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4. RIBAVIRIN FOR TREATMENT OF SUBJECTS WITH RESPIRATORY SYNCYTIAL VIRUS-RELATED INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Sofia Tejada[1,2], Raquel Martinez-Reviejo[1,2], Hanife Nur Karakoc[3], Yolanda Peña-López[2,4], Oriol Manuel[5], Jordi Rello[1,2,6,7]

[1]Instituto de Salud Carlos III, Madrid, Spain. [2]Vall d'Hebron Institut of Research (VHIR), Barcelona, Spain. [3]Karadeniz Technical University School of Medicine, Trabzon, Turkey. [4]Hospital Universitari Vall d'Hebron, Barcelona, Spain. [5]Lausanne University Hospital, Lausanne, Switzerland. [6]Universite de Nimes-Montpellier, Nîmes, France. [7]Universitat Internacional de Catalunya (UIC), Barcelona, Spain.

Introduction: Respiratory syncytial virus (RSV)-associated diseases have caused an estimated 1.8 million hospital admissions and 40,000 deaths among children. RSV can cause lower respiratory tract infections (LRTIs) in all age groups, adults with comorbidities, and immunocompromised patients.
Objectives: The aim was to summarize the evidence concerning efficacy and safety of ribavirin in subjects diagnosed with RSV associated with LRTI.
Methods: A systematic review and meta-analysis were performed. Eligible studies were observational (10 subjects) and RCTs of subjects with aerosol/oral ribavirin for RSV-LRTI. Comparator was supportive care/placebo. Systematic search on PubMed, Cochrane Library, and Web of Science databases was conducted between January 2001 and January 2022. PROSPERO register number: CRD42022308147.
Results: After retrieving 907 studies, 10 observational studies and 1 RCT were included (4/11 high quality of evidence). Seven studies included subjects with haematological malignancy/stem cell transplant, two lung transplants, and two healthy individuals. A total of 788 subjects diagnosed with RSV infection were included; 14.3% of them presented with only LRTI. Among 445 subjects treated with ribavirin, 195 (43.8%) received an aerosolized formulation. Pooled meta-analysis showed no differences in mortality (RR: 0.63; 95%CI: 0.28-1.42) in all subjects treated with aerosol/oral ribavirin compared to supportive care. In subgroup analysis, mortality was significantly lower in haematological subjects (RR: 0.32; 95%CI: 0.14-0.71), but did not differ significantly in lung transplant recipients (RR: 0.89; 95%CI: 0.31-2.56). Oral ribavirin (*vs.* supportive care) was associated with increased viral clearance (RR: 2.60; 95%CI: 1.35-4.99). Seventeen adverse events were reported among 119 subjects, but none were severe.
Conclusions: Ribavirin should be considered for treatment of RSV-LRTI in haematological subjects. There is a lack of evidence to support its use in lung transplant recipients. Oral formulation appears to be an easier, safe, and cost-effective alternative to aerosolized ribavirin. Further advances needs to focus on newer antivirals.
Funding: This work was funded by CIBERES, Instituto de Salud Carlos III, Madrid, Spain (Fondos FEDER: CB06-06-036). No funding or sponsorship was received for the publication of this article.

5. SOLID ORGAN TRANSPLANTATION FROM DONORS WITH RECENT OR CURRENT SARS-COV-2 INFECTION: A SYSTEMATIC REVIEW

Raquel Martínez-Reviejo[1,2], Sofia Tejada[1,2], Ana Cipriano[3], Hanife Nur Karakoc[4], Oriol Manuel[3], Jordi Rello[1,2,5,6]

[1]Instituto de Salud Carlos III, Madrid, Spain. [2]Vall d'Hebron Institute of Research (VHIR), Barcelona, Spain. [3]Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland. [4]Karadeniz Technical University School of Medicine, Trabzon, Turkey. [5]Universitat Internacional de Catalunya, Barcelona, Spain. [6]CHRU Nimes, Nîmes, France.

Introduction: Solid-organ transplantation (SOT) from SARS-CoV-2 positive donors could be a life-saving opportunity worth grasping. We perform a systematic review to evaluate the recipient outcomes of SOT from donors with recent or current SARS-CoV-2 infection.
Objectives: The aim of this systematic review is to evaluate the recipient outcomes of SOT from donors with persistent, resolved, or asymptomatic SARS-CoV-2 infection.
Methods: Search strategy was performed in PubMed, Cochrane COVID-19 Study Register, and Web of Science databases from the 1st of January 2019 to the 31st of December 2021. SOT adult recipients from a donor with past or current SARS-CoV-2 infection were elegible for inclusion. Outcomes were viral transmission, COVID-19 symptoms, mortality, hospital stay, and complications. PROSPERO Register Number: CRD42022303242.
Results: Sixty-nine recipients received 48 kidneys, 18 livers and 3 hearts from 57 donors. Six additional transplants from positive lungs were identified. IgG+ anti-SARS-CoV-2 titers were detected among 10/16 recipients; only 4% (3/69) recipients were vaccinated. Non-lung transplant recipients received organs from 10/57 (17.5%) donors with persistent COVID-19. In 18/57 donors, SARS-CoV-2 RNA was detected (median 32 Cycle threshold [Ct]) at procurement. Among non-lung transplant recipients, SARS-CoV-2 viral transmission was not documented. Four patients presented de-

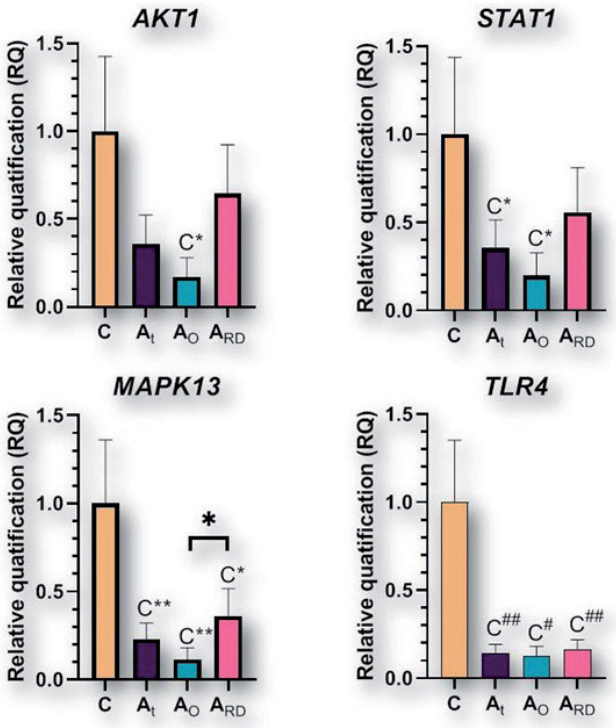
layed graft dysfunction, two patients acute rejection, and two patients died of septic shock. The median (IQR) hospital stay was 18 (11-28) days in recipients from symptomatic donors. Viral transmission occurred from three lung donors to their recipients, who developed COVID-19 symptoms. One of the recipients subsequently died.
Conclusions: Use of non-lung (kidney, liver and heart) organs from SARS-CoV-2 positive donors seem to be a safe practice, with a low risk of transmission irrespective of the presence of symptoms at the time of procurement. Low viral replication (Ct > 30) was safe among non-lung donors, even if persistently symptomatic at procurement.
Funding: This work was funded by CIBERES, Instituto de Salud Carlos III, Madrid, Spain. (Fondos FEDER) (CB06-06-036).

6. GENE EXPRESSION ANALYSIS OF FOUR POTENTIAL ASTHMA TRIGGERS IN LUNG TISSUE SAMPLES

Lucía Cremades-Jimeno[1], María López-Ramos[1], Selene Baos[1], Ignacio Mahíllo-Fernández[2], Joaquín Sastre[3,4], Blanca Cárdaba[1,4]

[1]Immunology Department, IIS-Fundación Jiménez Díaz-UAM, Madrid, Spain. [2]Biostatistics and Epidemiology Unit, Fundación Jiménez Díaz, Madrid, Spain. [3]Allergy Department, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain. [4]Ciber de Enfermedades Respiratorias, CIBERES, Madrid, Spain.

Introduction: Asthma is a chronic inflammatory respiratory disease. The treatments available nowadays are mainly symptomatic, needed for long periods and not all patients respond correctly to them, due to the high heterogeneity of asthma phenotypes that leads to a difficult classification of patients. With the aim of provide new diagnostic tools, we previously defined and theoretically prioritize, using systems biology, a group of potential biomarkers with the ability to differentiate asthma phenotypes in peripheral blood samples; and we identified four potential asthma triggers: AKT1, MAPK13, STAT1 and TLR4.
Objectives: Analyze the expression of the theoretically defined asthma triggers in lung samples of asthmatic patients to identify new possible therapeutic targets.
Methods: Lung tissue samples from 10 healthy controls (HC) and 19 asthmatic patients (A) were obtained from the CIBERES biobank. After mechanic homogenization, RNA and protein was extracted trough TRIzol method. Gene expression of the four genes was analyzed trough RT-qPCR and protein expression of p38 Δ/MAPK13 trough western blot. Statistically significant differences and sensitivity/specificity analysis (with ROC curves) was performed with GraphPad InStat program.
Results: All genes showed a lower expression in the A group compared to HC, with statistically significant differences for MAPK13 (RQ = 0.229; p = 0.0023), STAT1 (RQ = 0.354; p = 0.043) and TLR4 (RQ = 0.146; p < 0.0001). ROC curve analysis showed the best AUC values for TLR4 (AUC = 0.96; p = 0.0001) and MAPK13 (AUC = 0.85; p = 0.0033). We also analyzed separately the expression in patients with asthma only (AO, n = 9) from patients with asthma and other respiratory diseases (ARD, n = 10) and observed that the four genes showed statistically significant differences between the HC and the AO groups; while only MAPK13 and TLR4 were significantly different in the ARD group. The AUC values were higher for the discrimination of the HC with the AO patients than with the ARD group. Also, MAPK13 showed statistically significant differences between gene expression of both asthma subgroups, with good sensitivity and specificity for that discrimination (AUC = 0.81; p = 0.04). Finally, two bands were detected in the p38 Δ western blot that showed different behavior: one showed significant differences between HC and ARD, while the other between HC and AO (Figure).



Gene expression of the four potential asthma triggers in lung tissue samples of healthy control subjects and asthmatic patients. Relative quantifications (RQ) of the clinical groups compared to the control group are represented. Statistically significant differences between the C and the specified group are shown as: C*, C**, C# and C## (p < 0.05; p < 0.01; p < 0.001 and p < 0.0001 respectively). Statistically significant differences between two clinical groups are shown with * (p < 0.05). HC: healthy control subjects; At: all asthmatic patients; AO: patients with asthma only; ARD: patients with asthma and other respiratory diseases.

Conclusions: The gene expression of AKT1, MAPK13, STAT1 and TLR4 is different in lung tissue samples from asthmatic patients and healthy subjects, as well as he protein expression of p38 Δ/MAPK13, opening new possibilities in the search of asthma treatments.
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7. MONITORING PATIENTS WITH PULMONARY THROMBOEMBOLISM AFTER HOSPITALIZATION: ARE WE DOING IT RIGHT?

Celia de Dios Calama[1], María Ruiz Carretero[1], Celia Montaño Montaño[2], Carlos Cabanillas Díez-Madroñero[3], Amanda Paula Arenas Polo[4], Marta Cebrián Romero[4]

[1]Hospital Universitario San Pedro de Alcántara. CIBER de Enfermedades Respiratorias (CIBERES), Cáceres, Spain. [2]Hospital Universitario de Bellvitge, L'Hospitalet de Llobregat, Spain. [3]Hospital Universitario Doctor Josep Trueta, Girona, Spain. [4]Hospital Universitario San Pedro de Alcántara., Cáceres, Spain.

Introduction: A new multidisciplinary consensus on the management of pulmonary thromboembolic disease has recently been published (Lobo JL, *et al.* Multidisciplinary consensus for the management of pulmonary thromboembolism. Arch Bronconeumol. 2021), which analyzes aspects related to diagnosis, treatment and follow-up.
Objectives: The objective of our work is to analyze the follow-up protocol for patients hospitalized because of PTE in our area and its consistency with the data offered in the new consensus.

Methods: A retrospective descriptive study of patients followed up in our consultations after admission to our area with a diagnosis of PE and for a period of one year (November 2020-2021) is carried out. Epidemiological, clinical, diagnosis, treatment and prognosis variables are analyzed. The results are stored in a database and the statistical study is carried out using the PASW Statistics program.
Results: A total of 76 patients (62% men, 38% women) with an average age is 62 years old are included. 59.2% were classified as PESI I-II and 40.8% PESI III-IV. 39.5% corresponded to segmental PE, 35.5% in the main artery and 11.8% subsegmental. 13% of our patients were discharged with LMWH, 81.6% with Antivitamin K and the rest with DOACs (direct acting oral anticoagulants). The mean duration of treatment was 9 ± 3 months. The median duration until the first consultation was 4 ± 1.8 months. During follow-up no diagnostic test was performed (Scintigraphy in 58%, AngioCT 76%). In 74% of the cases no thrombophilia study was indicated. 13% of our patients presented PTE associated with cancer and 60% of these underwent treatment with LMWH. During follow-up, pulmonary hypertension associated with PE was diagnosed in 3.1% of patients.

	Consensus 2013 y 2021	HSPA
Tiempo hasta primera consulta	Not adress directly	4 months
Duración tratamiento	Least 3 months	9 months
Tipo de anticoagulante	Antivitamin K (need for reperfusion and contraindication ADOs)	Most Antivitamin K
	DOACs (most cases, expect contraindications)	5.3% DOACs
	LMVH (choise in cáncer)	-13.2% LMWH (60% of cancer patients)
Estudio trombofilia	Not recommended	Not done in 74%
Pruebas diagnósticas	AngioCT or scintigraphy is not recommended. Echocardiogram only if symptoms or signs of PHT-PTE.	-24% AngioCT -30% Scintigraphy

*Contraindications: Triple positive antiphospholipid syndrome, pregnant and lactating, or with severe renal insufficiency.

Conclusions: The multidisciplinary consensus for the management of PTSD (2013 and 2021) address some aspects related to the management and follow-up of patients diagnosed with PTSD. In our study, we observed a high level of concordance in the follow-up of patients with PE, particularly with regard to the minimum duration of anticoagulant treatment and the indication of different diagnostic tests. In our protocol there are clear differences with respect to the consensus in the choice of anticoagulant treatment. We did not find that the duration and time interval required are in line with the first and number of follow-up consultations in these patients.

10. FOLLOW-UP OF PATIENTS WITH PULMONARY THROMBOEMBOLISM AFTER HOSPITALIZATION: ARE WE DOING IT RIGHT?

Maria Estrella Ruiz Carretero[1], Celia de Dios Calama[1], Celia Montaño Montaño[2], Carlos Cabanillas Díez-Madroñero[3], Amanda Paula Arenas Polo[1], Marta Cebrián Romero[1]

[1]Complejo Hospitalario Universitario de Cáceres, Cáceres, Spain. [2]Hospital Universitario de Bellvitge, Barcelona, Spain. [3]Hospital Universitario doctor Josep Trueta, Gerona, Spain.

Introduction: A new multidisciplinary consensus for the management of pulmonary thromboembolism has recently been published (Lobo JL, *et al.* Multidisciplinary consensus for the management of pulmonary thromboembolism. Arch Bronconeumol. 2021), in which aspects related to diagnosis, treatment and follow-up are analyzed.
Objectives: The aim of our work is to analyze the follow-up protocol for patients hospitalized for PE in our area and its consistency with the data offered in the new consensus.
Methods: We have carried out a retrospective descriptive study of patients followed up at clinic after admission to our area with a diagnosis of PE and for a period of one year (November 2020-2021). Epidemiological, clinical, diagnosis, treatment and prognosis variables are analyzed. The results are stored in a database and they have been analyzed using the PASW Statistics 18 program.
Results: A total of 76 patients (62% men, 38% women) with an average age of 62 years are included. 59.2% were classified as PESI I-II and 40.8% as PESI III-IV. 13% of our patients were discharged with LMWH, 81.6% with vitamin K antagonist and the rest with DOACs (direct oral anticoagulants). The mean duration of treatment was 9 ± 3 months. The average duration until the first consultation was 4 ± 1.8 months. No diagnostic test was performed during the follow-up (scintigraphy in 58%, angioCT in 76%). In 74% of cases no thrombophilia study was indicated. 13% of our patients presented PE associated with cancer and 60% of these underwent treatment with LMWH (low molecular weight heparin).

	2013 and 2021 consensus	Our hospital
Time to first consultation	Not addressed directly	4 months
Duration of treatment	Minimum 3 months	9 months
Type of anticoagulant	Vitamin K antagonist (need for reperfusion and contraindication of DOACs)	Most ot them vitamin K antagonist
	DOACs (most cases, except contraindications)	5.3% DOACs
	LMWH (choice in cancer)	13.2% LMWH
Thrombophilia study	Not recommended	Not performed in 74%
Diagnostic tests	CT angiography and scintigrafphy are not recommended.	24% CT angiography 30% scintigraphy

Conclusions: On our research, we observed a high level of agreement in the follow-up of patients with PE concerning the consensus and particularly with regard to the minimum duration of anticoagulant treatment. In our protocol, there are differences in the choice of anticoagulant treatment and in terms of the indication of different diagnostic test.

11. REMDESIVIR DOES NOT REDUCE MORTALITY IN CRITICALLY ILL PATIENTS WITH COVID-19 REGARDLESS OF PATIENT AND VIRAL FACTORS

Catia Cilloniz[1,2], Anna Motos[1], Tatiana Castañeda[1], Juan Manuel Pericas[3], Albert Gabarrus[4], Andres Palomeque[4], Adrian Ceccato[4], Ferran Barbe[5], Antoni Torres[4,6]

[1]Hospital Clinic of Barcelona, Barcelona, Spain. [2]Facultad de Ciencias de la Salud, Universidad Continental, Huancayo, Peru. [3]Liver Unit, Internal Medicine Department, Vall d'Hebron University Hospital, Vall d'Hebron Institute for Research, CIBERehd, Barcelona, Spain. [4]Hospital Clinic de Barcelona, Barcelona, Spain. [5]Translational Research in Respiratory Medicine, Respiratory Department, Hospital Universitari Arnau de Vilanova and Santa Maria; IRBLleida, Lleida, Spain. [6]CIBERES, Barcelona, Spain.

Introduction: The role of remdesivir in the treatment of critically ill COVID-19 patients is an ongoing matter of controversy.
Objectives: We aimed to evaluate the effect of remdesivir on the outcomes of patients with severe COVID-19 admitted to the intensive care unit (ICU).

Methods: Multicentre, observational cohort study including consecutive COVID-19 patients admitted to 55 Spanish ICUs. The primary outcome was 90-day mortality. Subsequent analyses in clinically relevant subgroups by age, ICU baseline illness severity, organ damage, laboratory findings, respiratory support, serum levels of SARS-CoV-2 RNA and by timing of remdesivir initiation were performed.

Results: Between 5 February 2020 and 21 December 2021, 6225 patients were included. Of these, 795 (16%) received remdesivir during hospitalisation. Four percent (n = 34) of all population received remdesivir in < 3 days from initial symptoms and 15% (n = 114) in < 5 days from initial symptoms. Forty-seven percent (n = 361) received remdesivir before ICU admission, 35% (n = 269) received remdesivir on days 0-1 of ICU admission and 19% (n = 145) received remdesivir since day 1 of ICU admission. In the propensity-adjusted multivariate analysis, remdesivir use was not associated with 90-day mortality (HR 1.01, 95%CI 0.86 to 1.19; p = 0.895), nor with in-hospital (sHR 0.95, 95%CI 0.82 to 1.10, p = 0.47) and 30-day mortality (HR 0.88, 95%CI 0.72 to 1.07, p = 0.186). No effect was found regarding age, ICU baseline illness severity, organ damage, laboratory findings, respiratory support, SARS-CoV-2 viral load in plasma and timing of remdesivir initiation (Figure).

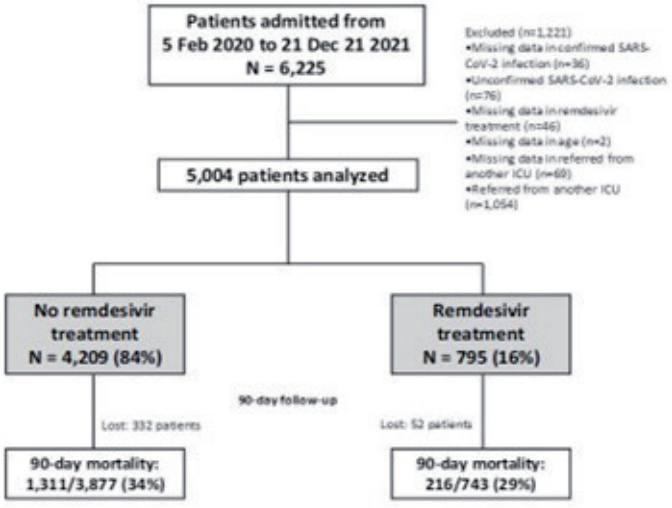
Conclusions: Remdesivir was not associated with improved survival in patients with severe COVID-19 patients requiring ICU admission, regardless of clinically relevant variables such as age, illness severity, organ damage, highly inflammatory status, respiratory support, SARS-CoV-2 viral load in plasma, or timing of remdesivir initiation.

Table 1. Characteristics of the study population

Variables	No remdesivir treatment (N = 4,209)	Remdesivir treatment (N = 795)	P-value
Age, median (Q1; Q3), years	64 (54; 71)	62 (52; 71)	0.009
Male sex, n (%)	2,930 (70)	562 (71)	0.568
BMI, median (Q1; Q3), kg/m²	28.7 (26; 32.2)	29 (26; 32.4)	0.676
Comorbidities, n (%)	1,956 (46)	349 (44)	0.182
Days since initial symptoms to ICU admission, median (Q1; Q3)	9 (7; 12)	8 (6; 11)	< 0.001
Glasgow Coma Scale, median (Q1; Q3)	15 (15; 15)	15 (15; 15)	< 0.001
APACHE-II score, median (Q1; Q3)	12 (9; 15)	11 (8; 14)	< 0.001
SOFA score, median (Q1; Q3)	5 (4; 7)	4 (3; 6)	< 0.001
Leucocyte count, median (Q1; Q3), 10^9/L	9 (6.4; 12.7)	8.2 (5.9; 11.4)	< 0.001
Lymphocyte count, median (Q1; Q3), 10^9/L	0.7 (0.49; 1)	0.72 (0.5; 1.06)	0.019
Neutrophil count, median (Q1; Q3), 10^9/L	7.7 (5.3; 11.3)	6.9 (4.7; 9.8)	< 0.001
D-dimer, median (Q1; Q3), ng/mL	987 (504; 2,300)	810 (425; 1,640)	< 0.001
C-reactive protein, median (Q1; Q3), mg/L	133 (66; 226)	120 (61; 195)	0.006
Serum creatinine, median (Q1; Q3), mg/dL	0.85 (0.69; 1.1)	0.79 (0.65; 1)	< 0.001
LDH, median (Q1; Q3), U/L	489 (365; 668)	442 (351; 602)	< 0.001
High-flow nasal cannula	1,150 (27)	340 (43)	< 0.001
Non-invasive mechanical ventilation	439 (10)	117 (15)	< 0.001
Invasive mechanical ventilation	2,291 (55)	280 (35)	< 0.001
Viral RNAemia in plasma, n (%)	477 (77)	107 (80)	0.521
Viral antigenemia in plasma, n (%)	268 (44)	67 (51)	0.158

Table 2.

Variables	No remdesivir treatment (N = 4,209)	Remdesivir treatment (N = 795)	P-value
Outcomes			
In-hospital mortality, n (%)	1,311 (31)	216 (27)	0.025
30-day mortality, n (%)	999 (25)	139 (18)	< 0.001
90-day mortality, n (%)	1,311 (34)	216 (29)	0.012
Length of ICU stay, median (Q1; Q3), days			
All patients	13 (7; 27)	13 (7; 29)	0.463
Surviving patients	13 (7; 27)	11 (7; 25)	0.141
Length of hospital stay, median (Q1; Q3), days			
All patients	23 (14; 40)	24 (15; 42)	0.063
Surviving patients	26 (16; 45)	24 (15; 44)	0.092
Ventilator-free days, median (Q1; Q3)	0 (0; 16)	0 (0; 16)	0.148
Mechanical ventilation length, median (Q1; Q3), days			
All patients	14 (8; 26)	17 (10; 30)	< 0.001
Surviving patients	14 (8; 26)	15 (9; 32)	0.008
ICU-free days, median (Q1; Q3)	3 (0; 19)	9 (0; 20)	0.003



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13. IMAGING ANALYSIS OF SARCOPENIA IN A COHORT OF STABLE PATIENTS WITH BRONCHIECTASIS

Mariela Alvarado- Miranda[1,2], Adriana Núñez[2,3], Maria Perez[2,3], Salvatore Marsico[4], Alberto Solano[4], Esther Barreiro[2,3]

[1]Universitat Pompeu Fabra (UPF), Department of Medicine, Barcelona, Spain. [2]Centro de Investigación en Red de Enfermedades Respiratorias (CIBERES), Barcelona, Spain. [3]Pulmonology Department-Muscle Wasting and Cachexia in Chronic Respiratory Diseases and Lung Cancer Research Group, IMIM-Hospital del Mar, Parc de Salut Mar, Health and Experimental Sciences Department (CEXS), Universitat Pompeu Fabra (UPF) Department of Medicine, Universitat Autònoma de Barcelona, Parc de Recerca Biomèdica de Barcelona (PRBB), Barcelona, Spain. [4]Radiology Department, Imatge Mèdica Intercentres-Parc de Salut Mar, Hospital del Mar, Barcelona, Spain.

Introduction: Chronic respiratory diseases are associated with systemic manifestations, characterized by alterations in the different body compartments. Sarcopenia, defined as loss of function and/or muscle mass, is present in diseases such as bronchiectasis.
Objectives: Our objective was to characterize by imaging (ultrasound-elastography and MRI) the degree of sarcopenia in the quadriceps of stable patients with non-cystic fibrosis bronchiectasis.
Methods: In stable adult patients (n = 20) with bronchiectasis and sarcopenia: fat-free mass (FFMI) < 16 kg/m^2 (men) and < 15 kg/m^2 (women) and quadriceps strength (QMVC) to fat-free mass ratio (QMVC/FFM) < 0.8 and in healthy control subjects (n = 10) the architecture and composition of the vastus lateralis (VL) of the non-dominant quadriceps muscle was assessed in a cross-sectional, prospective study. Patients and controls were clinically evaluated [lung and muscle functions, exercise (shuttle test) and general laboratory tests].
Results: Compared to control subjects, patients exhibited mild airflow obstruction, reduced upper and lower limb muscle strength and body mass index, without any nutritional alterations. In the patients compared to levels in the healthy controls, imaging tests showed a decrease in muscle thickness and pennation angle, while fatty infiltration and edema were increased. Quadriceps strength positively correlated with both exercise capacity (shuttle test) and VL muscle area, whereas negative correlations were observed between age and the variables quadriceps strength, exercise (shuttle), VL elasticity and quadriceps muscle area (Table 1 and 2).

Table 1. Anthropometric, functional and imaging characteristics

	Control Subjects n=10	Bronchiectasis n=20
Anthropometrics		
Age (years)	59(13)	62(13)
Body weight (Kg)	71(10)	56(9) **
BMI (kg/m2)	27(4)	22(3) *
FFM (kg)	47(9)	37(8) *
FFMI (kg/m2)	18(3)	14(3) *
Smoking history		
Never smoker (%)	0	13(65)
Ex-smoker, N (%)	0	7(35)
Lung function testing		
FEV1, %Predicted	100(13)	73(21) *
FVC, % predicted	98(12)	82(17) *
FEV1/FVC	81(4)	70(13) *
Exercise capacity and muscle function		
6-min walking distance(m)	522(70)	503(81)
Shuttle test(m)	576(190)	458(103)
QMVC (kg)	40(12)	24(7) **
QMVC (kg)/FFM (kg)	0.85(0.2)	0.68(0.18) *
Handgrip strength(kg)	28(9)	20(5) *
Muscle characteristics. MRI, ultrasound, elastography		
LV muscle thickness (cm)	2.60(1.70)	1.74(0.44)
LV fiber length (cm)	7.41(1)	7(1)
LV penation angle (º)	13(2)	11,27(3)
LV muscle area (cm2)	12(4)	9(3)
Quadriceps fat (%)	7(5)	9(7)
LV elastography (kPA)	21.23(7.10)	24.85(8.05)
Total quadriceps area(cm2)	149(34)	136(28)
Blood parameters		
Urea	32(3)	42(13) *
Total proteins(g/dl)	7(0.3)	7(0.4)
Albumin(g/dl)	5(0.1)	4(0.2) *
LDH(UI/l)	187(33)	185(32)
CRP (mg/dL)	0.14(0.09)	0.31(0,5)
Rheumatoid factor (UI/ml)	6.9(1.43)	16.2(20) *

Table 2. Correlations between the variables: age, quadriceps strenght, exercise capacity and radiological parameters

	Age	QMVC(Kg)	QMVC(Kg)/ FFM (Kg)	Shuttle test (m)	VL Muscle thickness (cm)	VL fiber length (cm)	VL Muscle area (cm²)	VL Elastography (kPA)	VL quadriceps fat (%)	Quadriceps total area (cm²)
Age	_	-.584**	-.398	-.469*	-.232	-.272	-.552*	-.538	-.437	-.407
QMVC(Kg)	-.584**	_	.649**	.611**	.272	.415	.493*	.283	.140	.352
QMVC(Kg)/FFM(Kg)	-.398	.649**	_	.214	-.073	.032	.095	.228	.372	-.103
Shuttle test (m)	-.469*	.611**	.214	_	.448*	.157	.261	.316	.135	.334
VL Muscle thickness (cm)	-.232	.272	-.073	.448*	_	.598**	.486*	.200	-.213	.746**
VL fiber length (cm)	-.272	.415	.032	.157	.598**	_	.646**	.234	-.296	.506*
VL Muscle area (cm²)	-.552*	.493*	.095	.261	.486*	.646**	_	.021	-.148	.391
VL Elastography (kPA)	-.538*	.283	.228	.316	.200	.234	.021	_	.506*	.356
VL quadriceps fat (%)	-.437	.140	.372	.135	-.213	-.296	-.148	.506*	_	.014
Quadriceps total area (cm²)	-.407	.352	-.103	.334	.746**	.506*	.391	.356	.014	_

Conclusions: These data show the presence of radiological alterations in the quadriceps of stable patients with bronchiectasis and sarcopenia without nutritional alterations. Muscle function should be routinely assessed in patients with bronchiectasis and periodic imaging monitoring will allow quantification of the degree of deterioration of the muscle architecture in these patients. These results have clinical implications for exercise training programs in patients with bronchiectasis.
Funding: FIS 21/00215 (FEDER, ISC-III), Intensificación INT19, CIBERES (ISC-III), y SEPAR Ayudas a la Investigación 2020.

16. POLYSOMNOGRAPHIC DETERMINANTS OF NONDIPPING BLOOD PRESSURE PATTERN IN OBSTRUCTIVE SLEEP APNEA

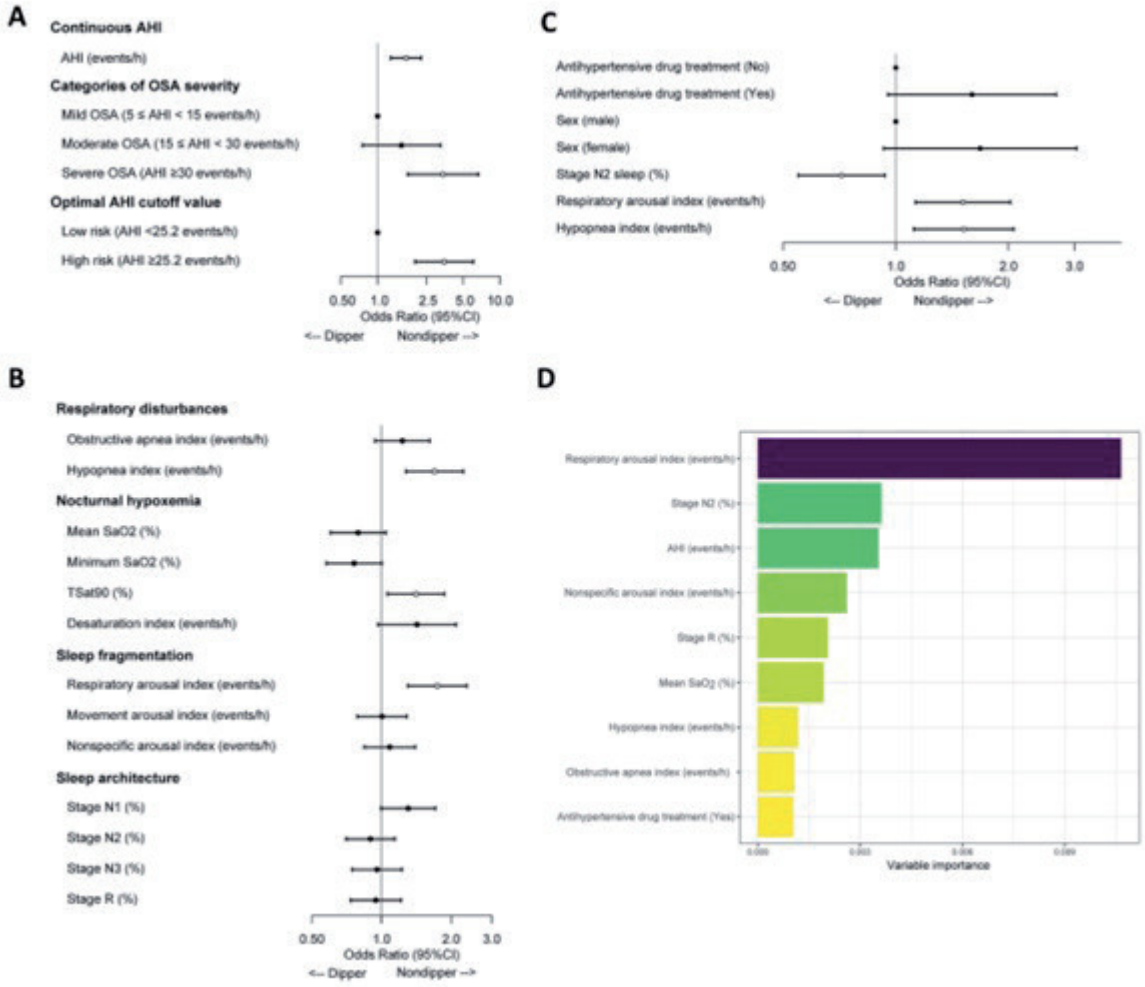
Lucía Pinilla[1,2], Ivan D Benítez[3,2], Esther Gracia-Lavedan[3,2], Gerard Torres[3,2], Olga Mínguez[3], Maria Aguilà[3], Adriano Targa[3,2], Mireia Dalmases[3,2], Olga Mediano[4,2], Juan F Masa[5,2], Maria J. Masdeu[6], Ferran Barbé[3,2], Manuel Sánchez de la Torre[1,2]

[1]Group of Precision Medicine in Chronic Diseases, Respiratory Department, University Hospital Arnau de Vilanova and Santa María; Department of Nursing and Physiotherapy, Faculty of Nursing and Physiotherapy, University of Lleida. IRBLleida, Lleida, Spain. [2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Group of Translational Research in Respiratory Medicine, Respiratory Department, University Hospital Arnau de Vilanova and Santa María; IRB Lleida, Lleida, Spain. [4]Pneumology Department, University Hospital of Guadalajara, Guadalajara, Spain. [5]San Pedro de Alcántara Hospital, Instituto Universitario de Investigación Biosanitaria de Extremadura, Cáceres, Spain. [6]Respiratory and Sleep Department, Parc Taulí University Hospital, Parc Taulí Research and Innovation Institute, Autonomous University of Barcelona, Sabadell, Spain.

Introduction: A close relationship between obstructive sleep apnea (OSA) and blood pressure (BP) impairments, including an increased risk for nocturnal nondipping, has been consistently observed. However, the precise mechanisms of this interaction are incompletely understood.
Objectives: Here, we characterized the polysomnography (PSG) parameters associated with alterations in the circadian BP pattern aiming to identify the main contributors to explain the nondipper profile in OSA.
Methods: Observational, prospective and multicentric study including subjects referred to the sleep unit for suspected OSA. Following a PSG, subjects with an apnea-hypopnea index (AHI) ≥ 5 events/h were included. Two groups were established based on the 24 h ambulatory blood pressure monitoring (ABPM) dipping ratio (DR; night/day BP ratio): dippers (DR ≤ 0.9) and nondippers (DR > 0.9). Adjusted logistic regression models were used to examine the individual associations of PSG parameters with the risk of nondipping. Multivariate variable selection processes based on least absolute shrinkage and selection operator (LASSO) and random forest were applied to identify the risk factors for nondipping.
Results: The cohort consisted of 299 patients: 131 (43.8%) dippers and 168 (56.2%) nondippers (Table 1). A significant increase in the risk of presenting a nondipper BP pattern was found along with AHI gain [odds ratio (OR) (95% (CI) = 1.71 (1.28-2.28)], with severe OSA patients (AHI ≥30 events/h) more likely to be nondippers than mild OSA patients (5 ≤ AHI < 15 events/h). The best AHI cutoff for predicting nondipper status was 25.2 events/h, increasing the OR (95% CI) to 3.50 (2.02-6.07) (Figure 1A). The hypopnea index [OR (95% CI) = 1.70 (1.27-2.26), TSat90 [OR (95% CI) = 1.41 (1.06-1.87)] and respiratory arousal index [OR (95% CI) = 1.74 (1.30-2.34)] were individually associated with the risk of a nondipping BP pattern (Figure 1B). LASSO regression identified 5 variables as predictors of nondipper status: respiratory arousal index, proportion of sleep time in stage N2, hypopnea index, use of antihypertensive drugs and female sex (Figure 1C). The top 5 predictive factors identified by the random forest model were related to macro- and microsleep structure, with the respiratory arousal index as the principal risk factor for the nondipper profile (Figure 1D).

Characteristics of the study cohort by categories of dipping ratio. Data are presented as the median [p25;p75] for quantitative variables and n (%) for qualitative variables. BP measurements from ABPM are expressed in mmHg. P values

	All (n = 299)	Dipper (n = 131)	Nondipper (n = 168)	p value
Clinical data				
Demographic/anthropometric				
Age (years)	52.0 [45.0;56.5]	50.0 [44.0;56.0]	52.0 [46.0;57.0]	0.149
Sex				0.177
Male	220 (73.6%)	102 (77.9%)	118 (70.2%)	
Female	79 (26.4%)	29 (22.1%)	50 (29.8%)	
BMI (kg/m²)	30.8 [27.4;34.9]	29.8 [26.9;34.0]	31.6 [27.9;35.7]	0.060
Smoking status				0.646
Never	110 (37.2%)	45 (34.9%)	65 (38.9%)	
Former	104 (35.1%)	49 (38.0%)	55 (32.9%)	
Current	82 (27.7%)	35 (27.1%)	47 (28.1%)	
Comorbidities				
Diabetes	43 (14.5%)	11 (8.46%)	32 (19.2%)	0.015
Hypertension	119 (40.8%)	40 (31.7%)	79 (47.6%)	0.009
Dyslipidemia	91 (31.2%)	29 (22.7%)	62 (37.8%)	0.008
Cardiovascular disease	50 (16.9%)	16 (12.4%)	34 (20.4%)	0.098
Medication use				
Insulin	14 (4.70%)	2 (1.53%)	12 (7.19%)	0.044
Any antihypertensive drug	114 (38.3%)	39 (29.8%)	75 (44.9%)	0.011
ACE inhibitors	69 (23.2%)	23 (17.6%)	46 (27.5%)	0.059
Beta-blockers	44 (14.8%)	16 (12.2%)	28 (16.8%)	0.350
Diuretic agents	45 (15.2%)	13 (10.0%)	32 (19.2%)	0.043
Calcium-channel blockers	30 (10.1%)	8 (6.11%)	22 (13.3%)	0.066
Angiotensin II receptor blockers	28 (9.46%)	11 (8.40%)	17 (10.3%)	0.721
Lipid-lowering drugs	67 (22.6%)	17 (13.0%)	50 (30.1%)	0.001
Hemodynamic data				
Dipping ratio	0.91 [0.85;0.97]	0.85 [0.81;0.87]	0.96 [0.93;1.01]	
24 h				
Mean BP	97.1 [90.2;105]	95.4 [90.0;102]	98.2 [90.6;108]	0.098
Systolic BP	128 [119;142]	127 [118;138]	130 [120;145]	0.055
Diastolic BP	79.9 [75.3;84.6]	79.5 [75.2;83.8]	80.3 [75.5;84.7]	0.424
24 h pulse frequency	70.8 [63.5;76.5]	69.5 [63.5;76.1]	71.2 [63.8;77.2]	0.468
Daytime				
Mean BP	99.0 [92.8;107]	99.8 [94.0;107]	98.9 [92.0;107]	0.187
Systolic BP	131 [122;144]	131 [123;144]	131 [121;145]	0.842
Diastolic BP	81.7 [76.9;86.5]	82.1 [78.1;87.5]	81.3 [76.2;85.3]	0.086
Daytime pulse frequency	71.1 [64.7;78.8]	70.3 [65.4;78.2]	72.2 [64.3;79.2]	0.681
Nighttime				
Mean BP	90.1 [82.1;99.0]	83.2 [78.2;90.8]	96.2 [86.5;107]	< 0.001
Systolic BP	120 [109;138]	112 [104;125]	129 [114;146]	< 0.001
Diastolic BP	74.4 [69.2;79.3]	70.4 [66.8;74.3]	77.5 [73.2;85.0]	< 0.001
Nighttime pulse frequency	65.4 [59.6;72.5]	64.9 [57.8;71.0]	66.0 [60.8;74.2]	0.069
Polysomnographic data				
Respiratory disturbances				
AHI (events/h)	34.7 [18.8;58.3]	25.4 [13.4;48.6]	41.4 [24.5;63.5]	< 0.001
Obstructive apnea index (events/h)	7.36 [2.15;23.4]	5.93 [1.57;16.3]	8.46 [2.54;28.3]	0.047
Hypopnea index (events/h)	18.5 [11.0;30.6]	15.3 [9.32;25.4]	21.9 [12.6;35.7]	< 0.001
Nocturnal hypoxemia				
Mean SaO2 (%)	93.0 [91.0;94.0]	93.0 [92.0;94.0]	93.0 [91.0;94.0]	0.022
Minimum SaO2 (%)	82.0 [73.0;86.0]	82.0 [75.0;87.0]	80.0 [72.0;85.0]	0.033
TSat90 (%)	3.23 [0.54;14.8]	2.40 [0.32;9.63]	4.50 [0.76;20.9]	0.010
Desaturation index (events/h)	8.02 [1.78;24.0]	4.86 [1.34;14.4]	9.75 [2.29;32.3]	0.057
Sleep fragmentation				
Respiratory arousal index (events/h)	23.4 [11.0;45.7]	18.5 [7.96;34.4]	27.7 [16.4;50.8]	< 0.001
Movement arousal index (events/h)	3.18 [1.30;6.60]	3.06 [1.58;6.48]	3.18 [1.14;7.02]	0.844
Nonspecific arousal index (events/h)	6.68 [3.14;11.5]	6.92 [3.01;9.96]	6.56 [3.28;13.1]	0.734
Sleep architecture				
Stage N1 (%)	12.4 [7.41;18.9]	11.7 [6.70;17.0]	13.4 [7.58;21.5]	0.121
Stage N2 (%)	43.3 [36.5;53.2]	44.6 [37.5;53.1]	42.4 [35.4;53.4]	0.191
Stage N3 (%)	26.6 [15.7;36.0]	26.6 [15.6;35.4]	26.6 [16.7;36.3]	0.787
Stage R (%)	13.8 [9.09;18.1]	14.1 [10.4;17.7]	13.0 [8.16;18.3]	0.351
Sleep quality				
Total sleep time (min)	340 [306;374]	344 [318;375]	336 [300;374]	0.252
Sleep latency (min)	14.8 [7.30;29.0]	12.5 [7.15;24.0]	17.0 [8.00;31.6]	0.082
Sleep efficiency (%)	84.8 [75.7;90.4]	86.5 [77.4;91.7]	82.8 [74.8;88.9]	0.014
Total wake time (min)	64.0 [40.4;103]	56.0 [34.0;92.0]	70.0 [48.9;108]	0.018
WASO (min)	45.5 [27.2;77.0]	40.0 [23.0;71.0]	50.0 [29.6;80.3]	0.127
Somnolence (ESS)	11.0 [7.00;14.0]	11.0 [6.00;13.5]	10.5 [7.00;15.0]	0.209



A. Individual associations of AHI with the risk of impaired nocturnal BP dipping. Logistic regression model for evaluating the individual association between the AHI and the risk of a nondipper BP profile. Data are presented as the OR (95% CI), which represents the risk of nondipping per 1-SD increase in the AHI, the AHI categorized by OSA severity, or the AHI categorized by the optimal cutoff value. B. Individual associations of OSA parameters with the risk of impaired nocturnal BP dipping. Logistic regression model for evaluating the individual associations between the OSA metrics and the risk of a nondipper BP profile. Data are presented as ORs (95%CI), which represent the risk of nondipping per 1-SD increases in the OSA parameters. C. LASSO analysis for identification of the key factors of nondipping BP. Predictive model for the detection of nondipper status constructed using a variable selection process based on LASSO regression. D. Random Forest analysis for identification of the key factors of nondipping BP. Ranking of the importance of each variable in the classification of the study groups (nondippers vs. dippers), based on RF analysis. Only variables displaying a minimum discrimination are shown in the graphic. Definitions of abbreviations: AHI = apnea-hypopnea index; BMI = body mass index; CI = confidence interval; LASSO = least absolute shrinkage and selection operator; OR = odds ratio; OSA = obstructive sleep apnea; R = rapid eye movement; RF = random forest; SaO2 = oxygen saturation; SD = standard deviation; TSat90 = time with SaO2 < 90%.

Conclusions: The respiratory arousal index is a key OSA-related parameter associated with the loss of nocturnal BP dipping. The results suggest that sleep fragmentation is an important mechanistic pathway underlying the relationship between OSA and circadian BP abnormalities.
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19. COMPARATIVE ASSESSMENT OF HETERORESISTANCE TO IMIPENEM BY ETEST AND DISC DIFFUSION METHODS IN *HAEMOPHILUS INFLUENZAE* RESPIRATORY ISOLATES FROM COPD PATIENTS

Celia Gil-Campillo[1,2], Aída González-Díaz[2,3], María Lázaro-Díez[1], Iris Elizalde[1], Sara Martí[2,3], Junkal Garmendia[1,2]

[1]Instituto de Agrobiotecnología (IdAB-CSIC), Mutilva, Navarra, Spain. [2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), ISCIII, Madrid, Spain. [3]Microbiology Department, Hospital Universitari de Bellvitge, IDIBELL-UB, Barcelona, Spain.

Introduction: Antibiotic resistance is a major Public Health challenge worldwide. Mechanisms other than resistance are being described as contributors to therapeutic failure. These include heteroresistance (a resistant subpopulation within the total bacterial population) and tolerance (ability of an antibiotic-sensitive bacterium to survive but not grow over prolonged periods of exposure to bactericidal antibiotics). Both phenomena escape standardized procedures used for antibiotic treatment decision making, as do not involve minimal inhibitory concentration (MIC) changes. *Haemophilus influenzae* causes chronic respiratory infection and is associated with exacerbations in COPD patients. Although resistance to imipenem is rare, heteroresistance has been reported and tolerance cannot be excluded, which altogether could facilitate decreased antimicrobial activity.
Objectives: To identify and characterise heteroresistance and/or tolerance in imipenem-susceptible H. influenzae strains in order to provide more accurate clinical treatment recommendations.
Methods: We used 90 whole-genome sequenced H. influenzae strains previously isolated from COPD respiratory samples at the Belltvitge University Hospital. Imipenem susceptibility was tested by Etest and disc diffusion using Mueller-Hinton fastidious (MH-F)

medium following EUCAST criteria. Next to establishing imipenem susceptibility, we evaluated: i) growth of colonies inside the growth inhibition halo in the presence of imipenem disc or Etest, as an indicator of heteroresistance; ii) growth of colonies inside the growth inhibition halo, after replacing the antibiotic disc with a glucose disc (an adaptation of the TD-test method for antimicrobial tolerance), as an indicator of susceptible non-heteroresistant but tolerant strains.

Results: Our results showed a signiticant proportion of imipenem heteroresistance by both methods. All disc diffusion heteroresistant strains had the same behavior upon Etesting, although coincidence between assays was moderate, 75.5% detected by Etest and 36.7% by disc diffusion. Heteroresistance was heterogeneous and two heteroresistant phenotypes were established by visual examination of the plates: i) Type 1, the growth inhibition halo covered of colonies; ii) Type 2, colonies at the edge of the zone of inhibition (Figure). Evaluation of TD-test in susceptible non-heteroresistant strains showed no tolerance to imipenem.

Conclusions: Using established susceptibility breakpoint values, we identified a significant proportion of imipenem heteroresistance but no tolerance among H. influenzae respiratory isolates with comparable reliability for Etest and disc diffusion methods.

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22. THE MYOSTATIN PATHWAY IN THE LOWER LIMB MUSCLES OF SARCOPENIC COPD PATIENTS

Adriana Núñez Robainas[1,2,3], Maria Guitart[1,2,3], Antonio Sancho Muñoz[1,2,3], Esther Barreiro[1,2,3]

[1]Pulmonology Department, Muscle Wasting and Cachexia in Chronic Respiratory Diseases and Lung Cancer Research Group, IMIM-Hospital del Mar, Parc de Salut Mar, Barcelona, Spain. [2]Medicine and Life Sciences Department (MELIS), Universitat Pompeu Fabra (UPF), Barcelona Biomedical Research Park (PRBB), Barcelona, Spain. [3]Group CB06/06/0043 of Centro de Investigación en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III (ISCIII), Barcelona, Spain.

Introduction: Skeletal muscle dysfunction, namely sarcopenia, is a common systemic manifestation in patients with chronic obstructive pulmonary disease (COPD). Myostatin regulatory pathway is involved in muscle mass maintenance. Whether the myostatin pathway is altered in the development of sarcopenia in patients with severe COPD remains to be fully elucidated. We hypothesized that the expression of markers of the myostatin pathway is altered in the lower limb muscles of sarcopenic severe COPD patients.

Objectives: In the vastus lateralis (VL) of the quadriceps muscle of 41 COPD patients (23 sarcopenic and 18 non-sarcopenic) and 13 healthy subjects (control group): 1) to evaluate gene expression levels of myostatin and downstream targets (ACVR2A, ACVR2B, SMAD2, SMAD3,

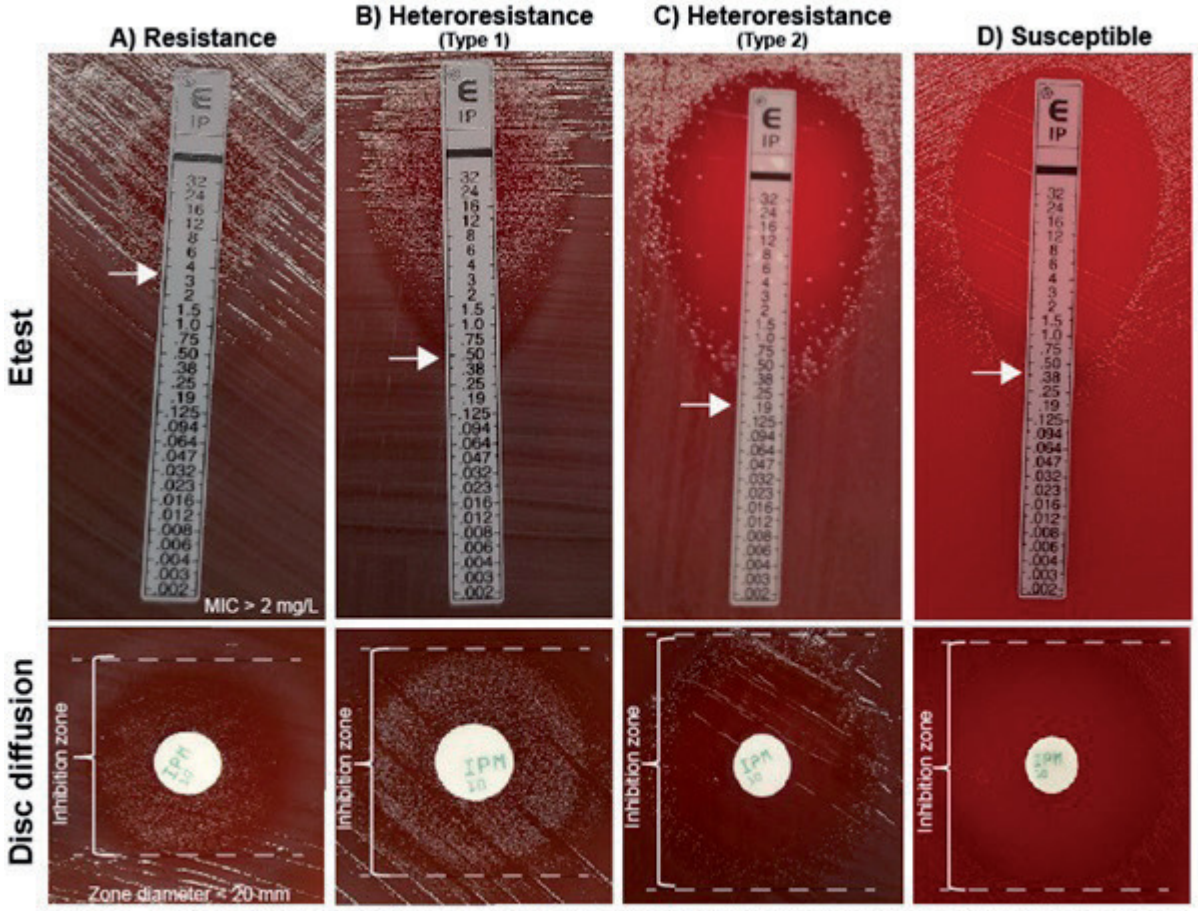

Figure 1. (A–D) Examples of imipenem Etest and disc diffusion assay results with *H.influenzae* COPD isolates (EUCAST criteria). White arrows indicate the MIC; white dashed lines show the diameter of the zone where bacterial growth is inhibited by imipenem, i.e. inhibition zone. (A) *H.influenzae* isolate resistant to imipenem; (B-C) Heteroresistant strains, growth inhibition around the antibiotic disc and Etest with appearance of colonies heavily covering the halo (Type 1) or at the edge of such inhibition halo (Type 2); (D) Susceptible isolate, growth inhibition around the antibiotic disc and Etest, and absence of colonies within the inhibition zone.

SMAD4, AKT1, PI3KR1) 2) to analyze protein expression levels of myostatin and downstream targets (SMAD2, p-SMAD2, SMAD3, p-SMAD3, SMAD4) 3) to quantify gene expression levels of follistatin, activin A, and IGF1.
Methods: Thirteen healthy subjects and forty-one patients with stable COPD (23 sarcopenic and 18 non-sarcopenic) were recruited. Nutritional and functional parameters of all the patients were evaluated. In the VL of all participants, mRNA expression of myostatin, activin A, ACVR2A, ACVR2B, SMAD2, SMAD3, SMAD4, AKT1, PI3KR1, IGF1 and follistatin were determined using qRT-PCR. Protein levels of myostatin, SMAD2/p-SMAD2, SMAD3/p-SMAD3 and SMAD4/p-SMAD4 were identified using immunoblotting.
Results: In sarcopenic COPD patients, nutritional and muscle function status were reduced compared to healthy controls. In muscle specimens of sarcopenic COPD, mRNA levels of myostatin, IGF1, and follistatin were greater, while those of activin A and SMAD3 were lower than in the controls and non-sarcopenic patients. Protein levels of myostatin, p-SMAD2, p-SMAD3 and SMAD4 significantly increased in sarcopenic COPD patients compared to control subjects.
Conclusions: In conclusion, both groups of COPD patients presented an upregulation of myostatin pathway. Nevertheless, downstream components of this pathway were found to be differentially expressed in sarcopenic compared to non-sarcopenic COPD patients. These findings may have potential implications in the management of sarcopenic COPD patients.
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25. OPTIMIZATION OF A WALL SHEAR STRESS IN VITRO MODEL OF CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH)

Ylenia Roger[1,2], Adelaida Bosacoma[1,2], Anna Sardiné[1,2], Àngela Vea[1], Ana Ramírez[1], Víctor I. Peinado[1,2,3], Isabel Blanco[1,2], Jordi Gracia[4,5], Manuel Castellà[6], Joan A. Barberà[1,2], Olga Tura[1,2,7]

[1]Department of Pulmonary Medicine, Hospital Clínic-University of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain. [2]Biomedical Research Networking Center in Respiratory Diseases (CIBERES), Madrid, Spain. [3]Department of Experimental Pathology, Institut d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC-IDIBAPS, Barcelona, Spain. [4]Liver Vascular Biology Research Group, IDIBAPS & Hospital Clínic de Barcelona, Barcelona, Spain. [5]Biomedical Research Networking Center in Hepatology and Digestive Diseases (CIBEREHD), Madrid, Spain. [6]Cardiovascular surgery service, Cardiovascular institute, Hospital Clínic-University of Barcelona, Barcelona, Spain. [7]Pneumology service, Hospital Dr. Josep Trueta, Hospital Santa Caterina de Salt, Institut d'investigació biomèdica de Girona (IDIBGI), Girona, Spain.

Introduction: Most of *in vitro* studies related to pulmonary vascular diseases, such as chronic thromboembolic pulmonary hypertension (CTEPH) are performed in vitro under static conditions which fail to entirely recapitulate the *in vivo* dynamics of pulmonary arteries. Exposing patient-derived endothelial cells (ECs) to a specific wall shear stress (WSS) could represent a better approach to modulate the in vivo environment and to achieve more representative and translatable results. Appropriate blood flow and hemodynamic signals are essential for directing the proper vascular development in humans, as mechanical forces influence the development of a balanced endothelial cell phenotype and function. The presence of an abnormal blood flow can alter gene and protein expression and lead to generation of endothelial dysfunction (ED) leading to abnormal vascular remodeling.
Objectives: The aim of this study is to compare the effect of WSS condition vs static condition on ECs derived from patients with CTEPH (EC-CTEPH) compared to healthy controls and to identify potential differences when ECs are subjected to different flow culture conditions.
Methods: WSS in both control and EC-CTEPH was assessed by culturing both cell lines for 24h in two different systems: a static and under the flow using Ibidi's perfusion system and a laminar WSS of 20 dyn/cm^2. Assessment of cell morphology, qRT-PCR for angiogenic and metabolic genes and supernatant (SN) analysis were performed using EC-CTEPH (n = 3). HPAE cells were used as control group (n = 3).
Results: Preliminary results show the maintenance of typical cobblestone morphology in ECs cultured under static conditions, but a more elongated morphology can be observed in ECs cultured at 20 dyn/cm^2. Due to changes in morphology and cytoskeleton rearrangements cell area appears increased under static conditions. Interestingly under flow condition control cells do align with the direction of the flow but do not in the case of EC-CTEPH. Glycolytic ratio of both cell types (control and EC-CTEPH) appears higher when cells are cultured under static conditions.
Conclusions: Culturing cells under static conditions is not sufficient to recapitulate the role of ED in the pathophysiology of CTEPH. Cells cultured at 20 dyn/cm^2, which is considered the physiological condition within the pulmonary arteries, behave differently than those cultured in vitro under static conditions. Whereas control cells align with the direction of the flow after 24h, EC-CTEPH do not. Further experiments will confirm these results.
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26. IRON DEFICIENCY ELICITS SYSTEMIC AND MUSCLE REDOX IMBALANCE IN COPD

Maria Pérez Peiró[1,2], Mariela Alvarado[1,2], Clara Martín Ontiyuelo[1,2], Xavier Duran[3], Diego Rodríguez Chiaradía[1,2], Esther Barreiro[1,2]

[1]Pulmonology Department-Muscle Wasting and Cachexia in Chronic Respiratory Diseases and Lung Cancer Research Group, IMIM-Hospital del Mar, Parc de Salut Mar, Department of Medicine and Life Sciences (MELIS), Universitat Pompeu Fabra (UPF), Barcelona Biomedical Research Park (PRBB), Barcelona, Spain. [2]Centro de Investigación en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III (ISCIII), Grupo CB06/06/0043, Barcelona, Spain. [3]Scientific, Statistics, and Technical Department, Hospital del Mar-IMIM, Parc de Salut Mar, Barcelona, Spain.

Introduction: Skeletal muscle dysfunction and nutritional abnormalities are prevalent systemic manifestations in chronic respiratory diseases including chronic obstructive pulmonary disease (COPD). Iron deficiency (ID) is one of the most common nutritional deficiencies worldwide, and up to 50% of COPD patients exhibit alterations in iron metabolism. Iron metabolism alterations may influence redox balance homeostasis in animals and patients. Whether the impact of ID on redox balance in COPD patients has not been evaluated. Thus, we hypothesized that severe stable COPD patients with ID may have an increased systemic and muscle oxidative stress due to the imbalance between oxidants and antioxidants production.
Objectives: In COPD patients with ID the objectives are the following: 1) to evaluate exercise capacity and limb muscle strength; 2) to assess blood iron metabolism parameters; 3) to analyze muscle and blood pro-oxidant and antioxidant markers; 4) to examine muscle fiber morphometry, composition and structural abnormalities; 5) to explore correlations between redox markers and clinical variables.
Methods: Forty patients with stable severe COPD (ID and non-iron deficiency (NID), n = 20/group) were recruited. In all patients, exercise capacity (6-minute walk test) and limb muscle strength (hand grip and quadriceps maximum voluntary contraction) were evaluated.

In the vastus lateralis (VL) of all patients, muscle fiber composition and morphometry (immunohistochemistry), TUNEL positive nuclei (TUNEL assay) and muscle damage (hematoxylin-eosin staining), were assessed. Pro-oxidant (protein carbonylation, MDA-protein adducts and protein tyrosine nitration) and antioxidant markers (catalase, superoxide dismutase (SOD), reduced glutathione and total antioxidant activity (TEAC)) were analyzed in serum (ELISA) and VL samples (immunoblotting) in all patients.

Results: In ID COPD patients, the distance walked in the 6-minute walk test decreased compared to NID COPD patients. Type I fibers were significantly decreased while type II fibers were significantly increased in ID COPD patients in comparison to those COPD patients with normal iron levels. In ID patients compared to NID COPD patients, muscle and serum protein tyrosine nitration significantly increased, while muscle protein levels of SOD-2 and serum TEAC significantly decreased. In muscles of ID COPD patients, SOD-2 levels negatively correlated with type II fibers proportion.

Conclusions: In ID COPD patients, exercise capacity decreased and a switch from type I toward type II muscle fibers was observed. ID elicits a differential systemic and muscle redox imbalance/balance in COPD patients. These findings contribute to the knowledge of iron's role in the redox balance within severe COPD patients.

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28. NEW MODEL FOR SUBJECTING 3D-CULTURED CELLS TO INTERMITTENT HYPOXIA MIMICKING SLEEP APNEA

Alicia Jurado[1], Anna Ulldemolins[1], Jorge Otero[1,2,3], Isaac Almendros[1,3,4], Ramon Farré[1,3,4]

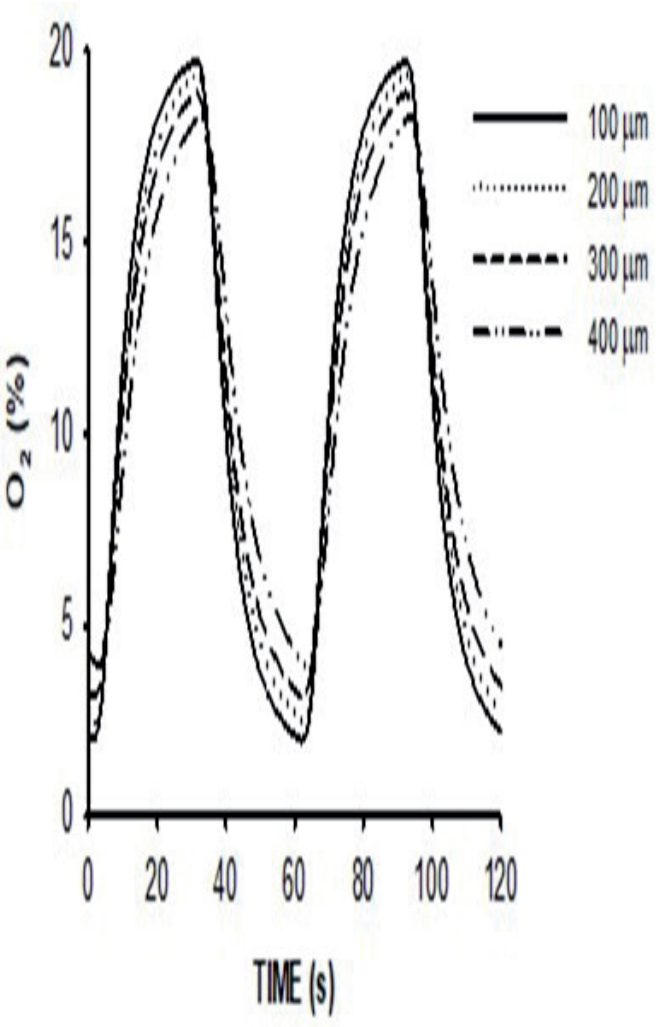
[1]Unitat de Biofísica i Bioenginyeria, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona, Barcelona, Spain. [2]The Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain. [3]CIBER de Enfermedades Respiratorias, Madrid, Spain. [4]Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain.

Introduction: Patients with obstructive sleep apnea (OSA) experience recurrent hypoxemic events with a frequency of up to 60 events/h. The intermittently hypoxemic arterial blood reaching the systemic capillaries induces transient hypoxia in the parenchymal tissue of all organs, which is the main cause of the pathological consequences of OSA. Whereas there are advanced experimental models to apply intermittent hypoxia to cells conventionally cultured in 2D plates (i.e. mimicking epithelial or endothelial monolayers), there is so far no well-characterized model to subject 3D-cultured cells to controlled intermittent hypoxia so as to study the effect of OSA on most cell types residing within tissues.

Objectives: To design and characterize an experimental setting to apply high-frequency intermittent hypoxia mimicking OSA to cells cultured in 3D hydrogel scaffolds made from native extracellular matrix (ECM).

Methods: The 3D scaffold was a 500-μm hydrogel made from lung ECM and placed on a 2 cm-diameter well bottom made consisting of a 165 μm-width permeable silicon membrane (as described in Front Pharmacol. 2022;13:945134). The 3D scaffold was covered with a 1 mm layer of cell culture medium and room air (20% O2). The chamber beneath the silicone membrane was subjected to a square wave of hypoxic/normoxic air composed of 30 s of 20% O2 and 30 s of 0%. The oxygen concentration at different depths within the hydrogel was measured with a 50-μm diameter, fast-response optical fiber oxygen sensor (Pyroscience, Aachen, Germany).

Results: The Figure shows examples of the oxygen concentration measured at 100, 200, 300 and 400 depth μm within the hydrogel scaffold for an intermittent hypoxia rate of 60 apneas/h, mimicking severe OSA. These data show that 3D-seeded cells within the ECM hydrogel would be subjected to well-controlled and realistic intermittent hypoxia patterns.



Conclusions: The new designed and characterized experimental model will be an excellent tool for investigating the effects of intermittent hypoxia in 3D-residing cells within the parenchyma of the different organ tissues in OSA.

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30. THE SOLUBLE GUANYLATE CYCLASE STIMULATOR BAY 63-2521 MEDIATES ITS VASCULAR ANTIREMODELING EFFECTS BY INHIBITING MAPK PATHWAYS SELECTIVELY IN SMOOTH MUSCLE CELLS

Adelaida Bosacoma Armora[1,2], Daniel Aguilar[3], Anna Sardiné[1,2], Ylenia Roger[1,2], Àngela Vea[1], Isabel Blanco[1,2], Olga Tura Ceide[1,2,4], Joan Albert Barberà[1,2], Victor Ivo Peinado[1,2,5]

[1]Servicio de Neumología, Hospital Clínic-Universidad de Barcelona; Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain. [2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Centro de Investigación Biomédica en Red Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain. [4]Department of Pulmonary Medicine, Dr. Josep Trueta University Hospital de Girona, Santa Caterina Hospital de Salt and the Girona Biomedical Research Institut (IDIBGI), Girona,

Spain. [5]Departamento de Patología Experimental, Institut d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC-IDIBAPS, Barcelona, Spain.

Introduction: Mitogen-activated protein kinases (MAPKs) are components of a number of signal transduction pathways that regulate processes such as cell proliferation, differentiation and apoptosis. MAP kinases can be grouped into three main families: ERKs (extracellular-signal-regulated kinases), JNKs (Jun amino-terminal kinases), and p38/SAPKs (stress-activated protein kinases), each with different functions and cellular localization, and can be activated by environmental stresses (ionizing radiation, oxidative stress, cigarette smoke, etc.), inflammatory cytokines, and/or growth factors. In a previous study performed in guinea pigs exposed to cigarette smoke (CS) (a model of COPD), treatment with a soluble guanylate cyclase (sGC) stimulator reversed pulmonary hypertension and vascular remodelling likely through normalization of the gene expression of some major genes in the MAPK pathway.
Objectives: The aim of the present study was to further characterize, in cultured primary lung cells exposed to CS extract (CSE), the effect of the sGC stimulator BAY 63-2521 (Riociguat) on the activation/inhibition of MAPK pathways.
Methods: Cultures of human pulmonary artery endothelial cells (HPAEC), smooth muscle cells (PASMC) and lung fibroblasts (NHLF) were exposed to CSE (diluted 1/5) alone and in combination with Riociguat (100 uM). The TaqMan™ Array Human MAPK Pathways was used to analyse the expression of 96 major genes representative of the three families of MAPKs. Validation by qRT-PCR under the same conditions was performed for the selected genes.
Results: The results show that the expression of p38 pathway genes were significantly reduced after exposure to CSE ($p < 0.05$), along with a significant increase in the expression of dual specificity phosphatases (DUSP), including DUSP1 and DUSP10. Treatment with Riociguat significantly reduced the expression of both phosphatases ($p < 0.05$) but also down-regulated MAP3K gene expression in the MAPK cascade of all three ERK, JNK and p38 pathways ($p < 0.05$). In contrast, NHLF cells showed no effect by CSE on MAPK pathways, but Riociguat significantly increased the expression of DUSP1 and DUSP5 and major genes in the JNK and ERK pathways ($p < 0.05$). Endothelial cells did not show significant changes by CSE or Riociguat.
Conclusions: In conclusion, these results show that the sGC stimulator Riociguat exert antagonistic effects on MAPK pathway expression depending on vascular cell type. While Riociguat appears in PASMC as an inhibitor of all 3 families of MAPKs, in fibroblasts it would have an activating effect on JNKs and ERK but not on p38. The inhibition of these pathways in PASMC could explain the vascular anti-remodeling effect of sGC stimulators.
Funding: Funded by FIS PI16/01147, SEPAR 888/2019.

37. TRENDS OF COPD IN SPAIN: CHANGES BETWEEN CROSS SECTIONAL SURVEYS 1997, 2007 AND 2017

Elena García Castillo[1,2], Tamara Alonso Pérez[1,2], **Adrián Peláez**[1,2], Patricia Pérez González[1], Joan B. Soriano[1,2], Ancochea Julio[1,2]

[1]Pulmonary Department, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria La Princesa., Madrid, Spain. [2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain.

Introduction: In Spain, the prevalence of COPD has been evaluated through three nation-wide epidemiological studies in the last twenty years. The Estudio epidemiológico de la EPOC (IBERPOC) was a population survey conducted in 1997 that found a prevalence of 9.1% in the

Demographic and clinical characteristics of participants in 1997, 2007 and 2017, with all age ranges available for each study

	1997	2007	2017	2007 (40-69 yrs)	2017 (40-69 yrs)
Subjects	4,035	3,802	9,092	3,191	6,978
Age yrs, mean (± SD)	53.4 (± 8.6)	56.6 (± 10.7)	60.2 (± 11.0)	53.3 (± 8.2)	55.5 (± 7.6)*
Age range, n (%)					
40-49 yrs	1,511 (37.4)	1,245 (32.7)	1,658 (18.2)	1,245 (39.0)	1,658 (23.8)*
50-59 yrs	1,304 (32.3)	1,114 (29.3)	3,146 (34.6)	1,114 (34.9)*	3,146 (45.1)*
60-69 yrs	1,182 (29.3)	832 (21.9)	2,174 (23.9)	832 (26.1)*	2,174 (31.2)
70-79 yrs	0	611 (16.1)	1,648 (18.1)	0	0
#> 80 yrs	0	0	466 (5.13)	0	0
Males, n (%)	1,976 (49.0)	1,797 (47.3)	4,311 (47.4)	1,502 (47.1)	3,355 (48.1)
Smoking history pack-yrs, mean (± SD)	27.8 (± 22.9)	26.0 (± 21.5)	26.3 (± 22.9)	24.4 (± 19.9)*	24.5 (± 20.7)*
Smoking status %, n (%)					
Never	2,025 (50.2)	1,635 (43.1)	4,182 (46.0)	1,264 (39.7)*	1,631 (23.4)*
Ex	987 (24.5)	1,174 (30.9)	3,112 (34.2)	997 (31.3)*	2,382 (34.1)*
Current	1,023 (25.4)	989 (26.0)	1,798 (19.8)	926 (29.1)*	2,965 (42.5)*
Height cm, mean (± SD)	161.8 (± 9.0)	164.0 (± 9.2)	165.3 (± 9.5)	164.7 (± 9.1)*	166.4 (± 9.4)*
Weight kg, mean (± SD)	72.8 (± 12.8)	73.9 (± 14.1)	75.4 (± 16.1)	74.0 (± 14.4)*	76.0 (± 16.7)*
BMI $kg*m^{-2}$, mean (± SD)	27.7 (± 4.3)	27.4 (± 4.5)	27.5 (± 5.0)	27.2 (± 4.5)*	27.3 (± 5.1)*
Universitary education, n (%)	476 (11.8)	912 (24.0)	4,940 (54.3)	832 (26.1)*	4,089 (58.6)*
Symptoms, n (%)					
Cough	546 (13.5)	510 (13.4)	1,320 (15.4)	412 (12.9)*	1,012 (15.3)*
Expectoración	430 (10.7)	446 (11.7)	1,075 (12.5)	358 (11.2)	784 (11.8)
Dyspnoea	421 (10.4)	375 (9.89)	1,060 (12.3)	248 (7.8)*	664 (10.0)
Wheezing	1,622 (40.2)	1,365 (36.0)	2,828 (32.5)	1,124 (35.3)*	2,215 (32.9)*
Previous diagnoses, n (%)					
Asthma	196 (4.9)	261 (6.9)	717 (7.9)	206 (6.5)*	563 (8.1)*
COPD		52 (1.4)	273 (3.0)	27 (0.8)	139 (2.0)*
Chronic bronchitis	193 (4.8)	152 (4.0)	209 (2.3)	103 (3.2)*	122 (1.8)*
Emphysema		18 (0.5)	45 (0.5)	14 (0.4)	30 (0.4)
FEV1 % predicted, mean (± SD)	87.8 (± 17.0)	102.1 (± 19.3)	100.7 (± 17.1)	103.8 (± 17.9)*	101.4 (± 15.6)*
FVC % predicted, mean (± SD)	88.4 (± 14.6)	96.8 (± 16.3)	101.0 (± 14.9)	98.7 (± 15.3)*	101.3 (± 13.9)*
Prevalence of COPD (FEV1/FVC < 0.7), n (%)	873 (21.9)	386 (10.2)	1077 (11.8)	246 (7.7)*	614 (8.8)*
Prevalence of COPD (FEV1/FVC < LLN), n (%)	621 (15.6)	214 (5.6)	544 (5.9)	144 (4.5)*	357 (5.1)*

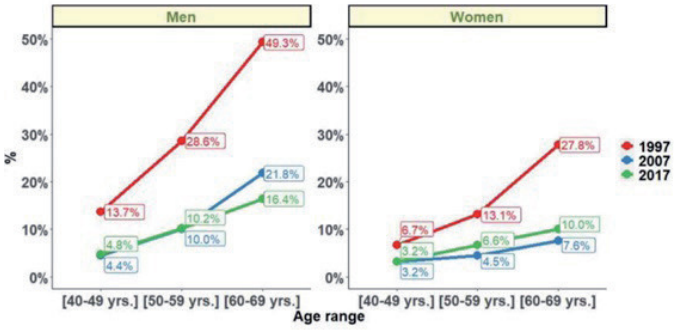
BMI: body mass index; FEV1: forced expiratory volume in 1 s; FVC: forced expiratory volume; % pred: % predicted; LLN: Lower limit of normal.

general Spanish population aged 40-69 years, using the 1995 European Respiratory Society guidelines to define COPD. The Epidemiologic Study of COPD in Spain (EPISCAN), conducted in 2007, found a prevalence of a postbronchodilator FEV1/FVC ratio < 0.70 of 10.2% in the general Spanish population aged 40-80 years. Finally, the second Epidemiologic Study of COPD in Spain (EPISCAN II), conducted in 2017 with a very similar methodology as the previous one, reported a prevalence of 11. 8% in the general Spanish population aged 40 years or older.

Objectives: We aim to describe the changes in prevalence and risk factors associated to COPD in Spain, comparing three population-based studies conducted in three timepoints.

Methods: We used data from all 17,270 participants from IBERPOC, EPISCAN and EPISCAN II. We applied a repeated cross-sectional study design to compare the COPD prevalence in these studies in 1997, 2007 and 2017. EPISCAN and EPISCAN II samples were recalculated including all subjects and defining the same age group (40-69 yrs) as the IBERPOC study. COPD was defined as a postbronchodilator FEV1/FVC ratio < 0.70, according to GOLD criteria.

Results: COPD prevalence in the population between 40 to 69 years of age decreased from 21.6% (95%CI 20.7-23.2%) in 1997 to 8.8% (95%CI 8.2-9.5%) in 2017, a 59.2% decline (p < 0.001). In 2007, the prevalence was 7.7% (95%CI 6.8-8.7%) with an upward trend of 1.1 percentage points in 2017 (p = 0.073). Overall COPD prevalence decreased in men and women, although a significant increase was observed in the last decade in females (p < 0.05) (Figure). Current smokers significantly increased in the last decades (25.4% in 1997, 29.1% in 2007 and 23.4% in 2017; p < 0.001). Regrettably, COPD underdiagnosis was constantly high, 77.6% in 1997, 78.4% in 2007, and to 78.2% in 2017 (p = 0.95), higher in younger ages (40-49 yrs. and 50-59 yrs.) and also higher in women than in men in all three studies (p < 0.05).



Changes in chronic obstructive pulmonary disease (COPD) prevalence from 1997 to 2017, by sex and age.

Conclusions: A significant reduction of 59.2% in the prevalence of COPD in Spain from 1997 to 2017 in subjects aged 40-69 years was observed. Our study highlights the significant underdiagnosis of COPD, particularly sustained in women and the younger populations.

Funding: The IBERPOC study funding was obtained from Boehringer Ingelheim, Spain, S.A. The EPISCAN study was a GSK sponsored GlaxoSmithKline Spain. The EPISCAN II study was a a GSK sponsored study, registered in ClinicalTrials.gov Identifier: NCT01122758.

40. CHARACTERIZATION OF DIFFERENT PHENOTYPES IN VENTILATOR INDUCED LUNG INJURY

Gema Sánchez[1], Raquel Herrero[2], Antonio Ferruelo Alonso[2], Raquel Murillo[1]

[1]Hospital Universitario de Getafe, Getafe, Spain.
[2]CIBER de Enfermedades Respiratorias, Getafe, Spain.

Introduction: Diffuse alveolar damage (DAD) is the histological correlate of the acute respiratory distress syndrome (ARDS) and is characterized by acute inflammation, increased alveolo-capillary permeability, apoptosis, and hyaline membrane formation in the lung. Mechanical ventilation is used as a life support treatment in ARDS, although it can cause ventilator induced lung injury (VILI). MicroRNAs (miRNAs) regulate gene expression and may be useful as biomarkers of this condition.

Objectives: To demonstrate that the presence of DAD in an animal model of VILI, as compared to the absence of DAD, is associated with a particular phenotype.

Methods: Male Sprague-Dawley rats were anesthetized and ventilated during 2.5 h using two ventilatory strategies: a protective strategy with low tidal volume (Vt, Vt = 9 ml/kg; PEEP = 5 cm H_2O, n = 10) and an injurious strategy with high Vt (Vt = 25ml/kg; PEEP = 0 cm H_2O, n = 16). We assessed the effect of mechanical ventilation in pulmonary histological changes, gas exchange, biochemical parameters, inflammation in serum and lung, apoptosis, and alveolo-capillary permeability in the lung. We analyzed miRNA expression in rat lungs by Next Generation Sequencing (NGS) and performed a pathway enrichment analysis by KEGG and REACTOME.

Results: Seven of 16 rats in the high Vt group developed DAD and showed, as compared to rats in the non-DAD group, (i) higher peak inspiratory pressure (PIP) and lower compliance; (ii) lower PaO_2/FiO_2 ratio and increased lactate concentration; (iii) higher levels of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase and glucose; (iv) increased alveolo-capillary permeability (IgM and total protein in bronchoalveolar fluid); (v) increased concentration of IL-1β, IL-6, MCP-1, MIP-2 and TNF-α in lung tissue; (vi) increased IL-6, MCP-1 and MIP-2 in serum; (vii) increased apoptosis (TUNEL) and lower caspase-3 activity in lungs; (viii) different miRNA expression profile in lungs (NGS). miRNA pathway enrichment analysis showed that the miRNAs differentially expressed were involved in pathways related to the coagulation process, immune response, inflammation, and apoptosis.

Conclusions: A phenotype associated to the development of DAD after a mechanical insult (VILI) is characterized, as compared to the absence of DAD, by higher PIP, impairment of oxygenation, acute systemic and pulmonary inflammation, increased permeability and apoptosis in the lung, and differential miRNA expression in the lung. The miRNAs differentially expressed between groups are involved in pathways thought to be important in the pathobiology of VILI and ARDS.

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46. PENICILLIN-BINDING PROTEIN AND SERINE/THREONINE KINASE ASSOCIATED (PASTA) DOMAINS FROM PNEUMOCOCCUS: LIGAND BINDING STUDIES BY NUCLEAR MAGNETIC RESONANCE

Jose Daniel Martínez[1], Francisco Javier Cañada[1,2], Beatriz Maestro[3], Jesús Miguel Sanz[3,4]

[1]NMR and Molecular Recogniton Group, Centro de Investigaciones Biológicas Margarita Salas, CSIC., Madrid, Spain. [2]CIBERES group CB06/06/1102, Madrid, Spain. [3]Protein Engineering against Antimicrobial Resistance Group, Centro de Investigaciones Biológicas Margarita Salas, CSIC, Madrid, Spain. [4]CIBERES group CB06/06/0003, Madrid, Spain.

Introduction: The pneumococcal cell wall is formed by a thick layer of peptidoglycan (PG). During cell growth and division, a complex machinery of proteins with different functions (elongation, division and regulation) is activated. In particular, S. pneumoniae displays a unique eukaryotic-like serine/threonine protein kinase named StkP,

which plays important roles in cell division, growth, PG synthesis and cell shape in bacteria. StkP is a transmembrane protein with an extracellular domain composed by 4 Penicillin-binding protein And Serine/Threonine kinase Associated (PASTA) units, of about 70 aa each.
Objectives: Although some evidence has been found that PASTA domains of certain bacterial species are able to bind synthetic fragments of PG there is a lack of a detailed description of how this interaction occurs as well as their role in the activation of StkP function. Moreover, some beta-lactam antibiotics might interact with PASTA repeats. A complete description of the molecular recognition of antibiotics by the extracellular domain of StkP remains elusive. To understand these interactions at atomic level is of uttermost importance, since it would provide answers to key questions such as whether these antibiotic-PASTA interactions are truly specific or if both the PG fragments and beta-lactam molecules bind the protein at the same site, which in turn may bring some light onto how PASTA repeats relates with StkP activation and function.
Methods: By applying NMR methodologies based in the selective spin saturation transfer from the proteins to their ligands when in contact, we analyzed the interaction of different combinations of expressed PASTA units, 123, 234 and 1234, with ampicillin, benzyl-penicillin and amoxicillin and peptides containing the D-Ala-D-Ala sequence using 1H- saturation transfer difference (STD-NMR) experiments.
Results: All the different PASTA constructs are able to bind these antibiotics, which compete for the binding to the PASTA domains studied. Results obtained by 1H-STD-NMR regarding the recognition by PASTA constructs of synthetic peptides containing the D-Ala-D-Ala sequence present in PGs will be also presented.
Conclusions: All combinations of PASTA domains are able to bind beta-lactam antibiotics, pointing to a multivalent interaction of StkP with peptidoglycan.
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48. *MYCOBACTERIUM ABSCESSUS* GENOMIC DIVERSITY AND LONGITUDINAL ANALYSIS OF MICROEVOLUTION AND REINFECTION

Sergio Buenestado-Serrano[1], Miguel Martinez-Lirola[2], Marta Herranz-Martín[1], Javier Martín-Escolano[1], Jaime Esteban[4], Paula López-Roa[5], Diego Domingo[6], Carlos Toro[7], Ester Zamarrón de Lucas[7], María Jesús Ruiz-Serrano[1,3], Patricia Muñoz[1,3], Laura Pérez-Lago[1], Darío García de Viedma[1,3] on behalf of the M. abscessus Madrid working team.

[1]Servicio de Microbiología Clínica y Enfermedades Infecciosas, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain. [2]Microbiología y Parasitología, Complejo Universitario Hospitalario Torrecárdenas, Almería, Spain. [3]CIBER Enfermedades Respiratorias (CIBERES), Servicio Madrileño de Salud, Madrid, Spain. [4]Servicio de Microbiología, Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain. [5]Microbiología y Enfermedades Infecciosas, Hospital Universitario 12 de Octubre, Madrid, Spain. [6]Microbiología, Servicio de Microbiología, Hospital Universitario La Princesa, Madrid, Spain. [7]Hospital Universitario La Paz, Madrid, Spain.

Introduction: *Mycobacterium abscessus* is a non-tuberculous mycobacterium, widespread worldwide, characterized by being an opportunistic pathogen and its resistance to a large number of antibiotics. There are still few genomic studies addressing its diversity.
Objectives: Our aim was to characterize *M. abscessus* strains from different institutions by whole genome sequencing to evaluate their diversity, inter-patient clusters and microevolution/reinfection events in prolonged persistences.
Methods: We compiled 39 isolates from 39 patients from 3 hospitals in Madrid and 53 sequential isolates from 15 patients (2-16 isolates per patient, obtained 2 months-11 years apart). Sequences were obtained by using the Nextera XT kit, run in a Miseq instrument and analyzed by a homemade pipeline.
Results: The Madrid isolates were distributed in 3 clades (20, 15 and 4 isolates; each one grouping subsp. *abscessus*, subsp. *masiliense* and subsp. *boletti*) with a high intraclade diversity (3-36248 SNPs). Some closer relationships were observed for several patients from the same or different hospitals (3-44 SNPs). Longitudinal intrapatient analysis revealed 7 reinfections (69-19143 SNPs; 3 months-4 years apart) and 11 microevolutions (2-24 SNPs; 2 months-4 years apart). The most complex case (cystic fibrosis; 16 isolates studied) involved 8 sequential reinfections along 11 years, together with a persistent strain transiently detected/underdetected.
Conclusions: In summary, genomic analysis of *Mycobacterium abscessus* allowed us to detect a wide genomic diversity in Madrid, with closer relationships between some isolates, and to reveal an unexpected intrapatient complexity, with involvement of reinfections and microevolution events.
Funding: 2021-II-PREDOC-IA-01 (IiSGM); PI21/01738 (ISCIII); PI21/01823 (ISCIII); ERDF (FEDER) Funds from the European Commission, "A way of making Europe".

51. SMOKING RULES AT HOME AMONG SPANISH ADULT SMOKERS IN 2021: FINDINGS FROM THE EUREST- PLUS ITC SPAIN SURVEY

Olena Tigova[1,2,3,4], Yolanda Castellano[1,2,3,4], Marcela Fu[1,2,3,4], Cristina Martínez[1,2,3,4,5], Geoffrey T. Fong[6,7], Esteve Fernández[1,2,3,4]

[1]CIBERES, Instituto de Salud Carlos III, Madrid, Spain. [2]Institut Català d'Oncologia (ICO), l'Hospitalet de Llobregat, Spain. [3]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), l'Hospitalet de Llobregat, Spain. [4]Universitat de Barcelona (UB), Barcelona, Spain. [5]University of California San Francisco (UCSF), San Francisco, United States. [6]University of Waterloo, Waterloo, Canada. [7]Ontario Institute for Cancer Research, Toronto, Canada.

Introduction: The home is the primary source of secondhand tobacco smoke (SHS) exposure for children, as well as for adult non-smokers. Voluntary smoking rules in a household, especially where smokers live, affect the amount of SHS that others in the home are exposed to and ultimately their health.
Objectives: To evaluate the prevalence and the determinants of smoking rules at home among Spanish adult smokers in 2021.
Methods: Data were drawn from the 2021 International Tobacco Control (ITC) Spain Survey, a nationally representative sample of adult smokers aged ≥ 18 years (n = 1,006). Data were collected either in-person or telephone between June and August 2021. Due to COVID-19 safety concerns, participants who preferred telephone interviews were able to do so. Study measures included demographics, household structure, smoking characteristics, and beliefs regarding SHS harm. We computed proportions and 95% confidence intervals (CI) as well as prevalence ratios (PR) of having a smoke-free home among smokers by means of generalised linear models adjusted for sex, age, and educational status.
Results: The prevalence of smokers' homes with complete smoking ban was 31.6% (95%CI: 27.8-35.2) while of homes with partial bans was 42.3% (38.5-46.2). The homes with complete smoking bans were more common among male respondents (33.2%; 28.7-37.9), those aged 55 or older (34.4%; 28.2-40.5), those with a medium educational level (34.7%; 28.9-40.4), those living with children (39.0%; 31.9-46.0), those without close friends who smoke (47.6%; 36.9-58.3), those who have recently quit (59.7%; 50.1-69.2), and those who believe that SHS is harmful to non-smokers (34.1%; 29.9-38.3). Among smokers, the following characteristics were significantly associated

with smoke-free rules: being 55 years or older *vs.* being 18-24 years old (PR = 1.76; 1.15-2.70), having a medium educational level *vs.* a lower one (1.29; 1.08- 1.54), living *vs.* not living with children younger than 18 years old (1.60; 1.31- 1.96), being a non-daily smoker (1.63; 1.23-2.16) or a recent quitter (1.67; 1.44-1.95) *vs.* daily smoker, having low *vs.* high nicotine dependence (2.21; 1.17-4.19), not having close friends who smoke (1.30; 1.09-1.55), and knowing vs. not knowing that SHS causes both lung cancer (1.72; 1.01-2.94) and heart attack (1.61; 1.08-2.38) in non-smokers.

Conclusions: The prevalence of smoke-free homes among Spanish smokers is relatively low. There is an association between smoke-free rules and older age, educational level, living with minors, low smoking habits (i.e., lower smoking frequency and lower nicotine dependence), not having close smoking friends and smokers' knowledge of the harmful health effects of SHS.

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54. PATHOGENICITY OF SEROTYPE 3 IN INVASIVE PNEUMOCOCCAL DISEASE

Covadonga Perez Garcia, Jose Yuste

Unidad de Neumococos, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain.

Introduction: Streptococcus pneumoniae is the major cause of respiratory tract disease. There are 101 serotypes described, where serotype 3 is associated with high mortality and morbidity rates in patients > 65 years old, as most infections occur as Invasive Pneumococcal Disease (IPD). In Spain, the 13-valent vaccine was introduced in 2010, and offers protection against this serotype. However, the number of cases of IPD due to serotype 3 has not decreased since its introduction.

Objectives: This study characterised the evolution of serotype 3 during the period 2009-2019. In addition, we analyze the relationship between genotypes, IPD and lethality in the adult population. Among genotypes, we also analysed if there were differences in the interaction with the lung epithelium and their ability to evade the immune response mediated by neutrophils and the complement system.

Methods: A total of 152 strains were selected from the Regional Public Health Laboratory of the Community of Madrid. The genotype was determined by PCR-MLST and the A549 and HL-60 cell lines were used for adhesion and phagocytosis assays. C3 deposition was studied by cytometry.

Results: The number of IPD cases by serotype 3 has remained constant since 2009 at > 65 years old. The molecular characterization of the strains demonstrated that genotypes ST180 (48.7%) and ST260 (23.4%) were the most frequent, followed by genotypes ST1220 (5.8%), ST505 (5.2%), and ST8561 (5.2%). Although, there is no association between lethality and any genotype, strains of ST180 had a high potential to produce mortality (50% of all-cause deaths). It had also been observed a higher mortality in patients with cardiac and respiratory pathologies. Pneumonia and sepsis were the most frequent clinical manifestation. Our results show that ST180 is the most frequent genotype affecting the respiratory tract. The interaction with lung and phagocytic cells showed that ST180 had increased adhesion rates and avoided more efficiently the immune responses including phagocytosis and interaction with complement component C3.

Conclusions: Pediatric vaccination has not generated herd immunity to adults against serotype 3, which is the second leading cause of IPD in adult population. As there is no association between ST and case fatality, this may depend on the presence of previous comorbidities in patients, such as respiratory or cardiac pathologies. The results seem to show an advantage of ST180 in evading the immune system and adhering to the lung epithelium compared to the other genotypes, which could explain its global and greater circulation.

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