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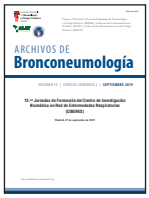

Abstracts

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

Madrid, 27 de septiembre de 2019

A NOVEL GENETIC ASSOCIATION AT 2P21 LOCUS FOR CHILDHOOD ASTHMA WITH SEVERE EXACERBATIONS IN ADMIXED POPULATIONS

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Introduction: Severe asthma exacerbations threaten the patient's life and constitute a major economic burden to healthcare systems. Although African ancestry being associated with a higher risk of asthma and asthma exacerbations, to date, no study has assessed the role of genetic variation for this phenotype in African-admixed populations.
Objectives: To identify genetic variants associated with severe asthma exacerbations in admixed populations.
Methods: Hispanic/Latino children and young adults with asthma from the GALA II study that had exacerbations in the last 12 months (defined as oral corticosteroids use, seek of emergency care or hospitalizations) were compared with non-asthmatic controls (1,283 cases and 2,027 controls). Logistic regression models were used to test the association between 9.7 million genetic variants with the presence of asthma with exacerbations, including as covariates age, sex, and principal components. Variants suggestively associated ($p \leq 5x10-6$) were examined for replication in African American from the SAGE study (448 cases and 595 controls). Variants showing evidence of replication were meta-analyzed with METASOFT, and in silico assessment of functional effects was assessed.
Results: A total of 171 variants were suggestively associated with asthma exacerbations in Hispanic/Latinos (lowest p = 6.8 x 10-8). From these, 41 replicated in SAGE, including previously reported variants at 17q12-q21 and novel associations at 2p21, 6q23.2, and 11q21. In the meta-analysis, several polymorphisms reached genome-wide significance at chromosome 17 (lowest p = 3.8 x 10-8), as well as one novel locus located at 2p21 (OR = 1.32, p = 3.8 x 10-8). The effect allele of this polymorphism was found to increase the expression of the uncharacterized LOC388942 in the lung.
Conclusions: We revealed a novel genetic association for asthma with severe exacerbations in Hispanic/Latino and African American children and young adults.
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A NOVEL INDIVIDUALIZED NON-INVASIVE TOOL TO IDENTIFY COMPLEX PATIENT-VENTILATOR INTERACTION DURING MECHANICAL VENTILATION IN CRITICALLY ILL PATIENTS

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Introduction: Asynchronies between the patient and the ventilator during mechanical ventilation may induce several deleterious effects that should be monitored and carefully managed
Objectives: To characterize and validate a novel individualize, non-invasive tool based on Entropy (E) analysis to detect Complex Patient-Ventilator Interactions (CP-VI). To study the distribution and clinical meaning of CP-VI in a cohort of 87 patients (27 self-extubated (Self-Ext) and 60 ready to wean pre spontaneous-breathing trial (pre-SBT)).
Methods: Paw and Flow signals were recorded using a dedicated software (Better Care). E was used to detected CP-VI upon derived values from Flow and Paw. Validation: Three experts reviewed Flow and Paw signals (gold standard) from a data-set of 92 segments of 15-minute, classifying if CP-VI were present or not. E detected CP-VI over tracings of Flow (E-Flow) and Paw (E-Paw) used before. Validaton (70% optimization / 30% testing) with 15 iterations of randomization for each part was done. Expert analysis and E detection were compared. Cohort analysis: 87 different patients were analysed o a data-set of 696

15-min segments. Data were transferred to MATLAB for processing and analysis.
Results: Experts identify 46 positive (22 in PSV, 24 in Assist-Control mode) and 46 negatives (23 in PSV and Assist-Control mode). Maximum change at a threshold of 25% respect to baseline in E-Flow (E-FlowMax25) was the most accurate combination to detect CP-VI (Sensitivity: 0.93, Specificity: 0.92, Accuracy: 0.92) irrespective the ventilatory mode (PSV: Sens: 1, Sp: 0.87, Acc: 0.93, Assist-Control modes: Sens: 0.83, Sp: 0.96, Acc: 0.89). In Self-Ext and pre-SBT, those who had more CP-VI were prone to avoid re-intubation, and prone to a successful SBT trial and extubation.

CP-VI events (15-min duration)	Pre-SBT cohort (n = 60)		Self-Ext cohort (n = 27)	
	SBT Failure n (%)	SBT Success n (%)	No Re-Int n (%)	Re-Int n (%)
0	3 (60)	2 (44)	3 (100)	0 (0)
1-2	9 (45)	11 (55)	5 (55.5)	4 (44.4)
≥ 3	8 (22.9)	27 (77.1)	9 (60)	6 (40)

Conclusions: Individualized, non-invasive analysis of Entropy can be used to detect CP-VI during mechanical ventilation. The greater the time spend with CP-VI in the two studied cohorts were associated with no need of MV.

ACUTE EXACERBATION OF BRONCHIECTASIS AND THE IMPACT ON THE DURATION OF ANTIBIOTIC THERAPY

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Introduction: The clinical history is important before starting an antibiotic treatment to be able to predict the optimal duration of the therapy.
Objectives: We aimed to describe the clinical, analytical and microbiological characteristics of exacerbated patients with non-cystic fibrosis bronchiectasis (BE), in order to evaluate their association with the duration of antibiotic therapy.
Methods: We performed a prospective bicentric study including adult patients with BE exacerbation divided into 2 groups according to the duration of antibiotic therapy (Group 1: less than or equal to 14 days, Group 2: from 15 to 21 days). A logistic regression analysis was performed to predict the long-term antibiotic treatment.
Results: 191 patients were included (72 [63;79] years); 108 (56.5%) females, 132 (69%) Group 1, 59 (31%) Group 2. Cylindrical and cystic BQ (p = 0.002), previous colonization of Pseudomonas aeruginosa (PA) (p = 0.003), long-term oxygen therapy (LTOT) (p = 0.001) and severe FACED scale (p = 0.029) were associated with a long-term duration antibiotic therapy. PA (non-mucoid) was the most frequent microbiological isolation in group 2 (p = 0.01) and Haemophilus influenzae in group 1 (p = 0.03). In the multivariable logistic regression model; LTOT, intravenous and sequential intravenous to oral route of administration, and number of initial antibiotics ≥ 3 were identified for predicting the long-term duration of antibiotic therapy [AUC 0.74 (95% CI: 0.66-0.81)].
Conclusions: Some clinical findings could be useful for predicting the long-term duration of antibiotic therapy during an exacerbation of BE.

UNIVARIATE AND MULTIVARIATE LOGISTIC REGRESSION ANALYSIS FOR PREDICTING THE LONG –TERM DURATION OF ANTIBIOTIC THERAPY

VARIABLE	UNIVARIATE			MULTIVARIATE		
	OR	IC 95%	*p-value*	OR	IC 95%	*p-value*
Previous colonization by PA	2.59	1.37-4.87	0.003	-	-	-
LTOT	3.75	1.6—8.78	0.002	3.43	1.35-8.69	0.009
Previous hospitalization due to BE exacerbation in the last year	2.02	1.08-3.79	0.029	-	-	-
Severe FACED	2.93	1.30-6.58	0.009	-	-	-
Route of administration			0.001			0.008
Oral	1	-	-	1	-	-
Intravenous	9.31	2.89-30	<0.001	7.68	2.01-29.4	0.003
Sequential intravenous to oral	5.86	1.94-17.72	0.002	6.72	1.90-23.73	0.003
Number of initial antibiotics ≥ 3	5.29	2.10-13.32	<0.001	3.89	1.45-10.46	0.007

AFRICAN AMERICAN CHILDREN AND YOUNG ADULTS SHOW DIFFERENT BACTERIAL COMPOSITION IN SALIVA BY ASTHMA STATUS

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Introduction: Asthma is a common and chronic disease that affects the lower airways. Among all the ethnicities, those with an African origin, such as African Americans, have been shown to be more susceptible to develop asthma disease, which is in agreement with the association between African genetic ancestry and asthma susceptibility. Aside from genetic predisposition, several studies have evidenced that respiratory microbiota plays an important role in disease susceptibility. The airways of asthma patients have been shown to contain higher diversity of bacteria, as well as being enriched in pathogenic species. Since asthma is one of the most prevalent diseases in childhood and sampling the airways in children is challenging, the study of non-invasive samples is needed.
Objectives: We aimed to identify differences in the salivary bacterial composition between African Americans children with and without asthma.
Methods: Saliva samples from 57 asthma cases and 57 healthy controls were analyzed by means of 16S rRNA sequencing. Measurements of bacterial diversity and genus relative abundance were compared between cases and controls using the non-parametric Wilcoxon test and multivariate regression models.
Results: A total of five phyla and a mean of 56 genera were identified. Among them, 15 genera had a relative abundance greater than 1%, being Prevotella, Haemophilus, Streptococcus, and Veillonella the most abundant genera. Differences between cases and controls were found in terms of diversity, as well as in relative abundance for Strep-

tococcus genus (13.0% in cases vs 18.3% in controls, p = 0.003) and Veillonella genus (11.1% in cases vs 8.0% in controls, p = 0.002). These genera remained significantly associated with asthma after correction for multiple comparisons and when potential confounders were considered.
Conclusions: We identified changes in the salivary microbiota associated with asthma among African Americans.
Funding: Supported by SAF2017-83417R by the Spanish Ministry of Economy, Industry, and Competitiveness (MINECO/AEI/FEDER, UE).

ANALYSIS OF MSR1 AS A BIOMARKER OF DIFFERENT RESPIRATORY DISEASES

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Introduction: MSR1 (macrophage scavenger receptor 1) has been described essentially in cells of different tissues (muscle, pulmonary and neuronal) (Kelley et al, 2014); nevertheless we have found a significant increase of its gene expression in PBMCs (peripheral blood mononuclear cells) in patients with severe nonallergic asthma when compared with healthy subjects (Baos et al, 2017).
Objectives: Define the cellular origin of MSR1 and analyze its importance as a possible biomarker of asthma and COPD and the role of methylation in its regulation.
Methods: Four groups of subjects were included in this study: healthy controls (n = 11), nonallergic asthmatic (n = 11), allergic asthmatic (n = 13) and COPD patients (n = 11). The expression of MSR1 on T cells (CD4+ and CD8+), B cells and monocytes was analyzed through confocal microscopy and flow cytometry, in whole peripheral blood and PBMCs samples. RNA was extracted from PBMCs to analyze the gene expression of MSR1 by RT-qPCR and the DNA, also from PBMCs, for the methylation analysis accomplished through the Sequenom EpiTYPER method. The statistical analyses were done with the unpaired T student test using the Graph-Pad InStat 3 program.
Results: MSR1 is present on the surface of the 4 cellular subpopulations analyzed. The total expression of MSR1 was relevant in all of the groups of subjects, in whole blood and PBMCs samples, with a greater presence in lymphocytes B and monocytes, but significant differences were only found in PBMCs. There were also differences in the gene expression between the 4 clinical groups. Methylation of the CpG islands analyzed will be discussed as possible regulatory targets of the expression of MSR1 in patients with these diseases.
Conclusions: The presence of MSR1 has been demonstrated in the cellular populations of peripheral blood samples, showing differences in its expression between the clinical groups. Methylation could be a possible regulatory mechanism of MSR1 in these pathologies.

ANALYSIS OF THE NASAL MICROBIOME IN LONG-TERM SURVIVORS AFTER LUNG TRANSPLANTATION WITH A GOOD ALLOGRAFT FUNCTION

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Introduction: Long-term survival after lung transplantation (LT) is limited by several complications, with infections being one of the main ones. Infections directly increase mortality and also stimulate the development of chronic lung allograft dysfunction (CLAD). Despite this fact, there is a small number of LT patients which are long-term survivors with good allograft function (LTS).
Objectives: To analyze the nasal bacterial microbiome in LTS and CLAD patients to determine if both groups of patients have a different microbiome in the upper respiratory tract.
Methods: Fifty one double lung transplant recipients were included in this multicenter cross-sectional study: 25 patients with CLAD and 26 patients with a stable allograft function 10 years after LT. A nasal swab was obtained from all of them and the bacterial DNA was extracted to prepare an amplicon library of the V4 16S rRNA gene by PCR to ultimately be sequenced. The bioinformatics and statistical analyses were performed using the pipeline QIIME 1.9.1 and the R 3.4.3 program.
Results: In figure 1A Alfa diversity Chao 1 index showed not significant differences between CLAD (left) and LTS (right) patients. Figure 1B represents a principal component analysis of beta diversity analyzed through "Weighted Unifrac" index. Both groups of patients present significant differences (p-value = 0.002) in bacterial composition and abundance. The figure shows two separated clusters (blue points = LTS patients, red point = CLAD patients). Regarding relative abundances, CLAD patients (left) show a significant increase of the Firmicutes phylum (green bars, FDR = 0.014) while LTS patients (right) have the Actinobacteria phylum increased (brown bars, FDR = 0.014). Each one of the columns represents a patient (figure 2).
Conclusions: The nasal microbiome in LTS patients is different from the CLAD patients' microbiome, being the LTS enriched with Actinobacteria and the CLAD with Firmicutes.

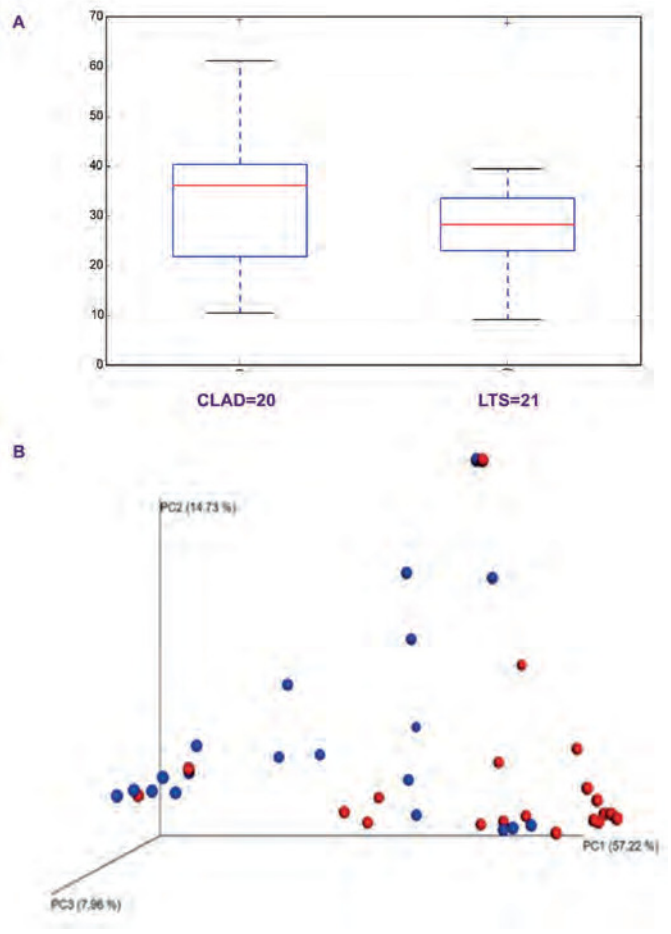

Figure 1.

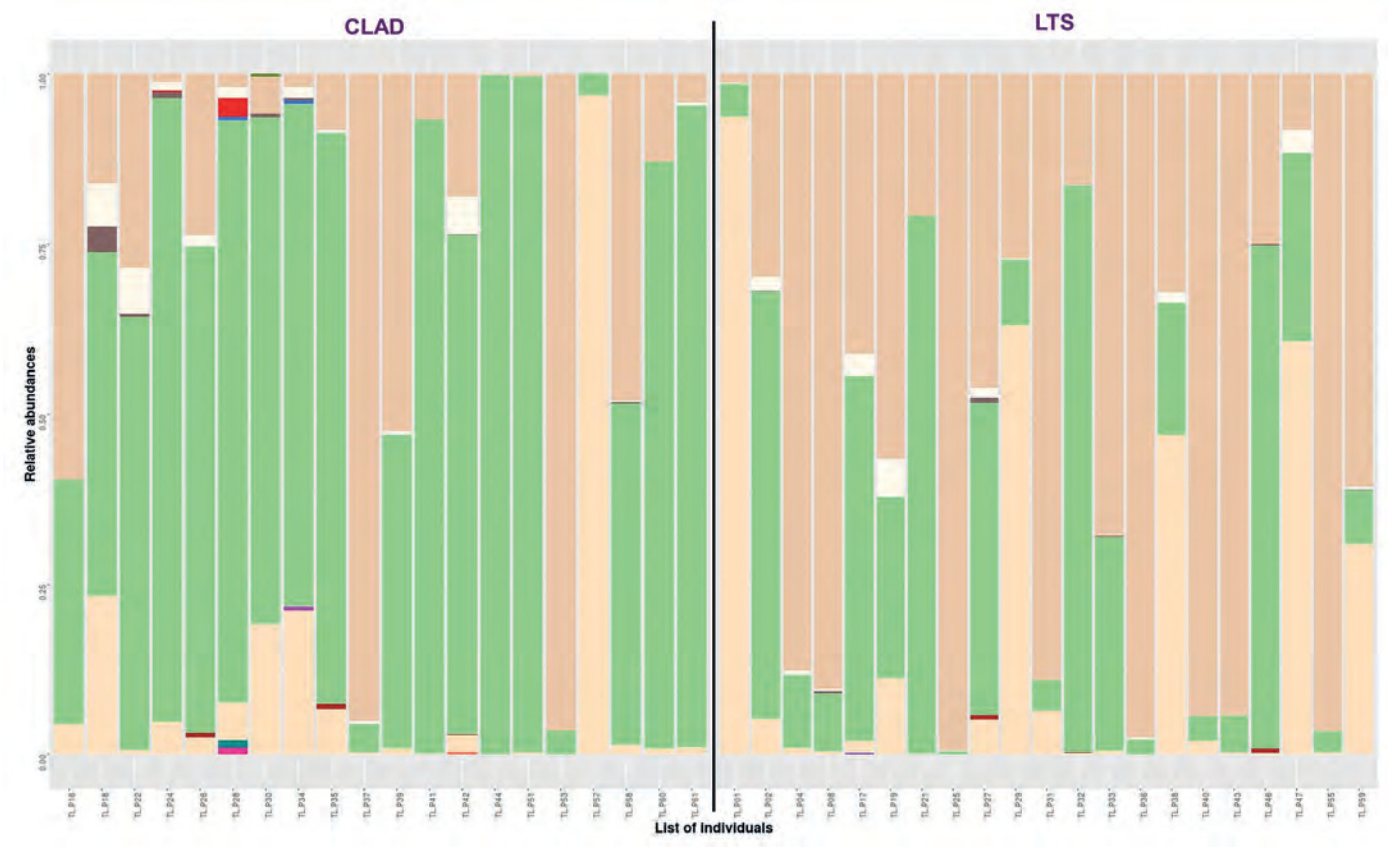

Figure 2. Results.

ANTIMICROBIAL SUSCEPTIBILITY AND MOLECULAR MECHANISMS OF RESISTANCE IN *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM PATIENTS WITH BRONCHIECTASIS

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Introduction: Non-cystic fibrosis bronchiectasis (BE) is a chronic structural lung condition that facilitates chronic colonization by differents microorganisms and courses with frequent exacerbations and recurrent infections. One of the main pathogens involved in chronic colonization and acute exacerbations is *Pseudomonas aeruginosa.* When not early eradicated during infection *P. aeruginosa* can accumulate high rates of resistance to the most antipseudomonal agents.
Objectives: To determine the antimicrobial susceptibility and the molecular mechanisms of resistance involved in *P. aeruginosa* strains isolated from patients with BE.
Methods: A prospective observational study was carried out in Hospital Clinic. A total of 44 strains of *P. aeruginosa* were isolated and characterized from sputum of BE patients. The antimicrobial susceptibility to: Aztreonam, ciprofloxacin, meropenem, imipenem, amikacin, tobramycin, pipertaz, ceftazidime and colistin was performed using the Kirby-Bauer method with the ATCC 27853 strain as a control. Interpretation of results was carried out according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Molecular characterization of each resistance mechanism was screened by PCR, electrophoresis in 2% agarose gels and sequencing.
Results: The frequency of *P. aeruginosa* resistant isolates was: Aztreonam (100%), ciprofloxacin (45.5%), meropenem (31.81%), imipenem (31.81%), amikacin (20.45%), tobramycin (20.45%), pipertaz (11.36%), ceftazidime (11.36%) and colistin (2.27%). The strains showed different antimicrobial profiles: MR (65.9%), MDR (25.1%) and XDR (9%). Mutations in gyrA, gyrB, ParC and ParE genes were found in ciprofloxacin resistant P. aeruginosa strains. The most frequent mutation in gyrA was A33G, in gyrB S466F, in parC S87W and in parE D539E. The presence of Beta-lactamase OXA50 was detected in all the strains. The aac(3)-Ia, aac(3)-Ic, aac(6'')-Ib and ant(2'')-Ia genes were related to aminoglycoside resistance.
Conclusions: The high level of resistance to first-line antimicrobial recommended in BE guidelines threatens the treatment of BE and the eradication of *P. aeruginosa.*

ASTHMA AND SEVERE OBESITY: GLUCOCORTICOID SENSITIVITY BEFORE AND AFTER BARIATRIC SURGERY

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Introduction: Asthma-obesity phenotype is characterized by more frequent exacerbations, poor control, and decreased response to glucocorticoids (GC). We hypothesized that alterations in anti-inflammatory pathways involving GC receptor activation and anti-inflammatory gene expression could be the reason for the poor response to GC treatment and that this condition could be reversed by bariatric surgery (BS).

Objectives: To compare the functional characteristics, response to GC and the role of vitamin D in this response among obese asthmatic patients (OA), with obese without asthma (O), non-obese asthmatics (A) and healthy subjects (H) before and after BS.
Methods: Severe obese (body mass index [BMI] ≥ 35 kg/m²) patients with asthma (OA) (n = 25) and without asthma (O) (n = 15) were evaluated and compared with non-obese asthma patients (A) (BMI < 30 kg/m²) (n = 15) and healthy subjects (H) (n = 19). GC sensitivity was determined in vitro through peripheral blood mononuclear cells (PBMCs) proliferation assay with dexamethasone (from 10-11 to 10-5M) and/or with vitamin D 10-7M. Forced spirometry was performed in all groups.
Results: The OA group had a mean age of 56 ± [SD] 7 years, BMI 40 ± 5 kg/m² and FEV1 80 ± 18%; the O group a mean age of 51 ± 8 years, BMI 45 ± 8 kg/m² and FEV1 92 ± 12%. The A group a mean age of 49 ± 15 years, BMI 24 ± 2 kg/m² and FEV1 91 ± 8% and the H group 40 ± 11 years, BMI 24 ± 2 kg/m² and FEV1 99 ± 11%. PBMCs from OA group showed a trend to a reduced GC sensitivity compared with healthy subjects and they improved their cellular response 6 months after BS. Dexamethasone IC50 value was significantly reduced in (OA) patients when vitamin D was added to the in vitro treatment ($p \leq 0.05$).
Conclusions: PBMCs proliferation was suppressed by dexamethasone. In obese patients with resistance to GC, PBMCs response to dexamethasone treatment was enhanced after BS. Vitamin D plays a relevant role in GC sensitivity in obese asthmatic patients.

BIOMARKERS TO PREDICT FEV1 DECLINE IN HEALTHY SMOKERS AND EARLY-ONSET COPD

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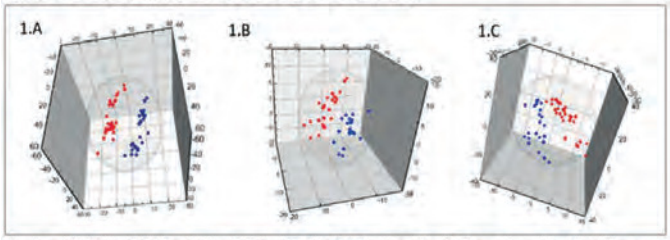
Introduction: Forced expiratory volume in 1 second (FEV1) is the standard clinical marker of chronic obstructive pulmonary disease (COPD), but biomarkers (BM) capable of predicting the onset or progression of disease are needed.
Objectives: Our study was designed to identify BM of clinical utility in the diagnosis and prediction possibilities in FEV1 decliners.
Methods: We conducted a metabolomic analysis in individuals from the CHAIN cohort (COPD History Assessment In SpaiN). For this purpose, blood and post-bronchodilator FEV1 were recorded. Two groups of comparisons were carried out in mild COPD (mCOPD) (n = 31) and smokers without COPD as control (C) (n = 31). Subjects characteristics are shown in table 1. The test by gas chromatography with a mass detector (GC-MS) and flow injection analysis (FIA) was performed. Them, the samples were evaluated in two FIA modes of acquisition (positive and negative ionization). The results were processed to construct the partial least squares analysis (PLS-DA) diagrams and compare the metabolomic profiles obtained. The altered metabolites were selected according to the variable importance in the projection parameter (VIP), considering a VIP > 1 indicative of significant differences between groups.
Results: The analysis with PLS-DA notably differentiates mCOPD and C (Fig. 1A, 1B, and 1C). We found 39 metabolites altered in both groups. Oleic acid, palmitic acid, urea, inositol, and glucose had the best area under the curve (AUC) values in the group with accelerated FEV1 decline defined as a loss ≥ 100 mL in the first two years (Table 2). Additionally, we observed a decrease in fatty acids and amino acids (phenylalanine, leucine, pyroglutamate, proline, threonine, and valine) in contrast to the increase in phosphocholine (Fig. 2A and 2B).

Table 1. General characteristic of study subject

	FEV_1 ≥ 100 mL per year^		FEV_1 < 100 mL per year^	
	mCOPD*	Control**	mCOPD*	Control**
N	12	16	19	15
Age, year	65.13 ± 8.0	59.13 ± 10.5	65.30 ± 8.3	59.26 ± 10.6
Male, n (%)	8 (66)	10 (63)	12 (63)	9 (60)
BMI (kg/m²), median	26.30 ± 4.4	27.76 ± 5.3	26.35 ± 4.3	28.25 ± 5.5
Smoking Status: never, n	0	0	0	0
Smoking Status: current, n	12	16	19	15
Pack year, median	41.05 ± 19.9	44.62 ± 27.1	40.52 ± 19.8	43.05 ± 27.3
FEV_1, mL, median	2208 ± 608	2662 ± 749	2180 ± 560	2663 ± 745
% predicted FEV1, median	89.72 ± 6.7	93.42 ± 13.8	90.03 ± 6.3	93.11 ± 13.5

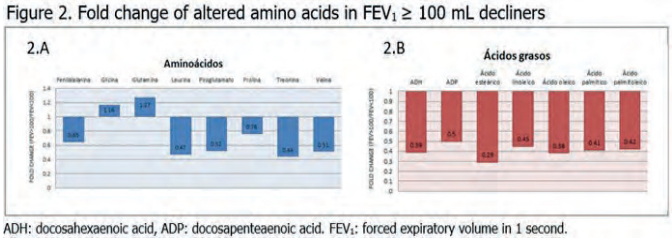
Mean, and SD (±) provided unless otherwise noted.
*COPD defined based on post-bronchodilator spirometry by FEV_1/FVC < 0.7. COPD severity subclass definition: mild% predicted FEV_1 > 80.
** Control defined as a smoker with a post-bronchodilator spirometry FEV_1/FVC ≥ 0.7.
BMI: body mass index; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; ^: decline of 100 mL year in the first two years of follow-up.

Figure 1. PLS-DA comparison samples with FEV_1 ≥ 100 mL vs FEV_1 < 100 mL decliners analyzed by the three methods used (gas chromatography with a mass detector; flow injection analysis with positive and negative ionization)



Samples with FEV_1≥ 100 (blue dots) and FEV_1 <100 (red dots) analyzed by: (1.A) gas chromatography with a mass detector (GC-MS); (1.B) flow injection analysis with positive ionization (FIA-ESI(+)-QTOF-MS) and 1.C: flow injection analysis with negative ionization (FIA-ESI(-)-QTOF-MS). FEV_1: forced expiratory volume in 1 second. PLS-DA: partial least squares analysis

Figure 2. Fold change of altered amino acids in FEV_1 ≥ 100 mL decliners



ADH: docosahexaenoic acid, ADP: docosapenteaenoic acid. FEV_1: forced expiratory volume in 1 second.

Table 2. Altered metabolites with best AUC value analyzed by gas chromatography with a mass detector and flow injection analysis with positive and negative ionization

Metabolites	VIP	FC	p	AUC	Methodology	Family
Docosahexaenoic acid	1.24	0.39	0.001	0.71	FIA (-) -QTOF-MS	Fatty acid
Stearic acid	1.83	0.41	0.006	0.73	GC-MS	Fatty acid
Oleic acid	1.93	0.38	0.02	0.73	FIA (-) -QTOF-MS	Fatty acid
Palmitic acid	2.06	0.41	0.04	0.70	FIA (-) -QTOF-MS	Fatty acid
Uric acid	3.13	0.45	0.02	0.72	GC-MS	Organic acid
Glucose	1.21	1.46	0.004	0.71	FIA (+) -QTOF-MS	Sugar
Urea	2.07	0.43	0.01	0.71	GC-MS	Organic compound
Inosine	1.77	0.58	0.002	0.76	FIA (-) -QTOF-MS	Nucleosides

AUC: area under the ROC (Receiver Operator Characteristics) curve; FIA(+)-QTOF-MS: flow injection analysis with positive ionization; FIA(-)-QTOF-MS: flow injection analysis with negative ionization; FC: Fold change; p: p-value in bivariate analysis ANOVA with fisher correction; GC-MS: gas chromatography with a mass detector (GC-MS); VIP: variable importance in the projection.

Conclusions: Healthy smokers can experience accelerated declines in lung function similar to those seen in mCOPD. Metabolomic analysis identified six metabolites associated with rapid functional decline.
Funding: Funded by Proyecto del PII de EPOC, AES16, AstraZeneca and Laboratorios Menarini.

CARDIOVASCULAR CHANGES INDUCED BY VITAMIN D DEFICIENCY IN BMPR2 MUTANT RATS

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Introduction: Pulmonary arterial hypertension (PAH) is a rare and progressive disease characterized by elevated pulmonary arterial pressure. Autosomal dominant mutations in the bone morphogenetic protein receptor 2 (BMPR2) gene are associated to the development of 70% of the cases of heritable PAH (hPAH) and 15-40% of those with idiopathic PAH (iPAH). The low penetrance (20%) suggests that a second hit is causing the development of the disease. Furthermore, Vitamin D (VitD) deficiency has been associated with cardiovascular diseases and a worse prognosis.
Objectives: In this study, the role of VitD deficiency was analyzed in relation with a possible development of PAH and heart alterations in BMPR2 mutant rats.
Methods: Sprague Dawley rats with a monoallelic deletion of 71 bp in exon 1 (Δ 71 rats) with decreased expression in BMPR2 and wild type (WT) rats (n = 40) were randomly allocated to a standard diet or a VitD free diet for 5 months.
Results: Pulmonary arteries from all BMPR2 mutants show lower relaxant response to acetylcholine and lower contraction to serotonin. In these animals, deficit of VitD also reduces the response to riociguat and KCl. Interestingly BMPR2 mutant rats fed a VitD free diet show right atrial hypertrophy. Calcification was also observed in BMPR2 mutants with VitD deficiency.
Conclusions: These preliminary experiments indicate that BMPR2 heterozygous mutation associated to VitD deficiency leads to heart structural changes and pulmonary vascular alterations.

COMBINED CELL THERAPY AND ANTICOAGULANTS AS A TREATMENT FOR ACUTE RESPIRATORY DISTRESS SYNDROME

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Introduction: The lungs of patients with acute respiratory distress syndrome (ARDS) are characterized by an acute inflammation and an increased procoagulant activity. Recent evidence suggests the beneficial effects of cell therapies and nebulized anticoagulants for ARDS. Considering the different mechanism through which cell therapies and antithrombin act in ARDS, we propose a combined administration of these therapies to potentiate their therapeutic properties and face the different pathways and processes involved in the pathophysiology of ARDS.
Objectives: Determine the therapeutic benefit of a combined cell therapy (alveolar type II cells (ATII), mesenchymal stem cells (MSC), supernatant (SN) ATII, SN MSC) with antithrombin in vitro in a coculture of ATII and alveolar macrophages injured with lipopolysaccharide (LPS).
Methods: Cocultured ATII and alveolar macrophages isolated from rat lungs were injured with LPS (10 ng/ml). Two hours after the injury a combined cell therapy (ATII, MSC, SN ATII, SN MSC) with antithrombin (0.1 ng/mL) was administered. The effect of the administered treatments was assessed by the analysis of proinflammatory mediators and coagulants factors via qRT-PCR at 18 h. Data are expressed as mean ± SEM. Statistical analysis was performed using One-Way-ANOVA and Newman-Keuls post-hoc test (statistical significance $p \leq 0.05$).
Results: Proinflammatory mediators and coagulant factors were significantly increased after LPS administration (Fig. 1). The expression of PAI1 was significantly reduced after administering the different treatments compared to the LPS group. The expression of IL1β, IL6, TF and Plasminogen was not as high as in the LPS group.

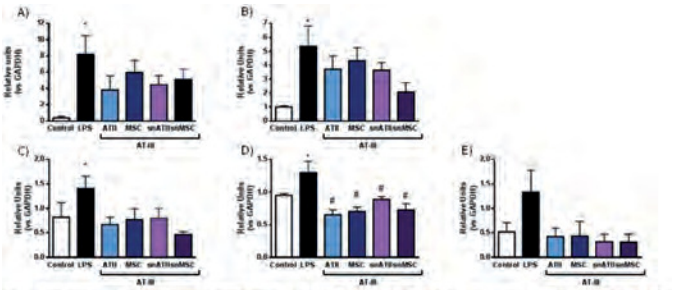

Fig. 1: Gene expression of proinflammatory mediators: A) IL1β. B) IL6 and coagulant factors: C) TF. D) PAI1. E) Plasminogen. evaluated by qRT-PCR at 18h. Data are expressed mean ±SEM in relation to GAPDH expression (n=6). *p≤0.05. vs control group; #p≤0.05 vs LPS.

Conclusions: The results indicate that combined cell therapy with antithrombin are able to attenuate inflammation and coagulation in vitro in a coculture of ATII and macrophages. Both treatments together are able to face different processes involved in lung injury.

COST-EFFECTIVENESS OF POSITIVE AIRWAY PRESSURE MODALITIES IN OBESITY HYPOVENTILATION SYNDROME

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Introduction: Obesity hypoventilation syndrome (OHS) is treated with either noninvasive ventilation (NIV) or continuous positive airway pressure (CPAP), but there are no long-term cost-effectiveness studies comparing the two treatment modalities.
Objectives: We performed a large, multicentre, randomized, open-label controlled study to determine the comparative long-term cost and effectiveness of NIV vs. CPAP using hospitalization days as the primary outcome measure.
Methods: Hospital resource utilization and within trial costs were evaluated against the difference in effectiveness based on the primary outcome (hospitalization days/year, transformed and non-transformed in monetary term). Costs and effectiveness were estimated from a log-normal distribution using a Bayesian approach. A secondary analysis by adherence subgroups was performed.
Results: In total, 363 patients were selected, 215 were randomized and 202 were available for the analysis. The median (IQR) follow-up was 3.01 [2.91; 3.14] years for NIV group and 3.00 [2.92; 3.17] years for CPAP. The mean (SD) Bayesian estimated hospital days was 2.13 (0.73) for CPAP and 1.89 (0.78) for NIV. The mean (SD) Bayesian estimated cost (€) per patient/year in the NIV arm, excluding hospitalization costs, was €2075.98 (91.6), which was higher than the cost in the CPAP arm of €1219.06 (52.3); mean difference €857.6 (105.5). CPAP was more cost-effective than NIV (99.5% probability) because the longer hospital stays in the CPAP arm were compensated for its lower costs. Similar findings were observed in the high and low adherence subgroups.
Conclusions: CPAP is more cost-effective than NIV; therefore, CPAP should be the preferred treatment for OHS patients with severe OSA.

DECIPHERING MOLECULAR SIGNATURES UNDERLYING PATHOLOGICAL MECHANISMS IN PATIENTS WITH COPD AND IPF

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Introduction: Chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) have great impact in the patient´s lifestyle and poor prognosis. The molecular mechanisms that trigger these pathologies have not been fully elucidated.
Objectives: To analyze surfactant proteins and lipids, markers of macrophage activation, and metabolic signatures in human lung biopsies from mild-COPD, severe-COPD, IPF, and control patients.
Methods: Surfactant proteins and M1 and M2 markers were analyzed in lung homogenates by western blot or ELISA. Metabolomic analysis of lung biopsies was done by high-resolution magic angle spinning NMR spectroscopy. Lipid levels and differences in metabolites were validated by enzymatic assays in tissue lysates.
Results: In IPF lung biopsies, surfactant proteins (SP-) A and D increased. Immature forms of SP-B and aggregates of SP-C were accumulated, and levels of phosphatidylcholine, the main lipid class in surfactant, decreased, suggesting an impairment of surfactant activity. With respect to macrophage markers, M2 markers were reduced but M1 markers did not change. Metabolomic analysis of lung biopsies revealed altered lipid, arginine, and polyamine metabolism and a remarkable increase of lactate levels. In severe COPD, we did not find changes in surfactant proteins and lipids. Markers of M2 macrophages decreased, whereas pro-inflammatory cytokines IFN-γ and IL-1β increased. Metabolomic analysis of lung biopsies revealed high glycogen and taurine levels. Increased taurine levels may indicate the activation of antioxidant mechanisms and elevated glycogen levels could reflect the inactivation of GSK3, with important consequences for promoting proliferation, metabolism and immune activation. Levels of lactate also increased in COPD samples.
Conclusions: Different biochemical profiles were found for the IPF, severe-COPD and control groups. COPD and IPF samples have high concentrations of lactate in common, which can promote aberrant repair mechanisms and pro-fibrotic responses.

DEVELOPMENT AND TESTING OF A NEW NON-TUBERCULOUS MYCOBACTERIA DIAGNOSTIC TEST USING SPECIFIC MYCOBACTERIAL CELL WALL ANTIGENS

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Introduction: Diagnosing non-tuberculous mycobacteria (NTM) infection is important to discard tuberculosis (TB) infection when test results are discordant and could be useful to handle patients with chronic pulmonary diseases (CPD) with NTM isolations of unclear clinical relevance.
Objectives: This study, describes a NTM-Interferon-gamma (IFN-γ) Release Assay (IGRA) that we have developed using specific NTM antigens to detect NTM sensitization.
Methods: We enrolled 334 patients: 90 latently TB infected (LTBI), 69 active TB, 55 NTM culture positive (20 lymphadenopathies, 31 CPD, and 4 disseminated infections), 13 NTM infection suspicion (positive TST, negative IGRA and no BCG vaccination record), 53 HIV-positive, 29 lymphadenopathies of unknown etiology, 3 non-NTM lymphadenopathies, and 22 uninfected controls with no TB exposure record. Peripheral blood mononuclear cells were isolated from whole blood samples. Cells were then stimulated overnight with NTM specific antigens. Detection of IFN-γ producing cells was evaluated by ELISPOT.

Results: NTM positive and suspicion groups had a significantly higher count of INF-γ-producing cells after NTM antigen stimulation. Using ROC curve analysis, a positivity cut-off of 6 spots was determined. The NTM suspicion, NTM positive and non-culture confirmed lymphadenopathies groups, had a higher number of positive results than LTBI, HIV positive, and active TB. CPD patients with NTM isolates considered as disease yielded a higher number of positive results than those considered as colonized (84.6% and 33.3%, respectively). Uninfected individuals and those with non-NTM lymphadenopathies yielded negative results.
Conclusions: The NTM-IGRA described in this study, could serve as an NTM infection diagnosis test capable of distinguishing NTM infection from LTBI. Moreover, it could enlighten the clinical relevance of NTM isolates from patients with CPD. Altogether, it would improve the handling of the patient and avoid unnecessary treatment. Ongoing studies are focused on characterizing the immune response against these antigens.

DIFFERENTIAL EFFECT OF INTERMITTENT HYPOXIA AND SLEEP FRAGMENTATION ON PD-1/PD-L1 UPREGULATION

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Introduction: Obstructive sleep apnea (OSA) is characterized by repetitive obstructions of the upper airway during sleep that result in intermittent hypoxia (IH) and sleep fragmentation (SF), and has been associated with a higher risk of cancer, as well as poorer cancer outcomes The Immunosurveillance is compromised in OSA patients as reflected by overexpression of the programmed death cell receptor and its ligand (PD-1/PD-L1) co-inhibitory axis. However, the contributions of intermittent hypoxia (IH) and sleep fragmentation (SF) are unclear.
Objectives: In this study we evaluate the effect of the IH and SF to modulates the expression of PD-1 and PD-L1 on immune cells from mice subjected to IH or SF, and in human cells exposed to IH, oxidative stress, or both conditions.
Methods: Six-week-old male C57BL/6J mice were exposed to either IH or SF using previously established in vivo models. Moreover, in vitro models using a human peripheral blood mononuclear cells (PBMC) were cultured overnight under normoxia, IH, hydrogen peroxide (H2O2) or both. Murine splenocytes and human PBMC were isolated, and labeled using surface-specific antibodies for flow cytometry analysis.
Results: Compared to control mice, IH induced higher expression of PD-L1 on F4/80 cells and of PD-1 on CD4 + and CD8 + T-cells, while no significant changes emerged after SF. In vitro models of IH and oxidative stress showed similar changes for expression of PD-L1 on human monocytes and PD-1 on CD4 + T-cells. Furthermore, H2O2 treatment increased PD-1 expression on CD8 + T-cells, compromising their cytotoxic capacity similar to IH.
Conclusions: No evidence of synergistic effects was apparent. Therefore, PD-1/PD-L1 upregulation reported in OSA patients appears to be preferentially mediated by IH rather than SF.

DISRUPTION OF THE TIGHT JUNCTIONS IN THE ALVEOLAR-CAPILLARY MEMBRANE IN PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME

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Introduction: Alveolar protein-rich edema is an early and critical event in Acute Respiratory Distress Syndrome (ARDS). The intercellular tight junction (TJ) complexes limit the passage of water and solutes through the epithelial and endothelial barriers.
Objectives: To determine whether the tight junction proteins of the alveolar-capillary membrane are altered in the lungs of patients with ARDS.
Methods: We performed a histological study of the lung of patients who died in the Intensive Care Unit and were autopsied in Getafe Hospital. We included patients with clinical criteria of ARDS (Berlin definition) and histological criteria of diffuse alveolar damage (DAD), as well as patients without ARDS nor DAD criteria (control group). All patients underwent mechanical ventilation for less than 10 days, and all ARDS/DAD patients died within the first 10 days of fulfilling ARDS criteria. Paraffin-embedded lung tissue sections were stained by hematoxylin-eosin. Expression of the tight junction proteins (ZO-1 and Occludin) was determined by immunofluorescence techniques. We used t-Student tests for clinical parameters ($p < 0.05$ was considered statistically significant). Project approved by our local ethical committee.
Results: The ARDS/DAD group (13 males/5 females) and the control group (3 males/5 females) showed no significant differences in SAPS II, age, shock, serum creatinine concentration, INR, platelets or PEEP. The expression of the tight junction proteins ZO-1 and occludin in the lungs of control patients was homogeneous in the alveolar walls and limited to the cell periphery. In contrast, the lungs of ARDS/DAD patients showed areas lacking ZO-1 and occludin, and areas in which these proteins had a diffuse cytoplasmic distribution.
Conclusions: The alveolar expression of tight junction proteins is altered early in the lungs of patients with ARDS and DAD. The disruption of the tight junction proteins may be mechanistically involved in the formation of protein-rich edema and may constitute a valuable therapeutic target in ARDS.
Funding: FIS PI15/01942. EXOHEP-CM S2017/BMD3727. FIS PI15/00482.

DNA METHYLATION SIGNATURE OF COPD AND AIRFLOW LIMITATION SEVERITY IN LUNG TISSUE

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Introduction: Tobacco smoking is the main environmental risk factor for chronic obstructive pulmonary disease (COPD), albeit not all smokers develop the disease. DNA methylation is one of the main epigenetic mechanisms that regulate gene transcription and is affected by smoke exposure.
Objectives: This study aimed to identify lung tissue DNA methylation changes associated to COPD and the grade of severity of airflow limitation independent of current smoking.

Methods: The lung tissue methylation e was assessed with the Infinium Methylation EPIC array (Illumina) in four study groups; 1) never-smokers (n = 26), 2) COPD former-smokers (n = 126), 3) current-smokers with normal lung function n = 11, and 4) COPD current-smokers. Differential methylated positions (DMP) were identified with the Champ pipeline. In a subset of samples (n = 111) the correlation of DMP levels with mRNA expression (Affimetrix arrays) was determined.
Results: Between never smokers and former smokers with COPD 117 DMP were identified, of them 14% (n = 17) were also associated to current smoking. DMPs were enriched in biological process ontologies as: cell shape, surfactant homeostasis and regulation of transcription in response to hypoxia. In never-smokers vs. COPD GOLD 1-2 we identified n = 105 DMPs (74 Genes), and in never-smokers vs. COPD GOLD 3-4 n = 976 DMPs (600 genes) were significant. Only 10% of DMPs were significant in both comparisons, and the proportion of overlap with the current smoking signature was higher in GOLD 1-2 (20%) than in GOLD 3-4 (4%). DNA methylation and mRNA expression levels in n = 21 DMPs were significantly correlated.
Conclusions: The lung DNA methylation profile is different in patients with COPD, especially in those with severe airflow limitation. The proportion of methylation changes associated to current smoking is high in patients with GOLD 1-2, and rare in GOLD 3-4, suggesting that these 'stages' could correspond to different diseases or being associated to different lung function trajectories.

DO DIFFERENCES EXIST IN THE MICRORNA EXPRESSION PROFILE IN PLASMA OF PATIENTS WITH LUNG CANCER AND COPD?

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Introduction: Lung cancer (LC) is a leading cause of cancer-related deaths worldwide. Chronic obstructive pulmonary disease (COPD) represents an important risk factor for LC development through the action of biological mediators. Our group has demonstrated in lung tumors the contribution of oxidative stress, inflammation, and epigenetic modifications, including microRNAs to the greater predisposition to LC development of COPD patients. Whether similar events might be identified systemically in these patients with LC remains to be elucidated.
Objectives: To explore whether microRNAs known to participate in lung tumorigenesis were differentially expressed in the blood of LC patients with and without COPD.
Methods: On the basis of an extensive literature search, the following microRNAs were analyzed in plasma samples (qRT-PCR) using specific primers: miR-451, miR-210, miR-126, miR-21, miR-Let7c, miR-145, miR-155, miR-200b and miR-223 in patients with LC-COPD (N = 84) and in LC patients (N = 25). Moreover, for quality control purposes identical analyses were also performed in COPD-only patients (N = 16) and healthy subjects (N = 20). All study participants were recruited from the LC Clinics at Hospital del Mar. The following comparisons were established: 1) any group of patients versus healthy individuals, 2) LC-COPD versus LC-only patients, and 3) LC-COPD versus COPD-only patients.
Results: 1) Compared to healthy controls, expression levels of microRNA-451 significantly decreased in plasma of LC-COPD and LC, while that of microRNA-210 increased in LC-only patients. 2) Compared to LC-only, plasma levels of microRNA-210 significantly decreased in LC-COPD. 3) Compared to COPD-only, plasma levels of microRNA-210 significantly declined in LC-COPD patients, whereas that of microRNA-155 increased.
Conclusions: Expression levels of microRNA-210 are differentially expressed in LC patients with underlying COPD. The downstream mechanisms regulated by this microRNA warrants further attention in future experiments. We conclude that the presence of COPD also induces systemic effects regarding the expression of certain microRNAs involved in lung tumorigenesis.

ECHOCARDIOGRAPHIC CHANGES WITH POSITIVE AIRWAY PRESSURE THERAPY IN OBESITY HYPOVENTILATION SYNDROME

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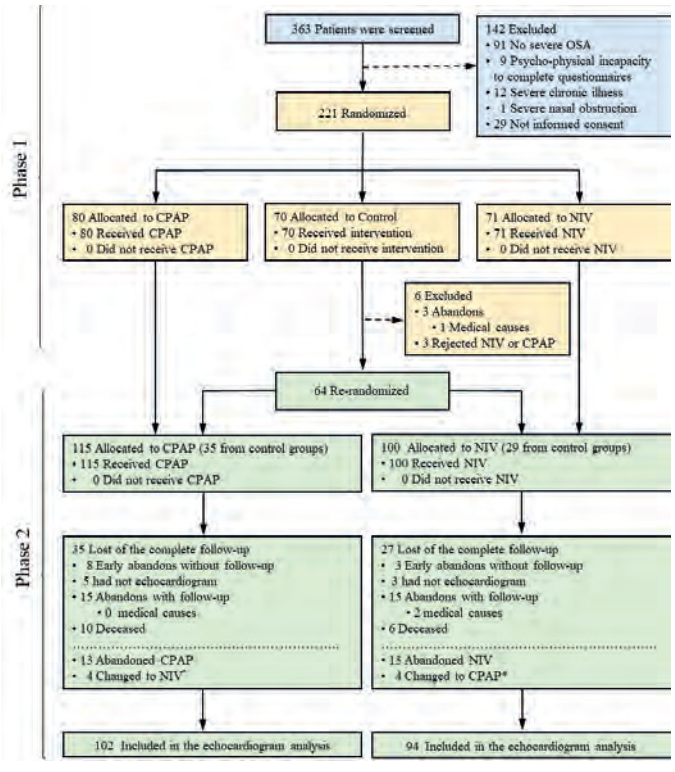
Introduction: Obesity hypoventilation syndrome (OHS) has been associated with cardiac dysfunction. However, randomized trials have assessing the impact of long-term noninvasive ventilation (NIV) or CPAP on cardiac structure and function assessed by echocardiography are lacking.
Objectives: In a pre-specified secondary analysis of the largest multicenter randomized controlled trial of OHS (Pickwick project, n = 221 patient with OHS and coexistent severe obstructive sleep apnea), we



compared the effectiveness of 3 years of NIV and CPAP on structural and functional echocardiographic changes.
Methods: At baseline and annually during 3 sequential years patients underwent transthoracic two-dimensional and doppler echocardiography. Echocardiographers at each site were blinded to the treatment allocation. Statistical analysis was performed using a linear mixed-effects model with a treatment group/repeated measures interaction to determine the differential effect between CPAP and NIV.
Results: 196 patients were analyzed, 102 treated with CPAP and 94 treated with NIV. Systolic pulmonary artery pressure decreased from 40.5 ± 1.47 mmHg at baseline to 35.3 ± 1.33 mmHg at 3 years with CPAP and from 41.5 ± 1.56 mmHg to 35.5 ± 1.42 with NIV ($p < 0.0001$ for longitudinal intragroup changes for both treatment arms). However, there were no significant differences between groups. NIV and CPAP therapies similarly improved left ventricular diastolic dysfunction and reduced left atrial diameter. Both NIV and CPAP improved respiratory function and dyspnea.
Conclusions: In patients with OHS who have concomitant severe obstructive sleep apnea, long-term treatment with NIV and CPAP led to similar degrees of improvement in pulmonary hypertension and left ventricular diastolic dysfunction.

EFFECT OF OBSTRUCTIVE SLEEP APNEA IN THE AGING PROCESS

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Introduction: Recent studies suggest that the obstructive sleep apnea (OSA) induced physiological events (intermittent hypoxia and sleep fragmentation) resemble to those induced by aging. Accordingly, younger individuals with OSA present cognitive and functional decline compared with age-matched controls. In this study, we hypothesized that OSA could induce age-related cellular and molecular alterations, anticipating or aggravating the aging process.
Objectives: To evaluate the effect of OSA on the age-related processes, and to determine whether this effect is age dependent.
Methods: Observational, cross-sectional and prospective study that included 418 patients referred to the sleep unit due to suspected OSA. The subjects were grouped according to the median of age (50 years) and OSA severity into the following groups: young patients without severe OSA; young patients with severe OSA; elderly patients without severe OSA; and elderly patients with severe OSA. Some of the main hallmarks of aging were evaluated: telomere attrition (leukocyte telomeric length), mitochondrial dysfunction (leukocyte mitochondrial DNA copy number), genomic instability (urinary 8-hydroxy-2-deoxyguanosine concentration), deregulation of nutrient sensing (insulin resistance) and alteration of intercellular communication (serum C-reactive protein concentration).
Results: In young patients, severe OSA was associated with a deregulated nutrient sensing ($p < 0.001$) and a trend towards greater genomic instability was identified ($p = 0.077$). No evidence was found in relation to the other parameters analyzed. Differently, severe OSA was only associated with mitochondrial dysfunction in elderly patients ($p = 0.027$). The differential effect of severe OSA between young and elderly patients was observed through a deregulated nutrient sensing ($p = 0.017$) and as a trend related to genomic instability ($p = 0.090$) (Table 1).
Conclusions: Severe OSA was associated with alterations in age-related processes such as mitochondrial dysfunction, genomic instability and deregulation of nutrient sensing, and this effect was dependent on the age of the patient.

	Age <50 years		Age ≥50 years	
	AHI <30 events/h	AHI ≥30 events/h	AHI <30 events/h	AHI ≥30 events/h
Subjects	111	81	96	130
Patient characteristics				
BMI kg/m2	29,1 [25,8;33,7]	31,2 [27,6;35,1]	29,1 [25,8;33,1]	33,2 [29,5;36,3]
Age	43,0 [38,0;47,0]	44,0 [41,0;47,0]	56,0 [54,0;59,2]	57,0 [53,2;63,0]
Male	71 (64,0%)	73 (90,1%)	54 (56,2%)	93 (71,5%)
AHI events/h	11,6 [5,90;20,6]	50,8 [39,0;81,0]	17,0 [10,7;22,5]	56,0 [42,8;72,4]
Telomere attrition				
Telomere length	0.99 (0.04)	1.00 (0.04)	0.99 (0.04)	0.99 (0.03)
Adjusted diference[1]	<0.01 (p=0.776)		<0.01 (p=0.615)	
Differential effect[2]	<0.01 (p=0.889)			
Mitochondrial dysfunction				
Mitochondrial DNA copy number	112 (49.3)	108 (46.8)	110 (49.1)	94.4 (41.8)
Adjusted diference[1]	-2.90 (p=0.680)		-14.38 (p=0.027)	
Differential effect[2]	-11.47 (p=0.215)			
Genomic instability				
8-OHdG concentration	10.2 (2.86)	10.7 (3.79)	11.9 (3.62)	10.9 (3.46)
Adjusted diference[1]	1.13 (p=0.077)		-0.35 (p=0.591)	
Differential effect[2]	-1.48 (p=0.090)			
Deregulated nutrient sensing				
Insulin resistance	1.84 (1.40)	3.57 (3.62)	2.42 (2.62)	3.38 (2.91)
Adjusted diference[1]	1.26 (p<0.001)		0.08 (p=0.822)	
Differential effect[2]	-1.18 (p=0.017)			
Altered intercellular communication				
CRP concentration	2.87 (3.12)	4.14 (4.09)	3.16 (3.39)	3.87 (3.82)
Adjusted diference[1]	0.20 (p=0.248)		0.03 (p=0.458)	
Differential effect[2]	-0.17 (p=0.734)			

[1] Adjusted difference between severe OSA (AIH ≥30 events/h) and non-severe OSA (AIH <30 events/h).
[2] Differential effect of severe OSA between young (age <50 years) and elderly patients (age ≥50 years).

Data are presented as n (%), median [interquartile range] or mean (standard deviation). AHI: apnea hypopnea index; OSA: obstructive sleep apnea.

Funding: PI14/01266 and PI18/00449 from ISCIII, FEDER, SEPAR. IRB-Lleida Biobank (B.0000682) and PLATAFORMA BIOBANCOS PT17/0015/0027.

EFFECTS OF DIESEL EXHAUST PARTICLE EXPOSURE ON A MURINE MODEL OF CHEMICAL ASTHMA

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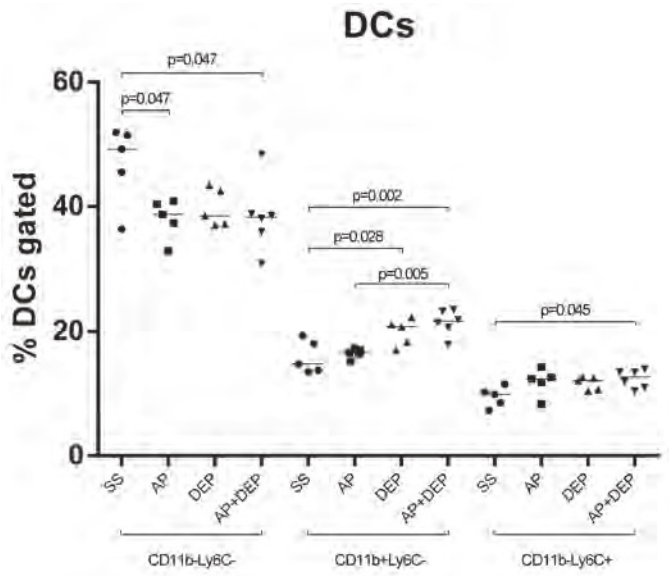
Introduction: Ammonium persulfate (AP) is a highly reactive salt that causes occupational asthma. Diesel exhaust particles (DEP) are implicated in the increase of respiratory sensitization to allergens, but no studies have been done on the role of DEP in asthma due to chemical agents.
Objectives: The present study aims to study the role of DEP in chemical asthma.
Methods: BALB/c ByJ mice were randomly divided into four experimental groups. On days 1 and 8, received dermal applications of 5% AP or vehicle. On days 15, 18 and 21, received 1%AP or vehicle via intranasal instillation (AP and SS groups, respectively). Two experimental groups received DEP on every of the three challenges (AP + DEP and DEP groups). Twenty four hours after the last intranasal instillation, the lungs were processed to characterize lung immune cells by flow cytometry: T and B cells, NK, neuthophils, eosinophils, inflammatory and resident monocytes, alveolar and interstitial macrophages (alvMacrophages and intMacrophages, respectively) and total, CD-11b+Ly6C+, CD11b+Ly6C-, CD11b-Ly6C+ and CD11b-Ly6C- dendritic cells.
Results: AP group exhibited an increase in eosinophils and NKs ($p = 0.026$ and $p = 0.014$, respectively) and a decrease in monocytes and CD11b-Ly6C-DCs ($p = 0.027$ and $p = 0.047$, respectively), while DEP group displayed an increase in neutrophils, NKs, CD11b+Ly6C-DC and intMacrophages ($p < 0.0001$, $p < 0.001$, $p = 0.028$ and $p < 0.001$, respec-

tively) and a decrease in monocytes and alvMacrophages ($p < 0.001$ and $p < 0.001$, respectively) compared to SS group. AP + DEP group experienced an increase in eosinophils, neutrophils, NKs, CD11b-Ly-6C+DCs, CD11b+Ly6C-DCs and intMacrophages ($p = 0.003$, $p = 0.009$, $p = 0.001$, $p = 0.045$, $p = 0.002$ and $p < 0.001$, respectively) and a decrease in monocytes, CD11b-Ly6C-DC, macrophages and alvMacrophages ($p = 0.001$, $p = 0.047$, $p = 0.002$ and $p < 0.001$, respectively) compared to SS group.



Lung Dendritic Cells levels from flow cytometry analysis. The experimental groups were SS: saline sensitized and saline challenged; DEP: saline sensitized and DEPs challenged; AP: AP sensitized and AP challenged; and AP + DEP: AP sensitized and challenged with a mixture of AP and DEP. Individual and median values of CD11b-Ly6C- dendritic cells, CD11b+Ly6C- dendritic cells and CD11b-Ly6C+ dendritic cells.

Conclusions: This study characterizes the immunological mechanisms underlying the exposure to DEP. The results demonstrated that DEP exposure activates the innate immune response and together with AP, exacerbates asthma immune hallmarks.

Funding: Study supported by Instituto de Salud Carlos III (PI15/01900 and PI18/00344), FEDER and FUCAP.

EFFECTS OF OBSTRUCTIVE SLEEP APNEA IN DIFFERENT PHENOTYPES OF PATIENTS WITH ACUTE CORONARY SYNDROME

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Introduction: Recent studies report a cardioprotective role for obstructive sleep apnea syndrome (OSA) in the context of acute myocardial infarction. In addition, the impact of continuous positive air pressure (CPAP) treatment on cardiovascular (CV) outcomes is controversial. An accurate characterization of the different cardiovascular phenotypes could be of great relevance in order to understand the effects of OSA and its treatment in the risk of CV events or deat.

Objectives: To evaluate the differential effect of OSA and its treatment in distinct CV phenotypes of patients with acute coronary syndrome (ACS).

Methods: Latent class analysis was performed to identify subgroups of patients in the ISAACC study (NCT01335087) based on 11 clinical variables associated with CV characteristics.

Results: Among the four clusters identified [patients with history of heart disease (HD), hypertensive (HT) patients who were admitted for their first CV event (ACS + HT), patients older than 60 years who were admitted for their first CV event (ACS + aged patients) and patients without comorbidities who were admitted for their first CV event (ACS)], we observed that OSA increased the risk of CV event in the ACS group (P-value: 0.068) but decreased it in patients with previous HD (P-value: 0.047). No relevant effects of CPAP were observed.

Conclusions: In summary, we observed a dual role for OSA in cardiovascular diseases: whereas OSA suggest a cardioprotective effect when considering the patients with a history of HD, OSA increased the CV risk in the ACS group.

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EFFECTS OF SUSTAINED AND INTERMITTENT HYPOXIA ON HUMAN LUNG CANCER CELLS

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Introduction: The relationship between lung cancer and COPD is well known. Recent clinical studies found a higher prevalence of obstructive sleep apnea (OSA) in patients suffering lung cancer. Although hypoxia has been suggested to be one of the main factors associated with lung malignancy, most studies addressing this topic have limitations due to the reduced number of patients recruited and because lung cancer has been analyzed avoiding its complexity in terms of different histotypes.

Objectives: To investigate the effect of different patterns of hypoxia on cells of the most prevalent histological sub-types of non-small cell lung cancer: adenocarcinoma (ADC) and squamous cell carcinoma (SC).

Methods: Four human lung cancer cell lines were exposed to normoxia (N) (13% O_2), sustained hypoxia (SH) (7% O_2), mild intermittent hypoxia mimicking OSA (MIH) (13%-7% O_2) and severe intermittent hypoxia (SIH) (7%-4% O_2) for 48 h and cell proliferation was quantified and HIF 1α expression was assessed.

Results: SIH pattern enhanced the proliferation of H520 (SC) (≈ 66%, $p < 0.001$), while the MIH did it in H520 (≈ 72%, $p < 0.001$) and H1437 (ADC) (≈ 40%, $p = 0.043$). SH increased tumor cell proliferation (≈ 56%, $p = 0.005$) in H1437. However, none of the hypoxic profiles elicited measurable changes in any of the two other ADC cells (H522, H1975). Only H1975 and H1437 showed increased HIF 1α expression in the nuclei (Table).

Nuclear/Cytoplasmic ratio of hypoxia-inducible factor 1a fluorescence for each cell line and hypoxic condition compared with 13% normoxia

	H522			H1437			H1975			H520		
	Mean	Standard error	P value	Mean	Standard error	P value	Mean	Standard error	P value	Mean	Standard error	P value
13% O_2	0.7	0.11		1.13	0.03		0.91	0.03		2.84	0.28	
13-7% O_2	2.63	0.86	0.098	1.38	0.10	0.059	1.27	0.3	0.319	3.18	0.37	0.266
7% O_2	0.97	0.16	0.261	1.35	0.16	0.208	1.72	0.35	0.078	2.7	0.15	0.629
7-4% O_2	0.92	0.06	0.090	1.36	0.09	0.022	2.39	0.55	0.047	2.78	0.26	0.883

Conclusions: Hypoxic-induced cell malignancy strongly depends on both the type of hypoxia (SH vs. MIH/SIH) and the cancer cell type. Therefore, our results provide critical insights for further clinical studies aimed at studying the relationship between lung cancer and respiratory diseases with different hypoxic patterns and severities such as COPD, OSA, obesity reduced ventilation, and overlap syndrome.

EFFICACY AND SAFETY OF DIFFERENT DOSES OF NEURAMINIDASE INHIBITORS FOR THE TREATMENT OF HOSPITALIZED ADULTS WITH INFLUENZA: SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: Efficacy and safety of different doses of neuraminidase inhibitors (NAIs) for hospitalized influenza patients have not been established.
Objectives: Our aim was to develop a systematic review and meta-analysis of randomized controlled trials (RCT) on the efficacy and safety of different doses of NAIs regimens in hospitalized adults with influenza.
Methods: The Cochrane collaboration searching methods was followed in Cochrane Library, PubMed and Web of Science databases (2008-2018). Eligibility criteria were limited to RCT comparing different regimens of NAIs in hospitalized adults (≥ 16 years) for influenza therapy. Primary outcomes were 28-day overall mortality and time to clinical resolution (TTCR).
Results: Three RCTs (with 902 patients) among 217 RCTs evaluated were included. Two RCT compared two different regimens and/or doses of intravenous peramivir and another compared two different doses of intravenous zanamivir with oseltamivir. The meta-analysis of mortality showed no significant differences with different dose regimens of systemic NAIs (Odds Ratio [OR]: 0.61; 95%Confidence Interval [CI]: 0.26–1.39). There were no virological or clinical (TTCR) advantages. No differences were observed (OR: 1.03; 95%CI: 0.66–1.61) in severe adverse events. The certainty of evidence (GRADE) was very low.
Conclusions: The evidence does not support the use of different dosages of systemic NAIs, even as salvage therapy.

EPIDEMIOLOGICAL RELEVANCE AND ROLE IN VIRULENCE OF THE NEW EMERGING SEROTYPES INCLUDED IN THE NEW 15-VALENT PNEUMOCOCCAL CONJUGATE VACCINE

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Introduction: *Streptococcus pneumoniae* is one of the most frequent causes of otitis media, community-acquired pneumonia and meningitis. Since the introduction of the 13-valent pneumococcal conjugate vaccine (PCV-13) serotype replacement by non-PCV13 serotypes has been reported.
Objectives: This study tries to analyze the epidemiological situation of serotypes 22F and 33F included in the next PCV-15 vaccine and characterize their pathogenicity mechanisms, analyzing the capacity to avoid the host immune response and biofilm formation.
Methods: Clinical isolates from four different origins affecting children and adults ≥ 65 years old were selected and MLST was determined. We constructed isogenic strains with capsules 22F and 33F derived from the non-encapsulated strain M11 with known genetic background and we also made isogenic strains with capsules of relevant serotypes that are included or not in the conjugate vaccine. We performed opsonophagocytosis assays with planktonic and biofilm cultures of these strains.
Results: Since the introduction of PCV-13, we found that serotypes 22F and 33F are rising in the risk populations of the study. The predominant clones were ST43322F and ST71733F. Furthermore, both serotypes were bad biofilm formers compared to other pneumococcal serotypes. Pediatric isolates of 22F and 33F from blood cultures, formed a better biofilm than adult's isolates, although there was a heterogeneous opsonophagocytosis evasion pattern, that was enhanced after biofilm formation. Compared to the prevalent serotype 19A and the non-encapsulated strain M11, serotypes 22F and 33F avoid the phagocytosis process very efficiently.
Conclusions: The increase of both serotypes, especially 22F, could be associated to the evasion of the immune system that is clearly increased in a biofilm state. Hence, we did not find a correlation between genotype and biofilm formation. Finally, pediatric clinical isolates of 22F and 33F, that form better biofilm than adults isolates, could have an increased capacity to colonize the nasopharynx of children.

HAEMOPHILUS INFLUENZAE GLUCOSE RESPIRATION ASSISTED FERMENTATION LEADING TO PRODUCTION OF THE IMMUNOMETABOLITE ACETATE HAS A KEY CONTRIBUTION TO THE HOST AIRWAY-PATHOGEN INTERPLAY

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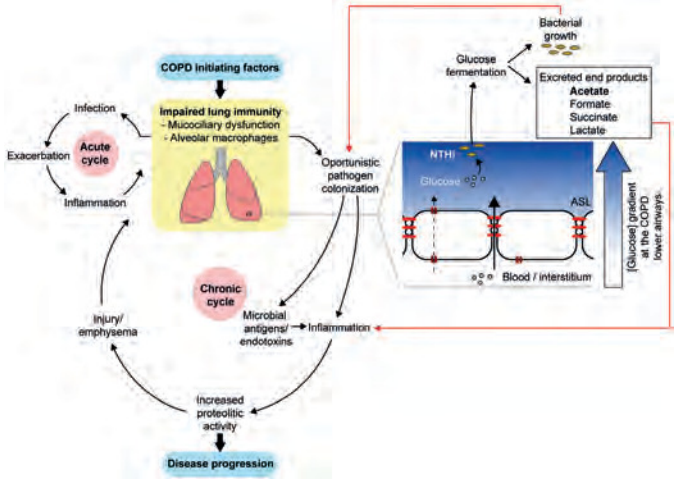
Introduction: Glucose homeostasis at the human lung lumen contributes to maintain a nutrient-depleted environment to limit the growth of pathogens. In healthy airways, glucose concentrations are maintained 3-20 times lower in the airways surface liquid (ASL) than in plasma. However, the ASL glucose concentration is elevated in sputum samples of patients with chronic obstructive pulmonary disease (COPD), which facilitates the proliferation of bacteria able to use glucose as carbon source. COPD is characterized by abnormal inflammatory responses and impaired airway immunity, which provides an opportunistic platform for nontypeable *Haemophilus influenzae* (NTHi) infection. This results in a vicious-circle where large inflammation due to multiple interactions between airway immune cells and NTHi results in worsening of the disease clinical status. NTHi glucose metabolism is a respiration-assisted fermentation. We hypothesised that such specialized glucose catabolism may be a bacterial pathoadaptative trait with a pivotal role in airway infection.
Objectives: Generation and characterization of bacterial mutants unable to produce acetate, formate or succinate, main products of NTHi glucose metabolism.
Methods: Inactivation of the NTHi ackA, pflA and frdA genes. In vitro and in vivo mutant phenotypic characterisation, in terms of bacterial fitness, growth, immunometabolite production and cell signalling, gene expression, and pulmonary infection.

Results: Inactivation of the ackA gene impaired acetate production, and led to slow bacterial growth, production of lactate under low oxygen tension, and bacterial attenuation in vivo. Bacterially produced acetate resulted in increased airway epithelial inflammatory responses, supporting that the COPD lung provides NTHi with elevated glucose concentrations, bacteria use it and produce fermentative end-products acting as proinflammatory metabolites at the site of infection.



Model proposing the biological significance of H. influenzae glucose catabolism during infection at the COPD lung.

Conclusions: This information has important implications for developing non-antibiotic antimicrobials, given that airway glucose homeostasis modifying drugs could help preventing microbial infection associated to chronic lung disease.

HIGH GENOMIC HOMOGENEITY AMONG *HAEMOPHILUS INFLUENZAE* SEROTYPE F

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Introduction: *Haemophilus influenzae* is an opportunistic pathogen highly adapted to the human respiratory tract which is often reported as the etiologic agent of infectious diseases. After the introduction of serotype b vaccine, non-typeable *H. influenzae* (NT-Hi) has become the most frequent cause of respiratory infection, followed in frequency by serotype f strains (Hi-f).
Objectives: To analyse by whole genome sequencing the genomic diversity among invasive and colonizing Hi-f isolates.
Methods: A total of 37 Hi-f isolated from Portugal (n = 3), The Netherlands (n = 18) and Spain (n = 16) were sequenced using Illumina technology. A core-based phylogenetic tree was constructed with Parsnp from the Harvest suite. The MLST was determinate and the core and accessory genome analysis was done by Roary and roProfile. The single nucleotide analysis was done through Snippy to detect polymorphisms (SNPs) among bacterial genomes.
Results: Thirty-one isolates were ST124 and the remaining six isolates were single locus variant of ST124 [ST106 (n = 1), ST1736 (n = 2) and a new ST (n = 3)]. Although all strains were closely related two major clusters were observed in the phylogenetic tree. The estimated core genome was 92% and 12,825 core-SNPs were detected. A total of 1,853 genes were predicted, of them, 1,691 were present in more than 95% of the strain and 162 were accessory genome (present in less than 95% strains). Regarding to allelic variation, 952 core genes were monoallelic. The fifteen most polymorphic genes were secA, mnmG, maeB, ftsH, emrA, hxuA, hgpC, HifGL_000293, dnaB, hgoB, HifGL_000294, HifGL_000920, proB, murD and alaS. This variation was mostly due to a cluster containing the colonizing Hi-f strains.

HUMAN SURFACTANT PROTEIN A BINDS THE HUMAN ANTIMICROBIAL PEPTIDE CATHELICIDIN INHIBITING ITS CYTOTOXIC EFFECT ON ALVEOLAR EPITHELIAL CELLS

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Introduction: Human cathelicidin (LL-37) is a multifunctional component of innate immunity with direct antimicrobial activity against several microorganisms. However, LL-37 also binds to host membranes causing a cytotoxic effect. Secretion of LL-37 by alveolar epithelial and immune cells is increased after infection or tissue injury. Surfactant protein-A (SP-A) is an abundant protein in the alveolar space with important immune defense functions.
Objectives: To evaluate whether SP-A binds to LL-37 and to analyze whether SP-A is involved in the local regulation of LL-37 activity.
Methods: SP-A binding to LL-37 was analyzed by tryptophan fluorescence and dynamic light scattering. Antimicrobial activity of LL-37 in presence or absence of SP-A was studied through killing assay. Cytotoxic activity of LL-37 was measured in alveolar epithelial cells by crystal violet staining and WST-1 cell viability assay.
Results: SP-A bound to LL-37 with high affinity (Kd = 0.01 ± 0.006 pM) in physiological conditions of pH and sodium chloride. SP-A/LL-37 interaction results in reduction of LL-37 cytotoxicity on alveolar epithelial cells at high LL-37 concentrations, without affecting LL-37 antimicrobial activity against the respiratory pathogens. However, at low LL-37 concentrations, SP-A significantly decreased LL-37 antimicrobial activity against nontypable Haemophilus influenzae, Klebsiella pneumoniae K2, and Pseudomonas aeruginosa O1, which is consistent with SP-A/LL-37 interaction that block LL-37 activity.
Conclusions: Our data indicate that SP-A protects lung epithelium from tissue injury caused by high LL-37 concentrations. These data suggest that in conditions of tissue damage or infection, when LL-37 secretion increases, SP-A protects from cytotoxic local high concentrations of LL-37, without interfering with LL-37 antimicrobial activity.

IDENTIFICATION OF NOVEL GENETIC ASSOCIATIONS WITH THE RESPONSE TO INHALED CORTICOSTEROIDS IN CHILDREN WITH ASTHMA

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Introduction: Inhaled corticosteroids (ICS) are the main medication used to control asthma symptoms and to prevent acute asthma exacerbations. Variability in response to ICS has been described among individuals and populations, which has been associated with a combination of environmental and genetic factors.
Objectives: We aimed to identify genes associated with ICS response in European children by means of a meta- genome-wide association study (GWAS).
Methods: A meta-GWAS of asthma exacerbations was performed across eight studies from the Pharmacogenomics in Childhood of Asthma (PiCA) consortium including 2,704 asthmatic children and young adults treated with ICS of European ancestry. A total of 8.1 million genetic variants with minor allele frequency ≥ 1% were meta-analyzed. Variants with $p \leq 5 \times 10\text{-}6$ were considered suggestively associated and followed up for replication in 363 asthma patients taking ICS from two independent studies of European ancestry (ALSPAC and BAMSE). The association with the change in FEV1 after ICS treatment was also assessed in 166 children from the SLOVENIA study.
Results: A total of nineteen SNPs at 10 loci were suggestively associated with asthma exacerbations despite use of ICS (minimum p-value = 6.7 x 10-7). A novel association with ICS response was found in several genes, including two genes previously associated with lung function measurements (WNT5A and CNTNAP5). A variant located at WNT5A showed evidence of replication in ALSPAC and BAMSE ($p = 0.025$). Moreover, variants in CNTNAP5 were also nominally associated with the change in FEV1 after ICS treatment (lowest $p = 0.025$), but not with baseline or post-treatment lung function measurements.
Conclusions: This study revealed novel associations of two genes with ICS response in children and young adults of European ancestry. Further validation will be explored in Hispanic/Latino, African American and Asian populations included in the PiCA consortium.
Funding: Study supported by Instituto de Salud Carlos III (AC15/00015; FI16/00136) and ERACoSysMed 1st Joint Transnational Call (SysPharmPedia).

IMMUNE PROFILE AND SURVIVAL IN LUNG CANCER PATIENTS: INFLUENCE OF UNDERLYING COPD

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Introduction: Immune microenvironment plays a role in lung cancer (LC) development. We hypothesized that immune profile of B and T cells may differ in tumors of LC patients with and without COPD and may also influence patients' survival.
Objectives: 1) To analyze levels of tertiary lymphoid structures (TLSs), B and T cells in tumor and non-tumor (control samples) lung specimens of LC patients with/without COPD and 2) to analyze the influence of those biological markers in the patients´10-year survival.
Methods: TLSs (numbers and area), B (CD20), and T (CD3) cells were identified in both tumor and non-tumor specimens (thoracotomy) from 90 LC-COPD patients and 43 LC-only patients (immunohistochemistry, double staining with specific antibodies). Survival was analyzed in all 133 patients.
Results: 1) Immune profile in tumors of LC-COPD versus LC: The number of TLSs significantly decreased in tumors of LC-COPD compared to LC patients. No significant differences were observed in tumors between LC-COPD and LC patients for B or T cells. In tumors compared to non-tumor specimens, a significant rise in TLSs was observed in LC (numbers and area) and LC-COPD (area), T cell counts declined in tumors of LC, while B cell counts increased in tumors of both LC and LC-COPD patients. 2) Survival: In LC-COPD patients: lower numbers of TLSs (cut-off: 0.9672) and greater numbers of B cells (cut-off: 85.18) were associated with longer survival rates. In LC patients: lower levels of T cells (cut-off: 8.607) were associated with longer survival rates. All patients together: lower numbers of T cells (cut-off: 8.554) and TLSs (cut-off: 0.9176) and greater numbers of B cells (cut-off: 91.45) were associated with longer survival rates.
Conclusions: TLSs, B cells and T cells are differentially expressed in tumors of LC-COPD from that in LC-only patients. Further analyses are required to identify the specific role of TLSs in LC development in patients with COPD.

IMMUNOMODULATION WITH MONOCLONAL ANTIBODIES OF PARP EXPRESSION AND ACTIVITY IN LUNG TUMORS OF MICE

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Introduction: Poly-ADP ribose polymerase plays crucial roles in DNA repair and other cell functions. Immunomodulators have proven to reduce tumor burden. PARP activity may modify the immune system response. We hypothesized that immunomodulators may modify the expression and activity of PARP-1 and PARP-2 enzymes in lung tumors of mice.
Objectives: To evaluate the selective expression of PARP-1 and PARP-2 and PARP activity in lung tumors of mice treated with immunomodulators (anti-CD-137, anti-CTLA-4, anti-PD-1, and anti-CD-19).
Methods: Three groups of wild-type BALB/C mice were established (N = 9/group): non-tumor control mice, tumor-bearing mice, and treated mice. Lung tumors (LP07 adenocarcinoma) were harvested from the lungs of mice treated with a cocktail of immunomodulators after one month. In lung tumor and non-tumor specimens, PARP-1 and PARP-2 expression (immunoblotting) and active PARP polymers (immunohistochemistry) were assessed with selective monoclonal antibodies in all mice.
Results: 1) PARP expression and activity in tumors compared to non-tumor lungs: Whereas PARP-1 expression was significantly lower in the tumors (both treated and non-treated mice) than in the non-tumor lung specimens, PARP-2 did not significantly differ among the groups. PARP activity did not significantly differ between tumors and non-tumor lungs. 2) PARP expression and activity in tumors between treated and non-treated mice: Whereas PARP-1 and PARP-2 expression did not significantly differ between tumors specimens in either treated or non-treated mice, immunomodulators elicited a significant rise in PARP activity in tumors of treated animals compared to non-treated mice.
Conclusions: In lung tumors of BALB/C mice, PARP-1 and PARP-2 were differentially expressed compared to non-tumor lungs. Treatment with the immunomodulators induced a rise in PARP activity in the tumors of the treated mice. These results suggest that PARP may have interfered with the immune profile of the tumor microenvironment. Future studies should be devoted to elucidating the specific biological mechanisms of PARP in response to immunomodulators.

IMMUNOPHENOTYPE AND GENE SIGNATURE OF LONG-TERM SURVIVORS WITH NORMAL ALLOGRAFT FUNCTION AFTER LUNG TRANSPLANTATION

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Introduction: The development of chronic lung allograft dysfunction (CLAD) is the leading limitation of long-term survival with good allograft function (LTS) after lung transplantation (LT).
Objectives: The aim of this study was to identify leukocyte subpopulations and to develop a gene model which allows the discrimination of LTS patients.
Methods: The cell markers and mRNA expression levels were compared between LTS (n = 30) and CLAD patients (n = 30) using flow cytometry and microarray techniques. Gene classifiers were built using supervised machine learning methodology and the expression of selected classifier-genes was confirmed by RT-qPCR.
Results: In LTS patients, the percentages of CD14high CD16- monocytes, CD56+ CD16- NK cells, CD4-CD8- alpha beta T cell subset and CD62L+ granulocytes were elevated whereas the percentage of Vd1+ gamma delta T cell subpopulation was significantly decreased. The computational process led to the identification of the 25 most relevant genes for LT recipients classification. LASSO method with the 25 classifier genes yielded the optimal results with an Area Under the Curve of 0.87 (sensitivity: 0.79 and specificity: 0.80). RT-qPCR analysis confirmed the differential expression of 17/18 of the genes selected from the microarrays. The combination of these markers in a model could ultimately improve classification performance.
Conclusions: This study identifies a set of lymphocyte subsets and genes associated with LTS after LT which should be validated in an independent LT cohort. The combination of these markers may have utility as a medical tool for safe immunosuppression minimization in LT population.
Funding: Study financed by ISC III (PI13/01076), FEDER, FUCAP, Astellas, Novartis and Chiesi.

IMMUNOTHERAPY WITH MONOCLONAL ANTIBODIES IN LUNG CANCER OF MICE: OXIDATIVE STRESS AND OTHER BIOLOGICAL EVENTS

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Introduction: Lung cancer (LC) is a major leading cause of death worldwide. Immunomodulators that target several immune mechanisms have proven to reduce tumor burden in experimental models through induction of the immune microenvironment. We hypothesized that other biological mechanisms may also favor tumor burden reduction in lung cancer-bearing mice treated with immunomodulators.
Objectives: To assess the levels of oxidative stress, antioxidant enzymes, apoptosis, autophagy, and cell proliferation rates in the subcutaneous lung adenocarcinoma tumors of BALB/c mice treated with a combination of immunomodulators (anti-PD1, anti-CTLA-4, anti-CD137, and anti-CD19 monoclonal antibodies).
Methods: Tumor weight, area, and immune cells (T, B, macrophages, and TNF-alpha levels, immunohistochemistry) and tumor growth, oxidative stress, apoptosis, autophagy, and signaling (NF-kB and sirtuin-1) markers were analyzed (immunoblotting) in subcutaneous tumor of BALB/c mice injected with LP07 adenocarcinoma cells treated with monoclonal antibodies (CD-137, CTLA-4, PD-1, and CD-19, N = 9/group) and non-treated control animals.
Results: Compared to non-treated cancer mice, in tumors of monoclonal-treated animals, tumor area and weight and ki-67 significantly reduced, while T cell counts, oxidative stress, apoptosis, autophagy, activated p65, and sirtuin-1 marker increased.
Conclusions: Immunomodulators elicited a reduction in tumor burden (reduced tumor size and weight) through decreased tumor proliferation and increased oxidative stress, apoptosis, autophagy, and signaling levels, which may have interfered with the immune profile of the tumor microenvironment. Future research should be devoted to the elucidation of the specific contribution of each biological mechanism to the reduced tumor burden.

IMPACT OF OBSTRUCTIVE SLEEP APNEA IN THE COGNITIVE EVOLUTION OF ALZHEIMER'S DISEASE PATIENTS

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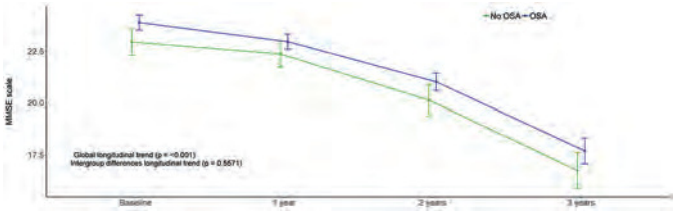
Introduction: During the last decades, the number of studies exploring the impact of obstructive sleep apnea (OSA) in the cognitive function increased substantially. In addition, the prevalence of OSA in Alzheimer's disease (AD) patients is up to 2-times higher compared to its prevalence in the general population. Considering this and the evidence present in the literature, we hypothesized that OSA could be worsening the cognitive evolution of patients with AD.
Objectives: To investigate the effect of OSA on the cognitive evolution of patients with AD.
Methods: In this prospective, single-center study (NCT02814045), patients with mild-moderate AD were evaluated at the baseline and after 12, 24 and 36 months of follow-up. OSA was defined as an apnea-hypopnea index (AHI) > 15/h. Primary outcome was measured by the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-cog) and Mini-Mental State Examination (MMSE).
Results: The cohort included 146 patients with 125 validated PSGs, from which 40 patients were diagnosed as non-OSA (32%) and 85 as OSA (68%). The median [IQR] age of the individuals was 75.0 [72.0; 80.0] years and the majority was composed of women (57.25%). In addition, the mean (SD) MMSE score at the baseline was 23.53 (2.23). There was a cognitive decline along the 3 years of follow-up according to the MMSE score ($p < 0.001$) (Figure), but no differences between the groups were observed. Differently, in the ADAS-cog score, the non-OSA group had a worse cognitive evolution. In fact, the mean

(SD) change at the 12 months of follow-up was 2.97 (5.73) and 0.29 (5.65) for the non-OSA and OSA groups, respectively. The estimated mean (95%) difference between the groups was -2.76 (0.12 to 0.16) (p = 0.033).



Cognitive evolution during the 36 months of follow-up according to the MMSE score.

Conclusions: OSA was not associated with a worse cognitive evolution after 36 months of follow-up. Further studies will be necessary to investigate the beneficial effect of OSA demonstrated by the ADAS-cog score.

INTEGRATED MRNA AND MIRNA EXPRESSION PROFILING OF LONG-TERM SURVIVORS WITH NORMAL ALLOGRAFT FUNCTION AFTER LUNG TRANSPLANTATION

A. Mendoza-Valderrey[1,2], S. Gómez-Ollés[1,2,3], R. Gonzalo[4], Á. Sánchez-Pla[4], M.P. Hernández-Fuentes[5], R. Escobar[1,2], C. Berastegui[1,2], B. Sáez[1,2], A. Solé[6], F. Zurbano[7], M. de la Torre[8], R. Laporta[9], J. Redel[10] and A. Román[1,2,3]

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Introduction: Long-term survival with good allograft function (LTS) after lung transplantation (LT) is mainly limited by the development of chronic lung allograft dysfunction (CLAD).
Objectives: The objective of this study was to identify genes and miRNAs which might contribute to a better understanding of the molecular mechanisms influencing LTS after LT.
Methods: The mRNA and miRNAs expression profiles were determined in blood samples from LTS (n = 30) and CLAD patients (n = 30) by microarray technology. Gene Ontology was used for enrichment analysis and the interactions between miRNAs and genes were studied by network analysis with the mixOmics R package.
Results: The analysis of mRNA expression revealed that 458 genes were differentially expressed between LTS versus CLAD patients. Genes related to neutrophil-granulocyte activation and neutrophil degranulation were downregulated in LTS, suggesting a dysregulation of the innate immune system in CLAD patients. Regarding miRNAs expression analysis, 36 mature miRNAs were differentially expressed between both groups. Of these 36 elements, 30 miRNAs had experimentally validated target genes enriched in the set of 458 differentially expressed genes. Biological significance analysis showed that miRNA target genes differentially expressed between LTS and CLAD patients were related to neutrophil degranulation.
Conclusions: Differences in both mRNA expression and upstream miRNA regulators have been found between LTS and CLAD patients. These results suggest that innate immune system may play a role in long-term survival after LT.
Funding: Study financed by ISCIII (PI13/01076), FEDER, FUCAP, SEPAR (138/2016), Astellas, Novartis and Chiesi.

INTEGRATIVE TRANSNATIONAL ANALYSIS TO DISSECT COMPLEX TUBERCULOSIS TRANSMISSION EVENTS INVOLVING MIGRANTS

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Introduction: In the current global scenario, a precise dissection of the tuberculosis (TB) transmission dynamics involving migrants is a challenging task. The epidemiology of tuberculosis may involve independent importations of Mycobacterium tuberculosis strains predominant at their countries of origin, transmission after arrival to the host country and earlier transmissions en-route. These options could overlap in settings with high proportion of migrants, as in Almeria, where in 2018 a 66% of the cases involved migrants.
Objectives: To characterize the transmission patterns of migrants from the Horn of Africa (HA) diagnosed in Almería. To combine Whole genome sequencing (WGS), epidemiological interviews and in vitro infection assays for an in-depth analysis of transmission dynamics. To develop simplified molecular tools to optimize the surveillance of transmission.
Methods: 24-loci-MIRU-VNTR analysis. WGS analysis (MiSeq-Illumina). U937-cells infection and CFU counting. Epidemiological interview (chronology of symptoms and migratory route). ASO-PCR design targeting strain-specific SNPs.
Results: TB was diagnosed in eight migrants from Somalia just after arrival to Almería, four sharing the same MIRU-VNTR pattern (Horn of Africa Cluster, HAC). This pattern was identified in seven HA-migrants at an asylum camp in The Netherlands. WGS split HAC in two branches: one involving 3 cases from Almería and 7 from the Netherlands (0-1 SNPs among them) and the other including 1 case accumulating 44 SNPs. The strain from the major branch did not exhibit higher success in the infection assay. The interview revealed a prolonged link along the migratory route for two of these cases. A specific ASO-PCR was optimized to target the successfully transmitted HAC strain.
Conclusions: An integrative transnational effort was key to determine the overlapping role of independent importation of strains, related variants and transmission en-route. Epidemiological, more than bacterial factors, may be responsible for the high number of patients infected en-route by the HA strain. The strain-specific PCR will allow an optimized surveillance.

IN VIVO CORRELATION OF RIGHT VENTRICLE HYPERTROPHIA AND LUNG METABOLIC REPROGRAMING IN A MONOCROTALINE RAT MODEL OF PULMONARY HYPERTENSION

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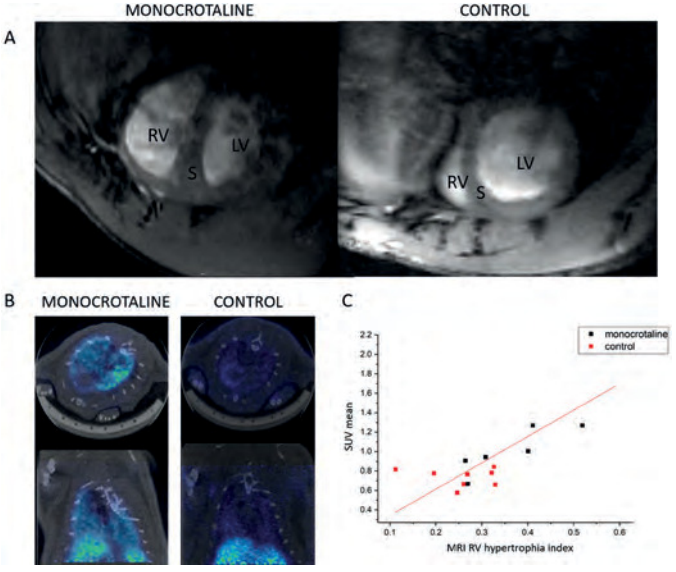
Introduction: Pulmonary arterial hypertension (PAH) is a rare form of pulmonary hypertension that primarily affects the lung microvasculature. In PAH, the gradual obliteration of the arterial lumen results in a progressive increase in pulmonary vascular resistance, leading to right ventricle (RV) hypertrophia and death. Recently, we identified a wider metabolic reprogramming in lung tissue in a rodent model of PAH (Izquierdo-García et al, Front Cardiovasc Med. 2018;5:110). Specifically, aberrant choline metabolism was identified as a feature of cell proliferation and metabolic change, representing a new possible biomarker for PAH characterization.
Objectives: *In vivo* monitoring of choline metabolism by Positron Emission Tomography (PET) imaging as a feature of PAH development.
Methods: PAH was induced in male Sprague Dawley rats (MCT group) by a single injection of monocrotaline. Three weeks after administration, MCT and controls rats were functionally characterized by cardiac MRI and 11C-Choline PET imaging. Afterwards, ventricular pressure was measured through right heart catheterization via jugular vein. The heart was removed, and right ventricle (RV) hypertrophy (Fulton's index) was measured by weighing the RV relative to the left ventricle plus septum. Lungs were fixed and embedded in paraffin.
Results: MCT rats showed a significant increment in ventricular pressure (111%, $p < 0.05$), lung arterioles medial wall thickness index (218%, $p < 0.05$) and Fulton's index (78%, $p < 0.05$) as is characteristic in PAH. RV versus Left ventricle volume ratios measured by In vivo Cardiac MRI (78%, $p < 0.05$) and lung [11C]Choline uptake (62%, $p < 0.05$) were significantly increased in MCT rats versus control animals (Fig. A & B). More importantly, MRI RV Index and lung choline standardized uptake value (SUV) mean are significantly correlated ($R = 0.66$, $p < 0.01$; Fig. C).



A. MRI transversal images of the heart from a MCT rat (left) and a control rat (right). B. Fused [11C]Choline positron emission tomography (PET)/computed tomography (CT) images of the lung parenchyma. C. Standardized uptake value (SUV) mean-MRI Fulton's index correlation (R2 = 0.657).

Conclusions: We demonstrated that RV hypertrophy and lung choline metabolism are correlated in our model of PAH, pointing the advantages of individual studies. [11C]Choline PET imaging is a potential *in vivo* biomarker for PAH diagnosis and therapeutic response in PAH.

LONG-TERM NONINVASIVE VENTILATION IN OBESITY HYPOVENTILATION SYNDROME WITHOUT SEVERE OBSTRUCTIVE SLEEP APNOEA

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Introduction: Noninvasive ventilation (NIV) is an effective form of treatment in obesity hypoventilation syndrome (OHS) with severe obstructive sleep apnoea (OSA). However, there is paucity of evidence in OHS patients without severe OSA phenotype.
Objectives: We performed a multicentre, open-label randomised controlled trial to determine the long-term comparative effectiveness of NIV and lifestyle modification with at least three years of follow-up using hospitalization days/year as the primary outcome measure.
Methods: In this multicentre, open-label parallel group clinical trial performed at 16 sites in Spain, we randomly assigned 98 stable ambulatory patients with untreated OHS and apnoea-hypopnoea index < 30 events/hour to NIV or lifestyle modification (control) using simple randomization through an electronic database. The primary end point was hospitalization days/year. Both investigators and patients were aware of the treatment allocation; however, treating clinicians from the routine care team were not aware of the treatment allocation. The study was stopped early in its 8th year due to difficulty identifying OHS patients with no severe OSA. The analysis was performed according to the intention-to-treat and per-protocol principles.
Results: 49 patients in the NIV group and 49 in the control group were randomised and 48 patients in each group were analysed. During a median [IQR] follow-up of 4.98 [2.98; 6.62] years, mean (SD) hospitalization days/year was 2.60 (5.31) in the control group and 2.71 (4.52) in the NIV group [adjusted rate ratio (95% CI) 1.07 (0.44; 2.59) ($p = 0.882$)]. Adverse events were similar between arms.
Conclusions: In stable ambulatory patients with OHS without severe OSA, NIV and lifestyle modification had similar long-term hospitalization days-year. Larger studies are necessary to better determine the long-term benefit of NIV in this subgroup of OHS.

LUNG CANCER PREVALENCE IN A LUNG CANCER SCREENING PROGRAM

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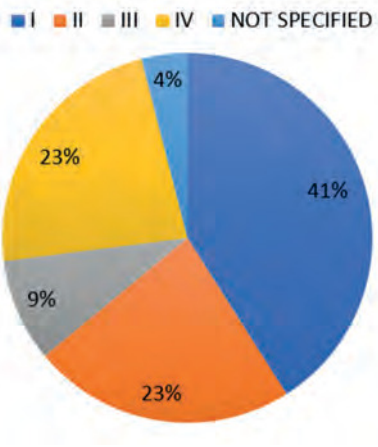
Introduction: Lung cancer is the second most frequent cancer and leading cause of cancer-related deaths. Lung cancer screening using low-dose computed tomography (LDCT) has demonstrated an improvement in survival in patients with this disease.

Objectives: Our objective is to describe lung cancer cases diagnosed with the early detection program at Fundación Jiménez Díaz Hospital in Madrid, between 2014 and 2018.
Methods: Prospective study with cross-sectional analysis of the lung cancer screening program cohort of 1005 patients. Included patients meet the NLST criteria (age between 55 and 74, age-pack history > 30) and have an additional risk factor of emphysema and/or CODP. 949 patients have a LDCT at the moment of the analysis. Different clinical features, pulmonary function tests, and LDCT findings were recorded. Patients were followed according to the I-ELCAP protocols. The statistical analysis such as mean, median, frequency and standard deviation was made with the SPSS 22.0 program.
Results: A total of 22 cases were diagnosed with lung cancer at the moment of the analysis, with a prevalence of 2.31%. The most frequent type of lung cancer was adenocarcinoma (64%), followed by squamous cell (22.7%). 64% of patients were stages I and II at diagnosis (Figure). Surgery was the most frequent treatment in 64% of the patients, most of them underwent lobectomy with lymphadenectomy. One-year, two-year and four-year survival was 100%; 90.9% and 90.9% respectively (median = 93.93%). Emphysema was present in 86.4% of patients and 80% were active smokers. CODP and emphysema association was observed in 72.7% of the patients. Only 2 deaths occurred, one in a patient with advanced stage IVB and another one with complications after treatment. The patients´ clinical features are described in the table.



Lung cancer stages.

Clinical features of patients with lung cancer

	N = 22
Age	65.08 ± 4.86
Men, n (%)	16 (72.7)
Smokers, n (%)	19 (86.4)
Pack -years	61.59 ± 18.12
COPD, n (%)	18 (81.8)
GOLD 1; 2; 3; 4. n (%)	2 (13.6); 13 (59.1); 2 (9.1); 0 (0)
LDCT	
Emphysema, n (%)	19 (86.4)
No emphysema, n (%)	3 (13.6)
Associations	
COPD and emphysema, n (%)	15 (72.72)
COPD without emphysema, n (%)	2 (9.09)
Emphysema without COPD, n (%)	3 (13.63)
No COPD no emphysema, n (%)	1 (4.5)

Conclusions: The prevalence of lung cancer in our cohort is 2.31%. The majority were diagnosed at early stages with a short-term median survival of 94%.

MIRNAS SIGNATURE IN SERUM: A BIOMARKER FOR DIFFERENTIATION OF ASTHMA AGAINST ACO AND COPD

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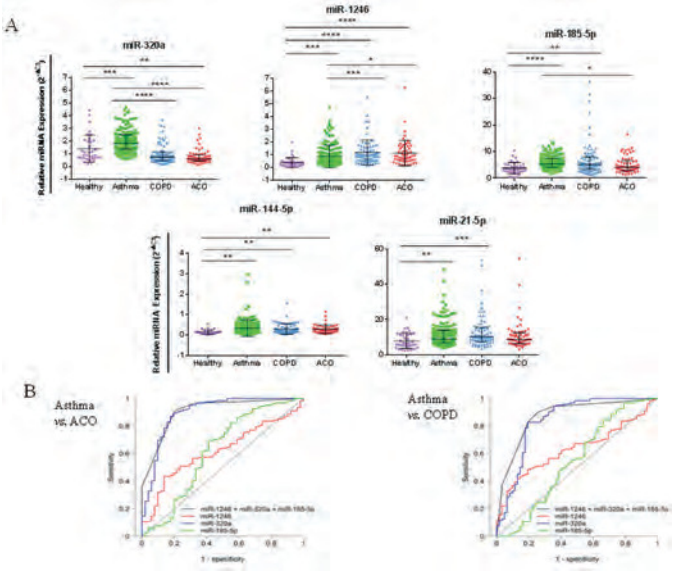
Introduction: Asthma and chronic obstructive pulmonary disease (COPD) are respiratory diseases that have some differences but can present concomitantly in the same patient (asthma-COPD overlapping or ACO). Novel disease specific biomarkers are needed to differentiate these similar pathologies. MiRNAs are small noncoding RNAs related to disease status that have been described as promising biomarkers.
Objectives: Evaluate if a previously described serum miRNA profile in asthma, is able to differentiate asthma against COPD and ACO.
Methods: Five coded miRNAs were studied in serum from 75 COPD patients, 63 ACO patients, 138 asthmatics and 39 healthy subjects by qPCR. Differences were calculated by Student T-test or Mann-Whitney U test. Analysis for biomarker determination was performed by ROC curve. Multivariate quantitative logistic regression models were performed using R.
Results: We found that miR-1 and miR-3 were differentially expressed in serum from asthmatics compared to patients with COPD and ACO; and miR-4 was upregulated in asthma versus ACO. Nevertheless, only miR-3 was able to differentiate asthmatics from COPD and ACO (AUC > 0.80). MiR-1, miR-2, miR-4 and miR-5 were differentially expressed between COPD and healthy subjects; besides, miR-1 and miR-2 relative expression may be used to differentiate between them. This was similar for ACO where miR-1, miR-2 and miR-3 were differentially expressed and can differentiate ACO from healthy controls. We did not find any differentially miRNA expressed between COPD and ACO patients. Combined expression of miR-1, mir-3 and miR-4 in qualitative logistic regression improves the differentiation



A. Relative miRNAs expression for each disease (Asthma, COPD and ACO) and healthy controls. B. Graphical representation of the ROC curves obtained for the individual miRNAs and the logistic regression models for asthma versus COPD and astha versus ACO.

Diagnosis capacity of individual miRNAs or in combination for disease differentiation

miRNAs individual ROC curve	Cutoff (2-ΔCt)		Sens	Spec	PPV	NPV	AUC (CI 95%)
miR-1246 healthy vs. ACO	0.41		0.84	0.78	0.86	0.76	0.85 (0.77-0.93)
miR-144-5p healthy vs. ACO	0.16		0.73	0.74	0.82	0.62	0.75 (0.64-0.86)
miR-320a healthy vs. ACO	0.64		0.58	0.80	0.83	0.55	0.72 (0.61-0.82)
miR-1246 healthy vs. COPD	0.51		0.81	0.87	0.92	0.71	0.87 (0.80-0.95)
miR-144-5p healthy vs. COPD	0.15		0.69	0.71	0.83	0.54	0.75 (0.65-0.86)
miR-320a asthma vs. ACO	0.99		0.82	0.89	0.77	0.92	0.90 (0.85-0.96)
miR-320a asthma vs. COPD	1.17		0.80	0.82	0.70	0.89	0.84 (0.78-0.91)
Qualitative Logistic Regression (miR-320a + miR-185-5p + miR-1246)	Sens	Spec	PPV	NPV	AIC	HL	AUC (CI 95%)
Asthma versus ACO	0.92	0.78	0.91	0.80	115.4	1.00	0.91 (0.86 - 0.95)
Asthma versus COPD	0.90	0.74	0.87	0.79	153.2	0.99	0.87 (0.82 - 0.93)
ACO versus COPD	0.44	0.77	0.69	0.52	158.7	0.99	0.62 (0.52 - 0.72)
Quantitative Logistic Regression (miR-320a + miR-185-5p + miR-21-5p)	Sens	Spec	PPV	NPV	AIC	HL	AUC (CI 95%)
Asthma versus ACO/COPD	0.90	0.93	0.93	0.91	117.2	0.79	0.96 (0.94 - 0.99)

of asthma from COPD (AUC = 0.90), while miR-3 alone is the most appropriate to differentiate asthma and ACO. Combination of miR-3, miR-4 and miR-5 in a quantitative regression model could differentiate asthmatics against ACO/COPD together with an AUC of 0.96.
Conclusions: We found that serum microRNAs can be used to differentiate asthma from COPD or ACO.

MUC1 BIOACTIVATION CONTRIBUTES TO LUNG FIBROSIS

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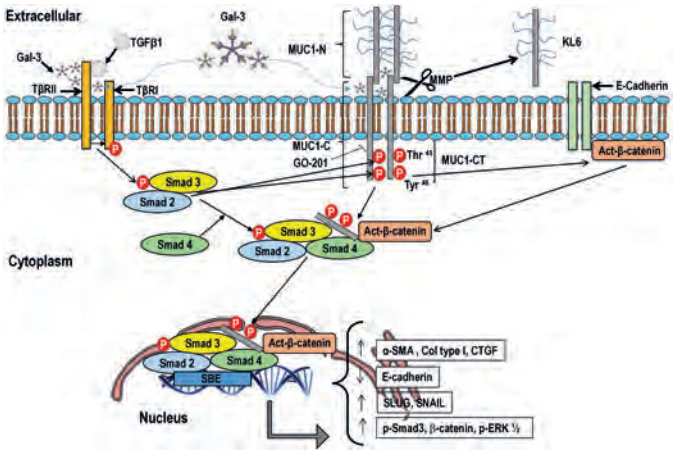
Introduction: MUC1 is a transmembrane mucin whose extracellular domain contains the KL6 epitope, which serves as serum biomarker in IPF. However, there is no evidence on the role of MUC1 intracellular bioactivation in the development of IPF.
Objectives: To characterize MUC1 intracellular bioactivation in IPF.
Methods: The expression of MUC1-CT and its intracellular phosphorylations at Thr41 and Tyr46 was analysed by western blot and immunohistochemistry in healthy and IPF lung tissue. Primary alveolar type II (ATII) cells and fibroblasts were stimulated with TGFβ1 and galectin 3 to evaluate the role of MUC1 on the epithelial and fibroblast to mesenchymal transition, as well as MUC1 intracellular interactions, which were also evaluated in lung tissue from human healthy/IPF. A model of bleomycin-induced lung fibrosis was used in MUC1-Knockout (KO) and Wild type (WT) mice. An inhibitor of MUC1-CT nuclear targeting (GO-201) was used to study in vitro and in vivo the effect of MUC1-CT nuclear translocation on IPF development.
Results: The expression of MUC1-CT and its phosphorylated forms was increased in lung tissue from IPF patients and bleomycin-induced fibrotic mice. TGFβ1 induced Smad3 phosphorylation and following it increased MUC1-CT Thr41 and Tyr46 phosphorylation's, thus increasing the expression of the active β-catenin to form a nuclear complex of phospho-Smad3/MUC1-CT and MUC1-CT/β-catenin. The nuclear complex activated ATII and fibroblast to myofibroblast transitions as well as cell senescence and fibroblast proliferation. The inhibition of MUC1-CT nuclear translocation reduced in vitro the fibrosis development. The pro-fibrotic galectin 3 directly activated MUC1-CT and served as a bridge between TGFβ receptors and MUC1-C terminal domain, indicating a TGFβ1 dependent and independent bioactivation of MUC1-CT. In vitro results were confirmed in vivo by the use of GO-201 inhibitor and MUC1-KO bleomycin-induced fibrotic mice.



Schematic showing novel evidence on the bioactivation of MUC1-CT in IPF. MUC1 cytoplasmic tail (MUC1-CT) and its phosphorylated forms at Thr41 (1224) and Tyr46 (1229) are overexpressed in IPF lung tissue. Thr41 (1224) and Tyr46 (1229) MUC1-CT phosphorylations are induced by TGFβ1. TGFβ1 binds to TGFβ1 receptor (TβRI/II) leading to recruitment and phosphorylation of Smad3. Phosphorylated Smad3 promotes the phosphorylation of MUC1-CT at Thr41 and Tyr46, thus increasing the active form of β-catenin. MUC1-CT forms protein complexes with phospho-Smad3 and active (act)-β-catenin in response to TGFβ1 and this stimulus promotes the nuclear translocation of phospho-Smad3/MUC1-CT and β-catenin/MUC1-CT complexes, required to activate Smad binding element (SBE) DNA sequence to promote pro-fibrotic gene expression, proliferation or cell senescence. Galectin 3 (Gal-3) binds to TβRI/II and reinforces TGFβ1 activation of Smad3 and MUC1-CT Thr41 (1224) and Tyr46 (1229) phosphorylations thus increasing the amount of act-β-catenin. Furthermore, Gal-3 binds to MUC1-CT, activating directly MUC1-CT Thr41 (1224) and Tyr46 (1229) phosphorylations and act-β-catenin. The MUC1-CT nuclear translocation is mediated by a mechanism that is dependent on its oligomerization. In this regard, GO-201 blocks the MUC1-C terminal CQC motif and abrogates oligomerization, TGFβ1-induced MUC1-CT nuclear translocation and MUC1-CT transcriptional function.

Conclusions: MUC1-CT bioactivation is enhanced in IPF and may lead to future strategies as a druggable target for IPF.

MUCOID *PSEUDOMONAS AERUGINOSA* ALTERS SPUTUM VISCOELASTICITY IN PATIENTS WITH NON-CYSTIC FIBROSIS BRONCHIECTASIS

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Introduction: *Pseudomonas aeruginosa* could acquire a mucoid phenotype due to mutations in mucA (mucoid *Pseudomonas aeruginosa*-mPA) that is a hallmark of poor prognosis in patients with bronchiectasis. Despite the higher prevalence of *Pseudomonas aeruginosa* in bronchiectasis, how mPA and non-mucoid *Pseudomonas aeruginosa* (non-mPA) phenotypes could affect viscoelastic properties of sputum is unknown.

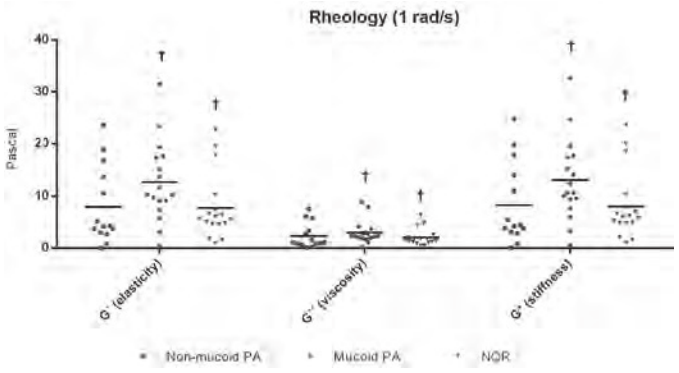
Objectives: Our aim was to determine the relationship between *Pseudomonas aeruginosa* phenotypes isolation, the viscoelastic properties of sputum and the clinical outcomes in patients with bronchiectasis.
Methods: A cross-sectional study was conducted of sputum samples obtained by spontaneous expectoration and sent for microbiology and rheology analysis. Elasticity and viscosity were measured at two oscillatory frequencies (1 and 100 rad/s). Socio-demographic and clinical data were recorded.
Results: We analyzed 17 patients with mPA, 14 with non-mPA and 17 with no organism reported (NOR). Compared with the NOR group, the mPA group showed higher elasticity (median 10.30 vs. 5.70, p = 0.023), viscosity (2.40 vs. 1.50, p = 0.039), and stiffness (10.70 vs. 6.00, p = 0.024). Values in the mPA group tended to be higher compared

Population baseline characteristics

	All patients	PA		NOR	p value
		mPA	Non-mPA		
	N = 48	N = 17 (35%)	N = 14 (29%)	N = 17 (35%)	
Baseline characteristics					
Female sex, n (%)	31 (64)	13 (76)	7 (50)	11 (65)	0.389
Age, mean (SD), years	68.5 (16)	61.7 (18.4)	71.8 (11.4)	72.5 (15.6)	0.096
BMI, mean (SD), Kg/m^2	24 (3.29)	24 (3.8)	23.8 (3.5)	24.2 (2.7)	0.925
Former smokers, n (%)	14 (29)	2 (12)	7 (50)	5 (29)	0.067
Smoking habit, mean (SD), packs/year	39 (32)	44.5 (51.6)	47.6 (29.8)	32.8 (34)	0.880
Chronic colonization, n (%)	36 (75)	15 (88)	13 (93)	8 (47)	0.003
Pseudomonas aeruginosa	34 (95)	15 (88)	13 (93)	6 (86)	< 0.001
Haemophilus influenza	2 (5)	0 (0)	0 (0)	2 (12)	0.419
Dyspnea (MRC Scale, 1-5), median [Q1; Q3]	2 [2; 3]	2 [2; 3]	3 [2; 3]	2 [2; 2.5]	0.118
Etiology, n (%)					0.114
Post-infectious	19 (40)	6 (35)	6 (43)	7 (41)	0.905
Idiopathic	13 (27)	8 (47)	2 (14)	3 (18)	0.081
Others	16 (33)	3 (17)	6 (43)	7 (41)	0.083
CCI Index, n (%)					0.357
Low (1-2 points)	36 (75)	12 (70)	13 (93)	11 (65)	0.179
Moderate (3-4 points)	8 (16)	4 (23)	0 (0)	4 (24)	0.144
High (≥ 5 points)	4 (8)	1 (6)	1 (7)	2 (12)	0.814
Therapy, n (%) [a]					
Oral Antibiotic	14 (25)	4 (23)	4 (28)	6 (86)	0.426
Nebulized Antibiotic	12 (21)	5 (29)	4 (28)	3 (17)	0.426
Bronchodilators	40 (83)	15 (88)	12 (86)	13 (76)	0.642
Nebulized Saline Solutions	6 (12)	2 (12)	1 (7)	3 (17)	0.751
Variables of severity					
Exacerbations last year, median [Q1; Q3]	2 [2; 3]	2 [2; 4]	2 [1; 3]	2 [1.5; 2]	0.031[b]
Hospitalizations last year, median [Q1; Q3]	0 [0; 1]	1 [0; 1]	0 [0; 0]	0 [0; 0]	0.021[bc]
Lobes affected (HRCT), median [Q1; Q3]	3 [3; 5]	5 [3.5; 5.5]	3 [2.75; 4]	3 [2; 4]	0.015[bc]
BE severity (BSI stages), n (%)					0.527
Mild: 0-4	4 (8)	0 (0)	1 (7)	3 (17)	0.172
Moderate: 5-8	11 (23)	5 (29)	3 (21)	3 (17)	0.691
Severe: ≥ 9	31 (65)	11 (65)	10 (71)	10 (59)	0.867
Sputum color, n (%)					
Mucoid	1 (1)	0 (0)	0 (0)	1 (5)	0.402
Mucopurulent	19 (39)	4 (23)	5 (35)	10 (59)	0.108
Purulent	28 (58)	13 (76)	9 (64)	6 (35)	0.048[b]
Pulmonary Function, mean (SD)					
FEV_1, % predicted	66 (23)	66 (20)	58 (19)	75 (28)	0.137
FEV_1, L	1.62 (0.72)	1.63 (0.59)	1.53 (0.72)	1.83 (0.86)	0.531
FVC, % predicted	76 (17)	77 (16)	71 (16)	81 (20)	0.317
FVC, L	2.62 (0.85)	2.54 (0.75)	2.57 (0.97)	2.74 (0.89)	0.793
FEV_1/FVC, %	70 (19)	73 (18)	62 (17)	75 (20)	0.128

Percentages calculated on non-missing data. [a]Could have more than 1 medication. [b]p < 0.05 for comparison between mPA and NOR. [c]p < 0.05 for comparison between mPA and Non-mPA.
BE: bronchiectasis; BMI: body mass index; BSI: bronchiectasis severity index; CCI: comorbidity Charlson index; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; HRCT: high-resolution computed tomography; mPA: mucoid *Pseudomonas aeruginosa*; MRC: medical research council; Non-mPA: Non-mucoid *Pseudomonas aeruginosa*; NOR: no organism reported; Q1: first quartile; Q3: third quartile; SD: standard deviation.

with non-mPA. Clinically, the mPA group showed greater hospitalizations during the previous year and greater affected lobes than the non-mPA and NOR groups.



Conclusions: The mPA phenotype is associated with increased elasticity, viscosity and stiffness of bronchiectatic sputum. Viscoelastic properties could be used as a marker of poor mucociliary clearance in mPA, with potentially important clinical implications.

MYCO-MIGE: OPTIMIZATION OF A NEW POWERFUL TOOL FOR MYCOBACTERIAL GENOME ENGINEERING

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Introduction: In 2009, Multiplex Automated Genome Engineering (MAGE) was described as a new powerful tool to introduce multiple mutations in *Escherichia coli,* using a cyclic automated system. MAGE uses the recombineering machinery to recombine ssDNA oligonucleotides (oligos) with the desired mutations into the *E. coli* chromosome. More recently, this technique has been improved following a co-selection strategy, in which, an oligo carrying an antibiotic resistant genotype is added together with the oligos carrying the desired mutations.
Objectives: With the objective to implement this technique in mycobacteria, we propose the Myco-MIGE (Multiplex Iterative Genome Engineering) methodology. In MIGE we manually performed the automation process in an iterative manner following several transformation cycles. *Mycobacterium smegmatis* mc2155 carrying the recombineering system in a plasmid is used as a reliable surrogate host to demonstrate proof-of-concept of this methodology.
Methods: In a first step of Myco-MIGE, we optimized the length and concentration of the mutagenic oligos. Oligos carrying a SNP that confers streptomycin resistance were designed to replace the wild type allele of the rpsL gene (which confers sensibility to streptomycin), thus providing a selectable phenotype. In a next step, we designed oligos to inactivate fourteen efflux pumps in *M. smegmatis,* by placing two consecutive STOP codons (missense mutations) in frame with the corresponding gene in the 5' end of the coding sequence. All these oligos were transformed in *M. smegmatis* together with the oligo conferring streptomycin resistance in a 100:1 ratio, in order to allow isolation of resistant bacteria and follow a co-selection strategy.
Results: After enumerating c.f.u. resulting from Myco-MIGE cycles, it is tempting to speculate that the desired mutations have accumulated in the bacterial population and these mutations are more abundant in the streptomycin resistant population.
Conclusions: Altogether, this tool hold promises to improve genome engineering in the Mycobacterium genus, opening new research avenues.

PREVALENCE OF SENSITIZATION TO AVIAN OR FUNGAL PROTEINS IN DIFFERENT WORK ENVIRONMENTS

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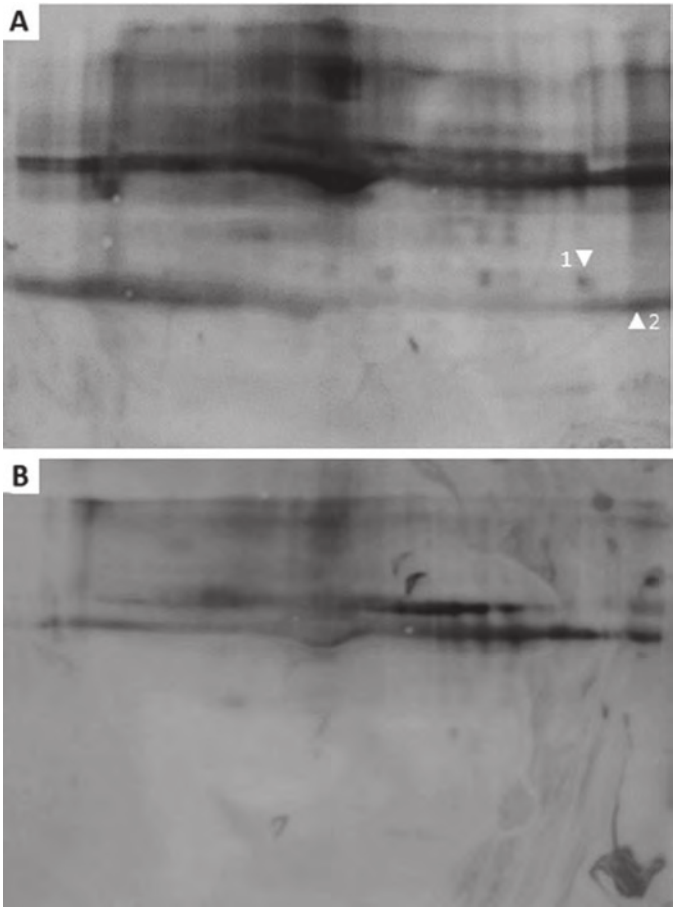
Introduction: Hypersensitivity pneumonitis (HP) is an interstitial lung disease usually caused by the inhalation of avian and fungal proteins. The present study assesses a cohort of Urban Pest Surveillance and Control Service (UPSCS) workers with high exposure to avian and fungal antigens.
Objectives: To identify the degree of sensitization to avian and fungal antigens and the potential risk of developing HP in UPSCS workers.
Methods: Study population: bird investigators and/or managers at UPSCS of the Public Health Agency, Parks and Gardens staff in Barcelona and employees of private urban pest control firms. Workers were divided according to their work activity: Pruning and Others. All individuals underwent a medical interview regarding exposure, pulmonary function tests and specific IgG antibodies. Antigenic proteins of



Western blots against pigeon serum proteins separated in 2D. A) Patient with HP due to pigeon exposure. B) Worker of a private urban pest control firm. Proteins recognised by patients but not by workers are indicated as 1▼ (Ig lambda chain) and 2▲ (Apolipoprotein A-I).

Demographic data, pulmonary function and specific IgG antibodies in the study population

			Pruning (n = 41)	Others (n = 60)	p
Age, median (range)			48 (27-64)	32 (20-62)	< 0.0001
Sex, M n (%)			33 (80)	43 (72)	0.3557
Smoking					0.6046
Smoker, n (%)			8 (23)	13 (23)	
Exsmoker, n (%)			12 (34)	14 (25)	
Non smoker, n (%)			15 (43)	29 (52)	
Pulmonary function	FVC%, median (range)		96,1 (70.2-121.6)	100.3 (76.1-126.8)	0.0386
	FVC < 80%, n (%)		6 (16)	3 (5)	0.1478
	FEV1%, median (range)		96.75 (70-137.4)	100.6 (74-143.6)	0.4616
	FEV1 < 80%, n (%)		3 (8)	2 (4)	0.3770
	TLC%, median (range)		101.3 (73.2-139.6)	95.35 (73.6-158.1)	0.1288
	TLC < 90%, n (%)		9 (24)	22 (38)	0.1862
	DLCO/SB%, median (range)		82.45 (60-110.3)	87.4 (62.4-118.8)	0.4533
	DLCO/SB < 80%, n (%)		14 (39)	14 (24)	0.1637
	DLCO/VA%, median (range)		79.65 (62.5-107.3)	87.9 (63.1-113.1)	0.0104
	DLCO/VA < 80%, n (%)		19 (53)	14 (24)	0.0072
Specific IgGs	Pigeon	median (range)	0.236 (0.031-0.843)	0.156 (0.044-1.802)	0.1574
		% positives	41.5	31.7	
	Parrot	median (range)	0.332 (0.046-1.161)	0.238 (0.049-1.900)	0.1534
		% positives	58.5	36.7	
	Small Parrot	median (range)	0.196 (0.057-1.048)	0.167 (0.052-1.434)	0.390
		% positives	51.2	41.7	
	Parakeet	median (range)	0.258 (0.060-0.757)	0.212 (0.036-1.715)	0.0306
		% positives	24.4	21.7	
	Penicillium	median (range)	0.697 (0.263-2.347	0.931 (0.180-2.387)	0.8919
		% positives	85.4	81.7	
	Aspergillus	median (range)	1.278 (0.346-2.623)	1.412 (0.168-2.545)	0.3035
		% positives	73.2	77.3	

pigeon sera were investigated using 2-dimensional immunoblotting with sera from patients with HP, asymptomatic exposed controls and healthy volunteers. Proteins of interested were sequenced by liquid-chromatography-mass scpectrometry (LC-MS).

Results: 101 workers have been recruited to date (76 men, average age: 42 yrs); 41 in the Pruning group and 60 in the others group. In the Pruning group, specific parakeet IgGs were higher (p = 0.03) and FVC% and DLCO/VA% were lower (p = 0.04 and 0.01, respectively). Two-dimensional immunoblotting showed protein bands of 20-30 KDa recognised by HP patients but not by workers. LC-MS analysis identified Ig Lambda chain and Apolipoprotein A-I as candidate proteins to distinguish HP patients from exposed workers.

Conclusions: A high degree of sensitization to avian and fungal antigens was observed in the study population. In the Pruning group, alterations in some pulmonary function parameters were observed. We identified two pigeon proteins that may play a role in the development of pathological differences between HP patients and exposed workers.

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PROTEIN CORONA INDUCED BY LUNG SURFACTANT INTERACTIONS DETERMINES THE *IN VIVO* FATE OF MICELLAR NANOSTRUCTURES DESIGNED FOR PULMONARY ADMINISTRATION

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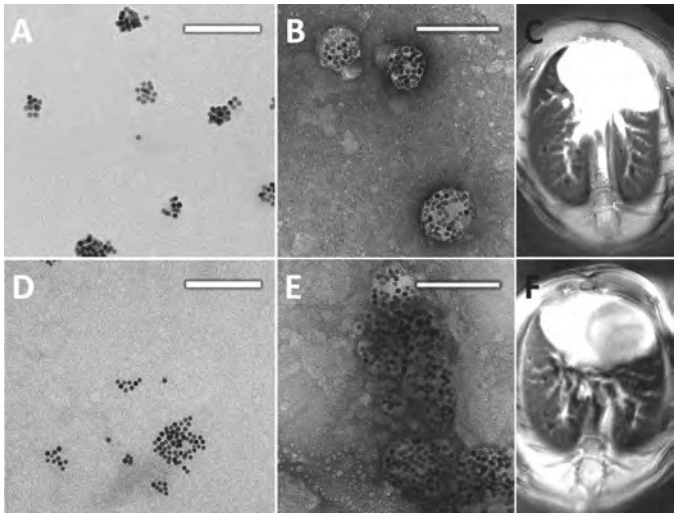
Introduction: The lungs are efficient targets for the administration via the airway of a wide variety of pharmaceutical ingredients. Micelle-based nanomedicines are on the list of investigational drugs. Their first barrier, when they are administered through the lungs, is the pulmonary surfactant. Its interaction with micelles will induce the adsorption of various lipid and protein components referred to as "corona". Recently, it has been shown that this corona determines the fate of many nanomedicines in the lungs.

Objectives: We attempt to: determine the in vivo fate after pulmonary administration of 2 different micelle-like iron oxide nanoparticles based on phosphatidylcholine (PC) and bovine serum albumin (BSA); analyze the effect of their coatings on the alveolar macrophage uptake, lung inflammatory response and lung clearance and explore the interactions between the micelles and one of the major proteins of the lung surfactant, the surfactant protein A (SP-A).

Methods: TEM and DLS were used for characterization. MRI and histopathology were used to analyse the *in vivo* fate and biodistribution. Cell uptake was determined by flow cytometry. Assays for aggregation and bacterial killing were performed.

Results: PC coated nanomicelles were retained within the lungs for weeks while BSA coated nanomicelles stayed only a few minutes. In both cases there was no inflammatory response. The interaction between SPA and micelles showed that SPA is rapidly adsorbed with high affinity on the BSA nanomicelles while in the case of the PC, it induces agglomeration which impedes the phagocytosis work of al-

veolar macrophages which stay mobilize up to 7 days after instillation.



TEM images of micellar BSA-SPION: A. BSA-SPIONs. B. Negative stained BSA-SPIONs after incubation with BAL. D. PC-SPIONs. E. And negative stained PC-SPION after incubation with BAL. MRI images of lungs after administration of C, BSA-SPIONs, and F, PC-SPIONs. Scale bars correspond to 200 nm.

Conclusions: We demonstrate the role of the binding interaction between surfactant proteins and micelles and how it modulates nanoparticle fate and clearance *in vivo* after direct pulmonary administration. This work provide useful information for the development of drug delivery systems based on PC to target alveolar macrophages with extended residence periods.

PULMONARY BIOBANK CONSORTIUM'S STRATEGY TO FIGHT AGAINST LOW REPRODUCIBILITY RATES IN BIOMEDICAL RESEARCH: HOW DO STABILIZATION METHODS AFFECT RNA QUALITY?

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Introduction: Low reproducibility rates have been documented in biomedical research, which undermine cumulative knowledge production and contributes to both delays and costs of biomarker discovery and drug development. Laboratory protocols, study design and biological reagents are identified as the main categories of errors contributing largely to irreproducibility.
Objectives: In that context, Pulmonary Biobank Consortium (PBC) carried out a study to assess the real impact of different stabilization methods on tissue quality and the integrity and functionality of derived biomolecules.
Methods: The influence of four stabilization methods [RNA Later (RNL), snap freezing (SF), snap freezing using Optimal Compound Tissue (SF-OCT) and formalin-fixed paraffin-embedded (FFPE)] on RNA quality and integrity was evaluated in paired samples of lung tissue. RNA integrity was evaluated through PCR-endpoint assays and amplifying seven fragments of different length of the HPRT1 gene, as well as with the RNA Integrity Number. Functionality was evaluated by RT-qPCR focusing on the differential expression of the HPRT1, SNRPD3 and Jun housekeeping genes among the stabilization methods tested.
Results: RNA from RNL and SF-OCT samples showed better integrity compared to SF and FFPE. However, only statistically significant differences were observed between the RNA from FFPE and other stabilization methods when gene expression of HPRT1, SNRPD3 and Jun housekeeping genes were evaluated. For the three mentioned genes, good correlation was obtained between its Cq and RIN values. The present work also describes, for the first time, the instability of SF samples at the moment just before RNA extraction.
Conclusions: Standardization of pre-analytic workflow can lead to improved reproducibility on biomedical research studies. The present study demonstrated clear evidences about the impact of the stabilization method on RNA derived from lung human tissue samples.

PULMONARY CIRCULATORY ALTERATIONS IN A MODEL OF PORTAL HYPERTENSION AND CIRRHOSIS INDUCED BY CCl4

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Introduction: Hepatopulmonary syndrome and portopulmonary hypertension are two pulmonary complications of liver disease responsible for high morbidity and mortality. Both diseases are linked with the development of portal hypertension. Hepatopulmonary syndrome is related to intrapulmonary vasodilation and hypoxemia, whereas portopulmonary hypertension is associated with vasoconstriction and vascular remodelling. The lack of validated models to perform studies is a big limitation to identify new therapeutic targets.
Objectives: The main aim of this project is to characterise the changes in the pulmonary circulation in an experimental model of portal hypertension and cirrhosis induced by the chronic administration of CCl4 in male Sprague Dawley rats.
Methods: Contractile responses were analysed in rat pulmonary arteries mounted in a wire myograph. The right lung was inflated in situ with formol saline through the right bronchus and embedded in paraffin. Lung sections were stained with haematoxylin and eosin and examined by light microscopy, elastin was visualized by its green auto-fluorescence and cross sectional area was measured using image-J software. The right ventricle and the left ventricle plus the septum were dissected and weighed.
Results: Pulmonary arteries of animals with advanced portal hypertension and cirrhosis have a lower response to each vasoconstrictor tested (potassium, serotonin, endothelin and hypoxia) although endothelial function is not significantly altered. These alterations in vascular function are accompanied by cardiac hypertrophy and pulmonary vascular remodelling. Finally, incubation of healthy pulmonary arteries with plasma from cirrhotic rats reproduces the lower response to 5-HT and hypoxia, suggesting the possible involvement of soluble mediators in the development of these pulmonary vascular alterations.
Conclusions: Our data suggests that the development of portal hypertension and cirrhosis after CCl4 treatment leads to a generalised hyporesponsiveness to vasoconstrictor factors which is compatible with the development of hepatopulmonary syndrome.

RADIOFLUORINATED GASES AS REGIONAL VENTILATION MARKERS: APPLICATION TO A RAT MODEL OF ACUTE LUNG INFLAMMATION

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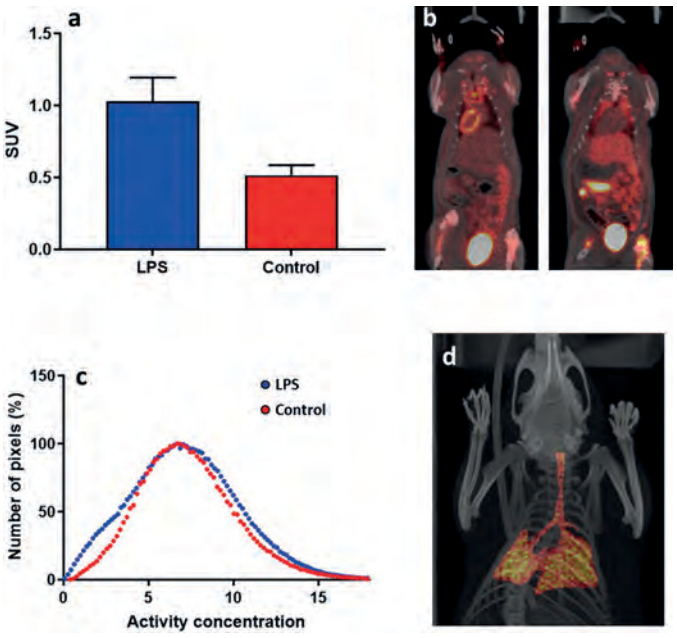
Introduction: Ventilation studies are currently performed with single photon emission computerized tomography (SPECT) using radiolabelled aerosols. However, these show central airway deposition and peripheral "hotspot" formation in patients with obstructive lung diseases. Recently, we have developed a method for the production of the radiofluorinated gas [18F]CF4, which proved efficient in the visualisation of lung ventilation in healthy rodents using Positron Emission Tomography (PET). Here, we describe the investigation of [18F]CF4 as a ventilation marker in an animal model of impaired lung ventilation and correlate the results with [18F]FDG-PET.
Objectives: To evaluate [18F]CF4 as regional ventilation marker in a rat model of lung inflammation.
Methods: [18F]CF4 was produced by a double irradiation process. Ventilation studies were carried out in a rat model of lung inflammation induced by intratracheal administration of lipopolysaccharide (LPS). Dynamic 10-min PET images were obtained at t = 4 hours after administration of LPS, during inhaled administration of the radiofluorinated gas. [18F]FDG-PET static images were also obtained for each animal 2 h after the finalisation of the first imaging study. Ventilation images were reconstructed by OSEM-3D iterative algorithm and voxel-based analysis was carried out to assess the non-uniformity of gas distribution. [18F]FDG-PET images were analysed to determine Standard Uptake Values in the lungs.
Results: Compared to controls, the [18F]FDG uptake in the lungs of LPS-treated rats was almost 2-fold higher at 4 h, with SUV values close to 1 (Figure a). PET images (Figure b) showed higher accumulation in the heart of healthy animals. Ventilation studies in control groups showed uniform distribution of the radiofluorinated gas and fast elimination of the radioactivity after discontinuation of the administration. No apparent ventilation defects could be observed in any of the groups by visual inspection (Figure d), although voxel-based analysis showed histograms with complete different profiles (Figure c).
Conclusions: [18F]CF4 is an appropriate marker of regional lung ventilation.



A. Lung SUV values obtained from PET-[18F]FDG images in LPS-treated and control animals. B.Representative coronal PET-CT images obtained after administration of [18F]FDG to LPS-treated (right) and control (left) rats. C. Histograms derived from voxel-based analysis of PET-CT images obtained after administration of [18F]CF4 to LPS-treated and control rats. D. Representative PET-[18F]CF4 images obtained in an LPS-treated rat.

ROLE OF IL-11 IN PULMONARY FIBROSIS ASSOCIATED TO PULMONARY HYPERTENSION

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Introduction: Pulmonary hypertension (PH) in idiopathic pulmonary fibrosis (IPF) portends a poor prognosis. Currently, no therapy can improve survival of patients diagnosed with this disease. IL-11 molecular pathway is over-expressed in proliferative disorders however, its role in PH- associated IPF is unknown.
Objectives: The aim of this study was to evaluate the expression of IL-11 in IPF patients with or without PH. Also we hypothesized that the stimulation of pulmonary artery smooth muscle cells (PASMCs) and human pulmonary artery microvascular endothelial cells (HMVEC-L) with IL-11 induced the transformation into invasive myofibroblast.
Methods: Human pulmonary artery rings, parenchyma tissue, broncho-alveolar lavage (BAL) and serum were obtained from control subjects (n = 32), IPF (n = 26) and IPF with PH patients (n = 22) to study the expression and predominant distribution of IL-11 and IL-11Rα. The effect of recombinant human IL-11 on pulmonary artery remodeling was evaluated in isolated PASMCs and HMVEC-L.
Results: IL-11 and IL-11Rα were over-expressed in pulmonary arteries, parenchyma, serum and BAL of IPF patients with PH and in a lesser extent in IPF patients compared with control subjects. The inmunostaining and inmunofluorescence revealed a predominant distribution of IL11 and IL-11Rα in remodeled pulmonary arteries of IPF patients and in a greater extent in IPF with PH patients and no expression in control subject. IL-11 induced morphological changes in isolated PASMCs and HMVEC-L characterized by myofibroblast phenotype. On the other hand, it was observed that IL-11 participated in proliferative and senescent processes in vitro. Finally, the main signaling pathways activated by IL11 were determined.
Conclusions: IL-11 and IL-11Rα are over expressed in pulmonary arteries of IPF + PH patients contributing to pulmonary artery remodeling. Pharmacologic modulation of this route may be a promising target for the treatment of this disease.

SALIVARY BACTERIAL LOAD IS ALTERED BY CHRONIC TREATMENT WITH HIGH-DOSE INHALED CORTICOSTEROIDS

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Introduction: Asthma is a common disease usually characterized by chronic airway inflammation. Pharmacological treatment is not always effective to control the disease symptoms, and some patients may develop life-threatening acute episodes called exacerbations. Recently, human microbiota has been associated as a risk factor for asthma and exacerbations. The advent of next generation DNA sequencing has allowed carrying out more sophisticated analyses of the microbiota genetic makeup (known as microbiome).
Objectives: The aim of this project is to analyse the bacterial DNA amount in different biological samples from asthmatic patients, and to determine changes caused by the development of exacerbations or by pharmacological therapies.
Methods: DNA was isolated from saliva samples (n = 99), pharyngeal swabs (n = 63) and nasal swabs (n = 63). Total DNA mass was assessed by spectrophotometry and fluorimetry. Bacterial DNA quantification was carried out simultaneously with the preparation of sequencing libraries of the V3-V4 region of the 16S ribosomal RNA gene by means of quantitative polymerase chain reactions (qPCR).
Results: Differences in the amount of bacterial DNA were found according to the type of biological sample ($p = 2.1 \times 10^{-27}$), with higher amount in saliva. The development of exacerbations or antibiotics/systemic corticosteroids therapies did not alter the bacterial DNA load. However, chronic treatment with high doses of inhaled corticosteroids was found to be associated with increased amounts of bacterial DNA in saliva samples ($p = 0.048$).
Conclusions: The amount of bacterial DNA was higher in saliva when compared to nasal and pharyngeal samples. Furthermore, chronic treatment with inhaled corticosteroids at high doses increases the amount of bacterial DNA in saliva.
Funding: Supported by grant SAF2017-83417R by the Spanish Ministry of Economy and Competitiveness (MINECO/AEI/FEDER, UE).

SETTING UP OF THE BRONCHI-OMICS PROJECT - BRONCHIECTASIS HETEROGENEITY AND SUSCEPTIBILITY TO INFECTION: HOST-MICROBIOME INTERACTIONS AND ITS PREDICTIVE POWER IN CLINICAL PRACTICE

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Introduction: Bronchiectasis (BE) is the third most common chronic respiratory disease characterized by impaired mucociliary clearance, chronic airway inflammation and bacterial colonization that causes an important impact both on the morbidity and mortality of patients and on the health system due to its high healthcare and economic burden. The relevance of BE as a public health problem justified this study to better understand the interaction between airway microorganisms and host and its relationship with severity, activity and progression of BE. In this sense, it's necessary to define variables and unify criteria to create a multicenter cohort.
Objectives: The first objective was the definition of the characteristics of the multicenter cohort and a broad spectrum of biological samples that allows the study of the bronchial microbiota and its interactions with the host by omics.
Methods: 1. Exhaustive review of the literature and definition of clinical and laboratory variables. 2. Expert agreement of the methodology for collection/storage of biological samples and development of protocols with the clinical analysis, microbiology and biobank laboratories. 3. Final consensus of the methodology in a multidisciplinary meeting (Pulmonologists, Microbiologists, Immunologists, Radiologists...), with investigators of the 11 centers involved. 4. Development of informed consent and ethical committees approval, of each center. 5. Final decision about circuits of sample collection, training and initiation visit, in each center.
Results: The recruitment process has started in February 2019 in HUGTiP. At this moment, there are 9/11 hospitals that have started the recruitment phase (about 90 patients).
Conclusions: Despite the complexity of what supposes a multicenter project, we believe that the objectives are being achieved and the problems successfully solved, and we hope finish the recruitment process at the end of this year and begin immediately the analysis of laboratory data (microbiology and immunology), in order to generate the first results of this multicenter cohort.

STUDY OF EVOLUTIVE ADAPTATION TO TUBERCULOSIS IN A *DROSOPHILA* MODEL

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Introduction: The progression form latent tuberculosis infection toward an active disease is related to massive neutrophil infiltration of lesions. However, the innate mechanisms capable of inducing the initial neutrophil response are not known. The *Drosophila melanogaster* model has served to understand the innate immune response against multiple infections, due to the high homology of the immune-related genes with humans.
Objectives: 1. To characterize the innate immune response against *Mycobacterium marinum,* which causes a tuberculosis-like disease in flies. 2. To characterize the response triggered by the oral administration of heat-inactivated *Mycobacterium manresensis* (hkMm) in the *D. melanogaster* model.
Methods: Survival of infected flies and bacillary load at different time points have been evaluated to assess the tolerance and resistance profiles considering sexual dimorphism. The response induced by the administration of hkMm in *D. melanogaster* subjected to *M. marinum* infection and other stress conditions (High Fat Diet) has also been assessed using the survival and the relative genetic expression of some relevant genes of the immune pathways.
Results: Tolerance but not resistance varied after *M. marinum* infection depending on sex and the reproductive status. The oral administration of hkMm reduced the mortality related to exposure to High Fat Diet, but did not affect the infection outcome. However, it triggered a systemic immune response immediately after their uptake, while *M. marinum* took up to 5 days to induce an immune response.
Conclusions: 1. There is a sexual dimorphism in tolerance, also influenced by reproductive status. 2. Oral administration of hkMm has an antioxidant effect, reducing mortality of flies exposed to High Fat Diet. 3. *M. marinum* is able to trigger a systemic immune response in the *Drosophila* model at late stages of the infection. 4. Oral administration of hkMm is able to trigger a faster and systemic immune response in the *D. melanogaster* contrary to *M. marinum* systemic infection.

STUDY OF THE INFLAMMATORY PHENOTYPE OF ASTHMATIC PATIENTS WITH BRONCHIECTASIS

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Introduction: Asthma is a heterogeneous disease with different inflammatory phenotypes. Eosinophilic inflammation is considered the most frequent subtype. However, subjects with bronchiectasis always present a neutrophilic inflammation feature.
Objectives: The aim of the present study is to analyze, in induced sputum, the inflammatory phenotype of a cohort of asthmatic patients with bronchiectasis.
Methods: Cross-sectional study, that included all moderate/severe asthma patients with bronchiectasis referred to our center during 2017-2018. We considered that a patient had eosinophilic inflammation when induced sputum eosinophil count was ≥ 3% and neutrophilic inflammation when neutrophil count was ≥ 65%. Asthma was considered to be controlled when the patient had an ACT ≥ 20.
Results: From 310 patients visited, 90 had bronchiectasis. Twelve patients were excluded. Sputum was obtained in 54 (32 women, mean age: 55 years, 3 smokers). 72% of these patients had an ACT < 20. Mean eosinophils and neutrophils in sputum were 12.2% and 63.1%, respectively. Fifteen patients (28%) presented eosinophilic inflammation, 19 (35%) neutrophilic inflammation, 9 (17%) mixed inflammation and 11 (20%) paucigranulocytic. A correlation was observed between the number of tobacco packs/year and neutrophils in sputum ($p = 0.015$) and between eosinophils in blood and sputum ($p < 0.01$).
Conclusions: In asthmatic patients with bronchiectasis, a high degree of inflammation in sputum was observed. The predominant inflammatory phenotype in these patients is neutrophilic. In most of these patients, asthma control is not achieved.

SURFACTANT LIPIDS ACT AS A BRAKE ON IL-4 + SP-A-MEDIATED M2 ALVEOLAR MACROPHAGE ACTIVATION THROUGH INHIBITION OF AKT-MTORC1 SIGNALING AXIS

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Introduction: Activation of macrophages by interleukin-4 (IL-4) is needed for tissue repair, although excessive activation causes pathology associated with fibrosis. We have recently shown that SP-A in the lung is required for complete IL-4-dependent activation (M2) of alveolar macrophages (aMφs) and successful repair of lung tissue (Science, 2017). However, the effect of surfactant lipids, which are continuously endocytosed by aMφs, on M2 activation is currently unknown.
Objectives: 1) To evaluate the metabolic profile of IL-4 + SP-A-stimulated aMφs incubated with and without lipids; 2) to analyze the effect of surfactant lipids on IL-4 + SP-A-dependent M2 activation; and 3) to investigate the mechanism through which surfactant lipids exert their effects.
Methods: Macrophages were pre-incubated with surfactant lipids to allow their endocytosis and then stimulated with IL-4 and/or SP-A. Metabolic profiles of resting and IL-4 + SP-A-stimulated aMφs with and without lipids were performed by using an XF24 extracellular flux analyzer. Analyses of aMφ activation, proliferation, and signaling were performed by flow cytometry, enzymatic assays, proliferation assays, and Western blot.
Results: aMφs stimulated with SP-A + IL-4 showed increased mitochondrial respiration and glycolysis, which support cell proliferation and the production of extracellular matrix. Surfactant phospholipids blocked the acquisition of the M2 metabolic profile. Consistently, surfactant lipids inhibited SP-A + IL-4-dependent M2 activation and proliferation of aMφs. The mechanism through which endocytosed surfactant lipids inhibited IL-4 + SP-A-dependent M2 activation is inactivation of the Akt-mTORC1 signaling axis.
Conclusions: Our results show that surfactant lipids act as a brake on IL-4- and IL-4 + SP-A–driven metabolic reprogramming of macrophages that results in M2 activation and allows proliferation. We suggest that a high SP-A/lipid ratio might be involved in shifting aMφs to repair mode, whereas a normalized SP-A/lipid ratio might allow macrophages to return to homeostasis. Thus, an unbalanced alveolar SP-A/lipid ratio might contribute to the pathogenesis of diseases caused by altered lung repair mechanisms.

SYSTEM BIOLOGY TO PRIORITIZE ASTHMA AND RESPIRATORY ALLERGY BIOMARKERS

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Introduction: Asthma and respiratory allergy are complex diseases, with a wide clinical spectrum. Due to their heterogeneity and the increase of prevalence in our society, it is necessary to define and evaluate candidate biomarkers which can be useful for diagnosis, prognosis and/or treatment. Previously, we defined and validated experimentally the ability of 94 genes, as molecular biomarkers to differentiate phenotypes of asthma and their severity.
Objectives: To prioritize the role of those biomarkers in nonallergic asthma (NA), allergic asthma (AA), and respiratory allergy (RA), using systems biology tools, based on their association with the disease, through a mechanistic explanation.
Methods: Anaxomics´ TPMS technology (Therapeutic Performance Mapping System) was used to generate a protein-protein interaction network, using our previous experimental results and information from external databases and scientific literature. This network was transformed into three mathematical models, one for each disease. The analysis of them revealed and prioritized potential molecular biomarkers.
Results: Molecular characterization of the pathophysiological processes of the AA defined 16 molecular motives: 2 specific for AA, 2 shared with RA and 12 shared with NA. The mechanistic analysis showed a total of 17 proteins with a high mechanistic relationship with AA. Likewise, 11 proteins were associated with RA, highlighting IL-5, and 16 proteins were related with NA. Specificity analysis showed that 12 proteins were specific of AA, 7 were specific of RA and 2 of NA. Finally, a triggering analysis revealed a relevant role for AKT1, STAT1 and MAPK13 proteins in the three conditions and for TLR4 in the asthmatic pathologies.
Conclusions: The generated models have allowed prioritizing the 94 biomarkers depending on the functionality associated with each disease.

TOWARDS GENOME REMODELLING IN *MYCOBACTERIUM* BY OPTIMIZATION OF A MINIMALISTIC CRISPR-CAS SYSTEM

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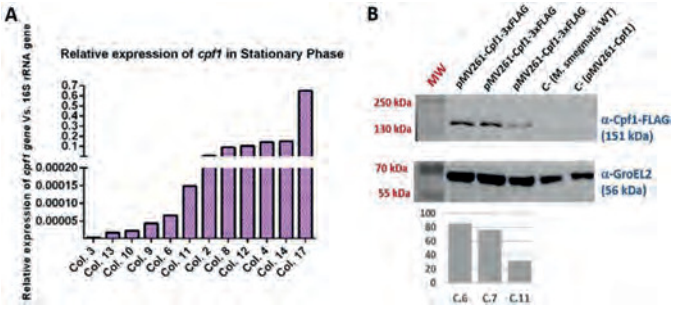
Introduction: Pathogens from *Mycobacterium* genus are causative of diseases as tuberculosis, leprae or Buruli ulcer, reinforcing the research priority in these pathogens. However, this research is handi-

capped by mycobacterial pathogenicity, long growth rates and the complex genetic manipulation of the *Mycobacterium* genus.

Objectives: With the aim of developing more effective genetic tools in *Mycobacterium*, we propose to implement the CRISPR-Cas editing technique using the most minimalist CRISPR system described to date: the Cpf1 protein of Francisella novicida. Cpf1 has demonstrated intrinsic ability to process pre-crRNA on mature crRNA and to generate a double-strand break in ssDNA.

Methods: Using *Mycobacterium smegmatis* (fast growing non-pathogenic species with high transformation efficiency) as a model bacterium, in a first step we optimized codons of the coding sequence of *F. novicida* Cpf1 for its use in mycobacteria. The optimized sequence of the protein was cloned under a strong promoter in the pMV261 replicative expression plasmid for mycobacteria, with all the regulatory elements required for its optimal expression.

Results: We verified by qRT-PCR the correct transcription of the optimized cpf1 gene in *M. smegmatis.* Further a 3xFLAG epitope was introduced in-frame with the C-terminal end of Cpf1, allowing its detection by Western-Blot using anti-FLAG antibodies (Figure).



Verification of the correct expression of the Cpf1 protein in M. smegmatis. A. Transcription of cpf1 relative to 16S rRNA gene analyzed in 12 bacterial colonies in stationary growth phase. B. Expression of Cpf1 protein in M. smegmatis detected by Western Blot. The densitogram shows the expression of Cpf1 normalized versus the expression of GroEL2.

Conclusions: Finally, DNAs coding for the desired crRNAs will be expressed from the pMV261-Cpf1 plasmid constructed. The functionality of the CRISPR-Cpf1 system will be analyzed by enumerating c.f.u. grown from bacteria transformed with a control plasmid (without crRNA) relative to bacteria transformed with plasmids bearing crRNA. Genomic editing and Knock-Out construction in selected genes will be evaluated by sequencing and phenotypic characterization respectively. As a long-term goal, we propose to extend this technology to the remaining species of the *Mycobacterium* genus to edit their genomes "à la carte".

ULTRAPROTECTIVE VENTILATION IN PATIENTS WITH CARDIOGENIC PULMONARY EDEMA AND ECMO

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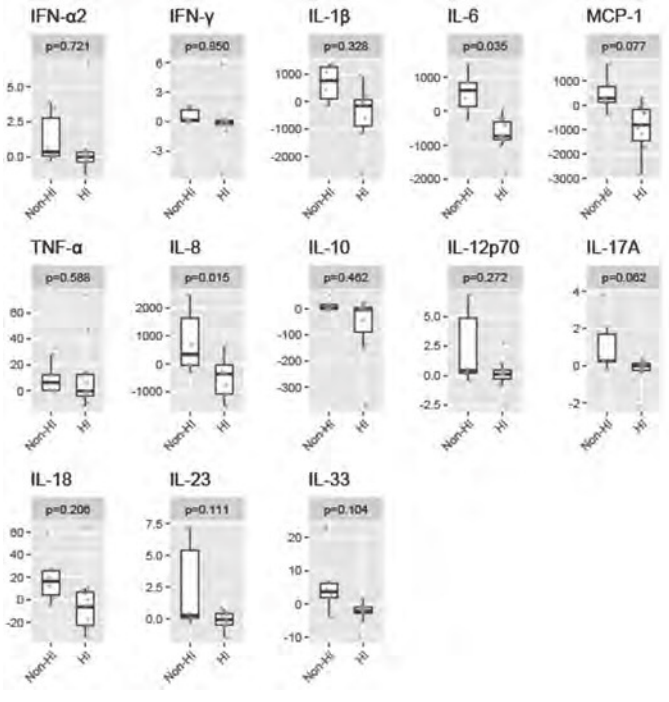
Introduction: Cardiogenic pulmonary edema (CPE) reduces functional residual capacity due to the alveolar occupancy and may facilitate ventilator-associated lung injury (VALI). Reducing tidal volumen (Vt) to 6 mL/kg of predicted body weight (PBW) has demonstrated to improve survival in acute respiratory distress syndrome (ARDS). Further reduction of Vt has been proposed to minimize VALI. In patients with cardiogenic edema, venous-arterial extracorporeal membrane oxygenation (va-ECMO) allows the application of ultraprotective ventilation.

Objectives: To evaluate the effects of ultraprotective ventilation on lung inflammatory response and respiratory mechanics during cardiogenic shock and va-ECMO.

Methods: Two ventilatory strategies (Vt 6 mL/kg or 3 mL/kg PBW) were applied in random order in patients meeting inclusion criteria. After 24 hours of each strategy, clinical, hemodynamic and respiratory parameters were recorded and bronchoalveolar lavage fluid (BALF) obtained. Inflammatory biomarkers were measured in BALF using a multiplexed assay. Data were compared using the Wilcoxon test for paired samples.

Results: 17 patients [29% female, age: 59 (53-65)] were recruited. Limiting Vt to 3 mL/kg resulted in lower airway pressures, end-expiratory lung volume (EELV) and compliance (Crs). There were no differences in hemodynamic parameters. Overall, there were no significant differences in BALF mediators according to Vt settings. The subgroup of patients with higher levels of IL6 during ventilation with 6 mL/kg (hyperinflamed) exhibited significant decreases in IL8 and IL6 levels after limiting Vt to 3 mL/kg. Conversely, Vt of 3 mL/kg was associated with a significant increase in these mediators in the non-inflamed subgroup.

	6 mL/kg PBW	3 mL/kg PBW	P
Tidal volume (mL)	400 (378-443)	215 (190-225)	< 0.001
Respiratory rate (rpm)	12 (10-13)	12 (10-14)	0.096
Plateau pressure (cmH_2O)	18 (17-20)	15 (13-17)	0.003
PEEP (cmH_2O)	6 (5-7)	8 (6-8)	0.034
Driving pressure (cmH_2O)	12 (10-14)	7 (6-9)	0.003
Crs (mL/cmH_2O)	35 (29-40)	27 (23-32)	0.013
EELV (mL)	904 (664-1043)	538 (494-827)	0.024
Strain	0.50 (0.43-0.59)	0.38 (0.24-0.50)	0.529
PaO_2 (mmHg)	100 (86-111)	97 (80-120)	0.782
$PaCO_2$ (mmHg)	36 (32-37)	39 (36-44)	0.074
pH	7.45 (7.43-7.50)	7.40 (7.34-7.45)	0.027
FiO_2	0.40 (0.35-0.50)	0.40 (0.35-0.50)	0.635
$FecmoO_2$	0.65 (0.60-0.80)	0.70 (0.60-0.80)	0.283
ECMO blood flow (L/min)	3.1 (2.9-3.7)	3.3 (2.9-3.5)	0.327
ECMO sweep flow (L/min)	3.0 (2.38-5.00) s	5.0 (3.5-6.5)	0.004



Conclusions: In patients with cardiogenic shock and va-ECMO, ultraprotective ventilation strategy is feasible and safe. There were no differences in lung inflammatory response according to the Vt. Baseline lung inflammatory response may modify the impact of the applied ventilation.

VIRULENCE FACTORS EXPRESSION IN *STAPHYLOCOCCUS AUREUS* DERIVED FROM LOWER RESPIRATORY TRACT INFECTIONS AND THEIR ROLE ON PATHOGENICITY

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Introduction: *Staphylococcus aureus* pathogenicity is multifactorial and has clinical implications.
Objectives: 1. To evaluate Fibronectin-binding proteins (FnBPs) expression on clinical isolates of *S. aureus* derived from lower respiratory tract infections (LRTI). 2. To investigate the correlation between FnBPs expression and the ability to adhere and invade epithelial cells. 3. To develop an immunochemical test to detect quorum sensing (QS) molecules.
Methods: Purified fibronectin (Fn) was used to cover 96-well culture plates to allow bacteria attachment and was then quantified using crystal violet. Strains (n = 63) were grown in tryptic soy broth till early exponential growth phase. To determine the capacity of cell adhesion and invasion, epithelial cells (A549) were incubated with different strains (n = 15). A microtiter-based ELISA has been developed and used to detect autoinducible peptide (AIP) in bacterial supernatants.
Results: The capacity of adhesion to Fn for the strains analysed are shown in Table 1. The strains with lower ability to adhere to Fn presented higher cell adhesion capacity in comparison to the ones that showed strong binding to Fn. Moreover, the latter strains had lower ability to be internalized by epithelial cells. AIP molecules were detected in supernatants, and its concentration increased over time.

Distribution of the adhesion to Fn depending on the study group

	Adhesion (%)		
	Low	Medium	High
Carrier (2)	0	2 (100)	0
Colonitzation (23)	7 (30.4)	14 (60.9)	2 (8.7)
Pneumonia (15)	10 (60)	6 (20)	0
Tracheobronchitis (23)	5 (21.7)	11 (47.8)	7 (30.4)

Conclusions: 1. The expression profile of virulence factors can be associated with clinical phenotypes. 2. The presence of FnBPs increases the internalization capacity and its absence might increase the expression of adhesion proteins, thereby promoting cell adherence. 3. The detection of QS molecules could provide more information about the disease status and their role as biomarkers of disease should be evaluated.
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VITAMIN D RECEPTOR IN PULMONARY HYPERTENSION

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Introduction: In recent years, a relationship has been reported between vitamin D deficiency and cardiovascular and respiratory diseases, including pulmonary hypertension (PH). PH-patients present a severe deficit of vitamin D and low vitamin D levels correlated with poor prognosis. Vitamin D actions are mediated by vitamin D receptor (VDR). However, currently the pathophysiological role of VDR in the lungs from PH-patients is unknown.
Objectives: The aim of this study is analyse the localization and expression of vitamin D receptor in pulmonary vasculature. And second, to examine the VDR regulation and its targets involve in PH.
Methods: VDR expression was analysed in lungs, pulmonary smooth muscle cells (PASMC) from controls and PH-patients by molecular techniques (qRT-PCR and Western Blot) and immunofluorescence. We also examined the VDR expression and localization in PASMC treated with calcitriol, the active form of vitamin D. Moreover, the expression of KCNK3, a VDR-target gene was analysed. The antiproliferative effect of calcitriol was examined PASMC treated with calcitriol for 48 hours by colorimetric assays, MTT and BrdU.
Results: VDR is expressed in the nucleus and the cytosol in PASMC. In PH patients, VDR expression was downregulated in lungs. In vitro, VDR expression is strongly upregulated after 48 hours of calcitriol treatment in PASMC from controls and PH patients, and VDR is mainly localized in the nucleus of PASMC after calcitriol treatment. Furthermore, calcitriol reduced the proliferation in PASMC from controls and PH patients and increased the KCNK3 mRNA expression.
Conclusions: All together, these data suggest that VDR is localized in the pulmonary vasculature and it is downregulated in PH patients. VDR, at least in vitro, can be rescued by calcitriol treatment, and exerts an antiproliferative role, maybe by KCNK3.

YKL-40 AND KL-6 IN SERUM AND SPUTUM SAMPLES OF PATIENTS DIAGNOSED WITH HYPERSENSITIVITY PNEUMONITIS

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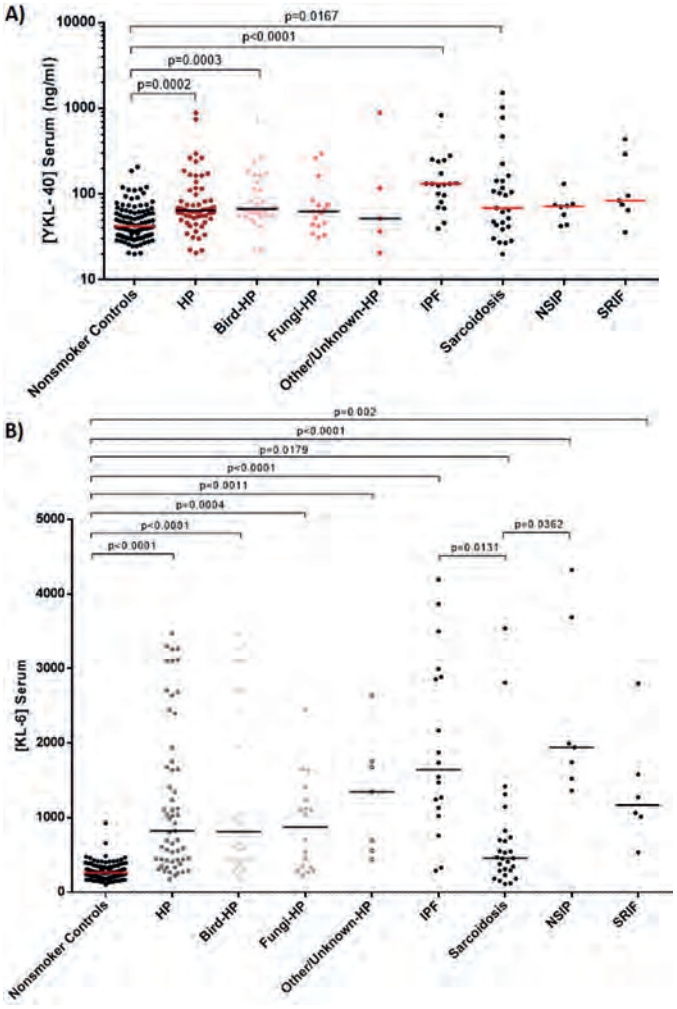
Introduction: YKL-40 protein is a biomarker of fibrosis and tissue remodeling produced by macrophages, neutrophils and epithelial cells. KL-6 protein is a biomarker of interstitial lung diseases (ILDs) mainly secreted by type II pneumocytes.
Objectives: This study assesses whether YKL-40 and KL-6 levels may play a role as biomarkers in hypersensitivity pneumonitis (HP) compared to other types of ILDs.
Methods: YKL-40 and KL-6 levels were determined in serum and/or induced sputum samples from 59 patients diagnosed with HP, 64 with other ILDs (idiopathic pulmonary fibrosis, sarcoidosis, nonspecific interstitial pneumonia and smoking-related interstitial fibrosis) and 122 healthy controls (22 smokers) using a commercial ELISA kit.
Results: Significant differences were found in YKL-40 serum levels between controls and HP patients due to bird exposure ($p = 0.0003$). For serum KL-6, these differences were found between controls and all HP patients ($p < 0.0001$). A cut-off level of 51.74 ng/mL for serum YKL-40 in HP due to birds had a sensitivity of 88% and a specificity of 63%. For KL-6, a cut-off level of 379.2 UI/mL had a sensitivity and specificity of 81%. YKL-40 serum levels were found to increase with

Demographic data and biomarker levels in the study population

	Controls (n = 122)	HP (n = 59)	IPF (n = 19)	Sarcoidosis (n = 28)	NSIP (n = 8)	SRIF (n = 7)
Age, median (range)	(20-65)	61 (33-85)*	65 (46-82)**	44.5 (29-79)*.**.***	63.5 (38-70)	75 (53-86)***
Sex, M n (%)	61 (50)	26 (44)	16 (84)	14 (50)	3 (38)	7 (100)
Smoking, n (%)	22 (18)	5 (8)	0	1 (4)	2 (25)	0
Serum YKL-40, ng/mL median (range)	43.33 (20.1-253.63)	64.5 (20.76-885.868)	132.27 (39.77-828.55)	69.06 (20.06-1512.92)	77.98 (42.54-131.81)	84.19 (35.95-436.27)
Sputum YKL-40, ng/mL median (range)	-	127.41 (34.74-753.92)	243.21 (70.26- 281)	162.69 (79.49-170.17)	289.25	42.671 (22.4-66.69)
Serum KL-6, U/mL median (range)	267.21 (102.69-927.3)	874.7 (177.6-6962.4)	1737.8 (289.85- 5518.2)	463.13 (111.5-5838)	1968.55 (1362.5-5156.8)	1274.2 (535.6-5651.6)
Sputum KL-6, U/mL median (range)	-	521.5 (14.1-8324.5)	984.9 (35.05-6283.2)	461.1 (37.75-4778.8)	848.67 (74.75-1622.6)	1426.8 (957.5-1781.6)

*p = 0.0385. **p = 0.002. ***p = 0.0016.



Serum YKL-40 (A) and KL-6 (B) levels in controls, HP, HP depending on the causal antigen and other ILDs (IPF [idiopathic pulmonary fibrosis], sarcoidosis, NSIP [nonspecific interstitial pneumonia], SRIF [smoking-related interstitial fibrosis]).

age in controls. No significant differences were observed in sputum levels in the different types of ILDs. In HP patients, a negative correlation was found between serum KL-6 and pulmonary function (DLCO and TLC). In HP patients, a correlation was observed between sputum YKL-40 and KL-6 levels (r = 0.411; p = 0.0045).

Conclusions: Serum YKL-40 levels show a relationship with age. Serum YKL-40 levels increase in HP patients due to bird exposure. In HP, KL-6 seems a better biomarker because is not modified by age, has high sensitivity and specificity and is related to pulmonary function. Longitudinal studies are necessary to establish the role of these biomarkers in the pathogenesis of HP.

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