	1.000	1 (20.0)	4 (80.0)	0.333
Pack-year index*	51 (42.5-78.0)	40 (30.0)	0.112	48.8 (45.0)	60.5 (42.8-79.0)	0.814
COPD, n (%)§	4 (80.0)	1 (20.0)	1.000	0 (0.0)	4 (100)	1.000
Respiratory functional tests, %						
FEV1*	86 (70.6-88.9)	100 (96.0)	0.052	74.5 (60.1)	96.4 (60.2-108.8)	0.643
FVC	97.3 ± 6.6	111.5 ± 7.1	0.280	93.2 ± 0.1	97.5 ± 9.2	0.773
FEV1/FVC*	74 (61.0-80.8)	74.6 (67.0)	0.782	63.1 (50.1-	76.3 (58.2-79.5)	0.355
DLCOsb	83.7 ± 5.13	78.1 ± 6.0	0.574	78.1 ± 14.5	71.3 ± 6.4	0.629
GOLD grade, n (%)			0.522			1.000
01-feb	2 (66.7)	1 (33.3)		1 (50.0)	1 (50.0)	
03-abr	2 (100)	0 (0.0)		0 (0.0)	0 (0.0)	
Acute coronary syndrome, n (%)§	3 (100)	0 (0.0)	0.509	1 (20.0)	4 (80.0)	0.333
OSA, n (%)§	2 (100)	0 (0.0)	1.000	0 (0.0)	2 (100)	0.467
GERD, n (%)§	3 (100)	0 (0.0)	0.509	1 (100)	0 (0.0)	0.333
Pulmonary hypertension, n (%)§	1 (100)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	NA
ILD classification, n (%)			0.406			0.553
Incipient DIP	5 (62.5)	3 (31.5)		1 (50.0)	1 (50.0)	
Not classifiable	1 (100)	0 (0.0)		0 (0.0)	1 (100)	
Probable UIP	2 (100)	0 (0.0)		0 (0.0)	1 (100)	
CPFE	1 (100)	0 (0.0)		1 (50.0)	1 (50.0)	
Emphysema index, %*	1.1 (0.1-8.4)	6.9 (4.09-7.38)	0.402	3.75 (1.0)	0.6 (0.1-7.3)	0.814
Emphysema grade, n (%)			0.248			0.282
Mild paraseptal	5 (100)	0 (0.0)		1 (33.3)	2 (66.7)	
Mild centrollobular	2 (66.7)	1 (33.3)		0 (0.0)	1 (100)	
Moderate centrollobular	1 (50.0)	1 (50.0)		1 (100)	0 (0.0)	
Enphysema lobar distribution, n (%)			0.455			1.000
Upper predominance	8 (80.0)	2 (20.0)		2 (40.0)	3 (60.0)	
Axial distribution emphysema, n (%)			*< 0.05*			1.000
Peripheral	5 (100)	0 (0.0)		1 (25.0)	3 (75.0)	
Central	3 (100)	0 (0.0)		1 (50.0)	1 (50.0)	
Reticulation degree, n (%)			0.233			1.000
Minor	5 (62.5)	3 (37.5)		2 (40.0)	3 (60.0)	
Reticulation lobar distribution,n (%)			0.400			1.000
Lower predominance	3 (60.0)	2 (40.0)		1 (25.0)	3 (75.0)	

Data presented as n (%) or mean ± standard deviation, unless otherwise stated. Abbreviations: FP = functional progression; COPD = chronic obstructive pulmonary disease; FEV1 = forced expiratory volume in the first second; FVC = forced vital capacity; DLCOsb = diffusing capacity for carbon monoxide with single breathing technique; GOLD = Global Initiative for Chronic Obstructive Lung Disease; OSA = sleep apnoea-syndrome; GERD = gastro-oesophageal reflux disease; ILD = interstitial lung disease; DIP = desquamative interstitial pneumonia; UIP = usual interstitial pneumonia; CPFE = emphysema-fibrosis syndrome; TBB = traction bronchiectasis; TBB = traction bronchiectasis. *Median (interquartile range). †All 21 patients who met the inclusion criteria in terms of number of functional respiratory tests and CT scans performed were analysed. Data were lost for 9 patients in year 1 and 15 in year 2. ‡p value for comparison of patients with functional progression with those without, calculated with #c^2, Fisher's exact test or likelihood ratio correction for qualitative variables, and with Student's t-test or Mann-Whitney U test for quantitative variables. $p < 0.05$ indicates statistical significance. §For the variables indicated, only the "yes" category of the dichotomous variable is represented. For these variables, the category "not applicable" or "none" is not represented. Neither are those categories not represented in the overall cohort. Categories with lower frequency have been removed.

52. HSA-MIR-320C, HSA-MIR-200C-3P AND HSA-MIR-449C-5P AS MICRORNA BIOMARKERS FOR DETECTING COPD PATIENTS

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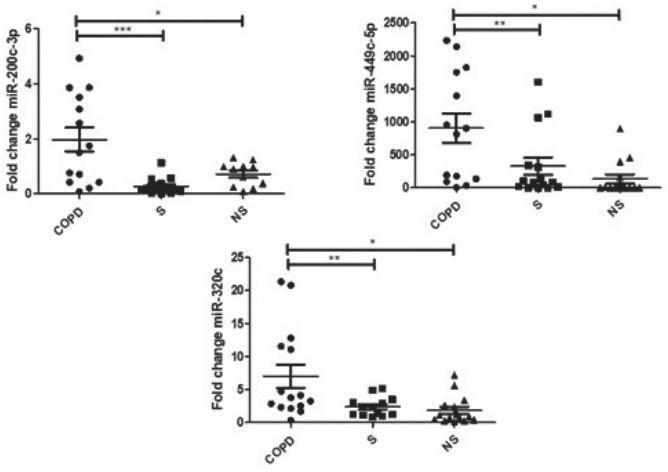
Background: Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death in the world, it is characterized by an abnormal inflammatory response of the lungs caused mainly by tobacco. However, only 20% of smokers develop COPD, suggesting that genetic susceptibility or alterations in the epigenetic machinery may be of importance in the development of COPD. Hypothesis: The expression of miRNAs can be altered in COPD patients, therefore could be a potential biomarker for early detection of this disease.
Objectives: To develop a panel of miRNAs in bronchoalveolar lavage (BAL) that allows the detection and differentiation of COPD patients from smokers and non-smokers with normal lung function.
Methods: 45 subjects (21 men and 24 women) with a mean age of 61.61 ± 12.95 years, BMI 25.72 ± 3.82 kg/m^2, FEV1/FVC 68.37 ± 12.00%, FEV1 80.07 ± 23.63% with clinical indication of bronchoscopy, divided into 3 groups: COPD, smokers, non-smokers. All of them underwent anamnesis, forced spirometry, blood extraction and BAL. MiRNAs (miRNeasy Serum/Plasma Advanced) were extracted from the BAL samples.

Results: The COPD and smoking groups were compared, and it was observed that there was a total of 144 differentially expressed miRNAs: 93 up-regulated and 51 down-regulated. From all, it was observed that miR-449c-5p, hsa-miR-320 and hsa-miR-200c-3p differentiated COPD patients from the rest of the groups.

Data represents mean and SD. COPD: chronic obstructive pulmonary disease

	COPD	Smoker	Never smoker
Group	1 (n = 15)	2 (n = 15)	3 (n = 15)
Age (years)	67.07 ± 12.24	55.66 ± 12,41	62.46 ± 12.38
Gender (M/F%)	53.33/46.66	66.67/33.33†	20/80
BMI (kg/m²)	26.12 ± 4.07	26,10 ± 3,10	24.58 ± 4.57
Pack-years	51.61 ± 42.16†	36.66 ± 27.38†	0,0
FEV1/FVC (%)	57.26 ± 8.78*†	76.9 ± 4.63	76.83 ± 6,36
FEV1 (% Ref)	61.23 ± 18.17*†	94.81 ± 17.10	97,00 ± 10.86



Expression of 3 miRNAs compared between groups. COPD: chronic obstructive pulmonary disease. S: Smoker. NS: Nonsmoker. *p < 0.05, **p < 0.01, ***p < 0.001

Conclusions: MiR-449-5p, miR-320c and miR-200c-3p can be considered as potential BAL biomarkers to distinguish COPD from smoking and never smoking patients.

Funding: This study was funded by grants from IdISBa (Pilot projet 2017) and SEPAR (1052-2020 and 056/2015).

53. MIRNA DELIVERY THROUGH CALCIUM CARBONATES NANOPARTICLES

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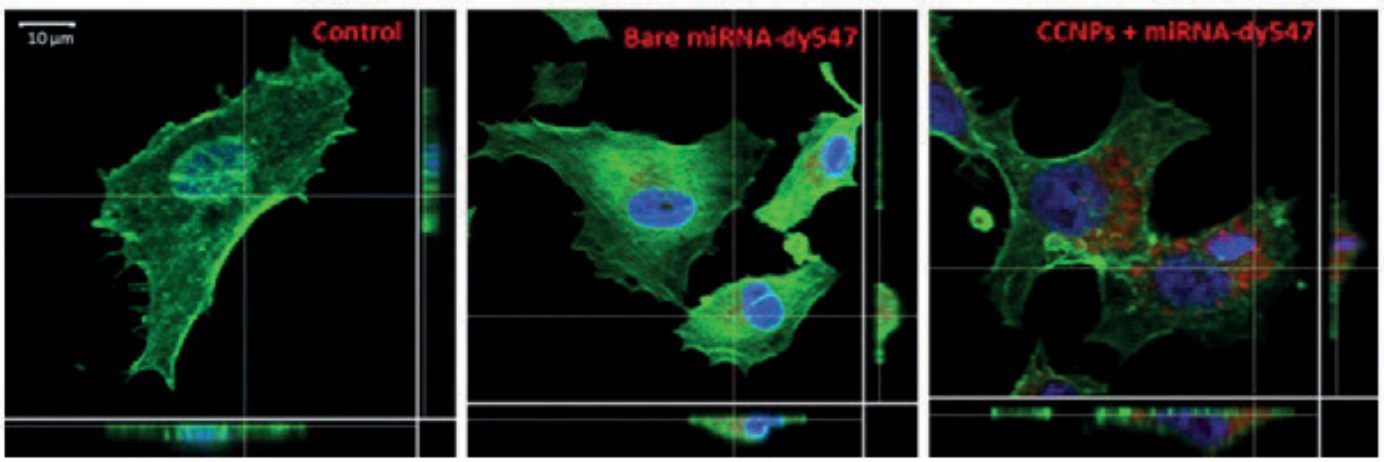
Background: Idiopathic pulmonary fibrosis (IPF) have treatments that slow the disease progression, but they do not reverse it. MicroRNAs (miRNAs) have been studied recently as novel therapeutic agents for IFP due to their central role in gene expression. However, the efficacy of these small RNAs is limited since bare miRNAs have poor cellular uptake and targeting ability, short circulation time, and off-target effects. Nanoparticles have been proposed to overcome the main issues of miRNAs delivery.
Objectives: This study aims to develop robust, cost-efficient, and biodegradable calcium carbonate-based nanoparticles for the efficient transfection of miRNAs to improve current therapies against IPF.
Methods: Amorphous calcium carbonate nanoparticles (CCNPs) have been used to encapsulate a fluorescent miRNA (miRNA-dy547). CCNPs are synthesized with a mixture of calcium carbonate and polymers while the miRNA is entrapped within the CCNPs using a post-loading methodology. CCNPs have been characterized using fluorescence spectroscopy, DLS and XPS. The cellular uptake of CCNPs+miR-dy547 has been studied at 3 hours (h) and 24h with confocal microscopy using EA.hy926 cells.
Results: Our initial results with XPS show phosphorus, carbonyl and amino groups (Table) in CCNPs+miRNA-dy547 indicating the presence of nucleotides. Regarding the in vitro studies, the results reveal more miRNA-dy547 in the cells incubated with CCNPs+miRNA-dy547 than cells incubated with bare miRNA-dy547 (Figure).

Atomic concentration table of the XPS measurement

	C [%]	Ca [%]	Gd [%]	N [%]	Na [%]	P[%]	C = O ev [%]	NH+ ev [%]
CCNPs	72,2	1,7	0,2	0,7	1,0	-	-	-
CCNPs + miRNA-Dy547	64,5	2,3	1,4	6,1	2,0	2,0	290,6 eV [5,0]	402,1 eV [75]

Conclusions: We demonstrate that CCNPs can encapsulate the miRNA-dy547, which improves the miRNA-dy547 cellular uptake compared to bare miRNA-dy547. Therefore, these cost-efficient biocom-



An orthogonal view from different planes (x/y, x/z or y/z) of CLSM images shows the co-localization of miRNA (red) within the cytoskeleton (green) of endothelial EA-hy926 cells.

patible nanoparticles could be a potential tool to deliver therapeutic miRNAs.
Funding: This work was supported by grants from the Ministerio de Economía, Industria y Competitividad (SAF2017-84494-C2-R) and from La Caixa Foundation (Health Research Call 2020: HR20-00075). 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.

54. SMOKE-FREE HOME REGULATION IN 12 EUROPEAN COUNTRIES: TACKSHS SURVEY

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Background: While smoking and exposure to second-hand tobacco smoke (SHS) in most public places is addressed by smoke-free regulations, the exposure to SHS at private places, such as homes, is still prevalent and represents a significant public health challenge. The smoking rule voluntarily adopted in a household has an important impact on exposure of bystanders at SHS at home.
Objectives: To evaluate prevalence and correlates of smoke-free home regulations in 12 European countries; additionally, to explore whether reported exposure to SHS at homes by non-smokers varied by type of the rule implemented.
Methods: A cross-sectional survey in 12 European countries was conducted within the TackSHS Project (www.tackshs.eu), in 2016-2018. Approximately 1,000 participants representative of the general population aged ≥ 15 years were interviewed face-to-face in each country. The survey included questions regarding smoking rule and SHS exposure at home. The frequencies and 95% confidence intervals (95%CI) as well as prevalence ratios were calculated.
Results: Among 12 countries, the prevalence of homes with a total smoking ban was 70.2% (95%CI: 68.6-71.8) and 17.5% (16.7-18.3) of homes had a partial ban. The proportion of smoke-free homes varied by countries and ranged from 44.4% (43.1-45.8) in Greece to 84.5% (82.7-86.3) in England. Among respondents living with children 72.1% (70.7-73.6) reported having adopted smoke-free rule and 46.8% (45.2-48.4) of families with at least one smoker. Among non-smokers, the prevalence of SHS exposure at home was 13.1% (12.4-13.8); it was 5.0% (4.6-5.4) among those who adopted a complete smoking ban; 45.1% (43.8-46.4) with partial and 56.8% (55.3-58.3) no ban.
Conclusions: The prevalence of smoke-free homes is high, and there is a high variability in its prevalence among the countries studied. SHS exposure at home is of concern, particularly in households that don't have any smoking rule.
Funding: TackSHS Project was funded by European Union's H2020 Program (Grant N.681040). OT is a CIBERES predoctoral reseacher.

55. IDENTIFICATION OF HIGHLY IMMUNOGENIC EPITOPES IN THE SARS-COV-2 SPIKE PROTEIN TO PRODUCE NEUTRALIZING ANTIBODIES FOR TREATMENT

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Background: In this project we screened 27 predicted SARS-CoV-2 Spike epitopes against convalescent serum samples from 509 COVID-19 patients to identify those epitopes with the highest IgG reactivity.
Objectives: The final goal to produce monoclonal antibodies for COVID-19 treatment.
Methods: A computational pipeline based in predictive models was designed to predict the SARS-CoV-2 Spike viral protein epitopes most likely to be recognised by neutralizing antibodies from COVID-19 convalescent patients. This pipeline included linear and structural epitope predictions, considering several variables that influence their binding to antibodies: glycosylation status, localization in the viral membrane and accessibility in the 3D conformation of their parental protein. COVID-19 patients were prospectively recruited after 30-40 days post symptom initiation, positive PCR or hospital discharge. Lateral flow immunoassays to detect IgG to SARS-CoV-2 were performed to all study participants. Epitope mapping with predicted epitopes was performed through Luminex technology (Luminex Corp. EEUU) to select those epitopes with highest IgG reactivity in the convalescent serum samples. The seropositivity cut-offs for each peptide were calculated using a set of negative controls. Generally, and especially with home-made ELISAs, cut-off values are estimated using known independent negative sera.
Results: Twenty-seven epitopes of SARS-CoV-2 Spike protein were predicted by our pipeline and included in the Luminex panel. 126 pre-pandemia were included in the epitope mapping to perform cutt-offs. Two epitopes exhibited significantly higher MFIs compared to the rest of epitopes. The percentages of patients above the seropositivity cut-offs based on MFI of NC were between 46-48%.
Conclusions: The availability of monoclonal antibodies against SARS-CoV-2 is a potentially treatment strategy for the management of COVID-19 moderate and severe cases. The two epitopes with highest IgG reacitvities detected in this study are good candidates for the generation of new monoclonal antibodies.
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