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SP-A ENHANCES INTERLEUKIN-4-MEDIATED ACTIVATION OF ALVEOLAR MACROPHAGES BY AMPLIFYING INTERLEUKIN-4-INDUCED SIGNALING AND METABOLIC REPROGRAMMING

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Introduction: The phenotype of alveolar macrophages (aMπs) is determined in part by the alveolar environment. We recently reported that aMπs switch their phenotype by integrating IL-4 and lung-specific signals that lead to activation of tissue repair programs (Science 2017). Surfactant protein SP-A is a lung factor that amplifies IL-4Rα-dependent activation and proliferation of aMπs via the myosin18A receptor. However, the mechanism by which SP-A and IL-4 synergistically enhance activation and proliferation of aMπs remains elusive. Understanding the mechanisms that govern aMπ type 2 activation is important since deregulation of this response is involved in lung diseases caused by exaggerated repair, such as fibrosis, COPD, or even cancer.
Objectives: To analyze the signaling pathways and metabolic changes activated by IL-4 in the absence and presence of SP-A in aMπs.
Methods: The IL-4 signaling pathway (with/without SP-A) was studied by: 1) Quantification of covalent modification of proteins (pAkt, p-4E-BP1, pGSK3, pFOXO3, pPKCζ, pSTAT6). 2) Blockade of the myosin18A receptor. 3) Pharmacological inhibition of enzymes (PI3K, AKT, mTORC1, mTORC2, PKCζ) and its effects on proliferation and alternative activation of aMπs as well as on covalent modification of proteins. On the other hand, the metabolic profile of resting and IL-4-driven alternatively activated aMπs (with/without SP-A) was studied using an XF24 extracellular flux analyzer to measure the oxygen consumption rate, glycolysis reserve, and extracellular acidification rates. In addition, we analyzed glucose uptake by aMπs (with a fluorescently labeled glucose analog and flow cytometry).
Results and conclusions: SP-A activated PI3K through binding to the myosin18A receptor; accordingly, blocking PI3K activity or the myosin18A receptor abrogated SP-A's effects on IL-4 signaling. SP-A-dependent activation of PI3K and subsequent phosphorylation of its downstream effectors Akt, mTORC1, GSK3β, and PKCζ amplified IL-4-mediated aMπ proliferation and/or alternative activation. On one hand, SP-A sustained the PI3K-Akt-mTORC1 signaling pathway triggered by IL-4. Both alternative activation and proliferation of aMπs induced by SP-A+IL-4 are inhibited by Akt inhibitor VIII, torin1 (mTORC1/mTORC2 inhibitor), and rapamycin (mTORC1 specific inhibitor). Our results also indicate that SP-A increased Akt-dependent phosphorylation of GSK3β, which abrogates its role in inhibiting proliferation. On the other hand, the SP-A/Myo18A/PI3K/PKCζ axis was involved in enhancing IL-4-dependent STAT6 activation and arginase activity. PKCζ inhibition was able to reduce IL-4+SP-A-driven p-STAT6 and alternative activation, but not proliferation. With respect to aMπ metabolic profile, IL-4 increased basal mitochondrial respiration and enhanced glycolysis, and SP-A significantly enhanced IL-4 effects on aMπ metabolism. These results are consistent with the IL-4+SP-A-driven activation of the PI3K-Akt-mTORC1 axis, since glucose uptake and an active anabolism are dependent on Akt and mTORC1 activation, respectively. In conclusion, SP-A activates PI3K-dependent coordinated signaling pathways that amplify IL-4 actions in cell proliferation, metabolic reprogramming, and in the acquisition of effector functions of aMπs.

EARLY DETECTION OF SKELETAL MUSCLE BIOENERGETIC DEFICIT BY NON INVASIVE ^{31}PMR SPECTROSCOPY STUDY IN MICE EXPOSED TO CIGARETTE SMOKE

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Introduction: Skeletal muscle dysfunction is a common feature in chronic obstructive pulmonary disease (COPD) which is associated with intrinsic muscular abnormalities. Approximately half of all COPD patients present with cachexia, a condition in which disease-associated metabolic changes lead to a severe loss of skeletal muscle mass.
Objectives: Using a mouse model of chronic cigarette smoke exposure, we tested the hypothesis that chronic exposition promotes mitochondrial uncoupling and causes reduction in adenosine triphosphate (ATP) synthesis in distal skeletal muscle.

Methods: We employed in vivo 31P nuclear magnetic resonance spectroscopy in intact mice to assess the rate of ATP synthesis, and characterized the concomitant gene expression patterns in skeletal muscle in emphysema versus control mice. We specifically explored whether experimental COPD model may alter mitochondrial respiratory chain complexes in peripheral muscles. We evaluated complex I and IV enzyme activities (specific activity assays) in gastrocnemius of emphysematous mice. Whole-body was also assessed in all rodents at baseline and after every month until 6 months.
Conclusions: Compared with control animals, emphysematous mice showed a significant reduction in body weight gain and decreased activity of complexes I and IV. By using this approach, we found that rate of muscle ATP synthesis was reduced by 50% in mice exposed to cigarette smoke compared with air-exposed control mice. Furthermore, ATP synthesis rate was directly related to mitochondrial enzyme activity. Taken together, these data support the hypothesis that decreased muscle ATP synthesis, in part, may be caused by low mitochondrial enzyme activity, which may explain some aspects of muscle weakness observed in COPD patients. These findings may prove relevant to elucidating the mechanisms underlying skeletal muscle wasting observed in other chronic diseases, as well as in aging.

SLEEP DISORDER BREATHING AND NOCTURNAL HYPOXEMIA ARE ASSOCIATED WITH AN INCREASED RISK OF LUNG CANCER

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Introduction: Obstructive sleep apnea (OSA) and nocturnal hypoxemia may predispose to lung cancer.
Methods: We conducted a cross-sectional case control study including individuals participating in two prospective studies of home sleep apnea testing (HSAT) in the setting of newly diagnosed lung cancer (NCT02764866) and lung cancer screening (NCT02764866). HSAT, imaging studies, epidemiologic data, and a sleep questionnaire were available for all subjects. Patients with OSA defined as an AHI > 15 (Group A; n = 129) were compared with controls with an AHI < 15 (Group B; n = 173). Propensity score matching was used in order to evaluate the association between OSA, nocturnal hypoxemia, and lung cancer.
Results: 302 patients were included by combining both cohorts. Salient patient characteristics are summarized in Table. Lung cancer was more prevalent in Group A when compared to Group B. The difference reached statistical significance when measured by propensity score matching and nearest neighbor matching (P = 0.015 and P = 0.041 respectively). Binary logistic regression adjusted for confounders revealed an association between nocturnal hypoxemia and lung cancer, including the ODI3% (P = 0.026) and the T90% (P = 0.038). The association between AHI (P = 0.075) and ODI4% (P = 0.082) with lung cancer did not reach statistical significance, although a trend was seen.
Conclusions: Sleep disordered breathing and nocturnal hypoxemia are associated with an increased risk of lung cancer.

Variable	
Age, mean ± SD	64.7 ± 7.7
Sex female, n (%)	125 (41.4)
BMI, mean ± SD	28.5 ± 5.2
Neck circumference in cm, mean ± SD	39 ± 5.1
Visceral fat, median (IQR)	12 (8-17)
T90 %, median (IQR)	5.5 (0.8-30.2)
Lung cancer, n (%)	64 (21)
History of smoking, n (%)	255 (98)
Pack-year index, median (IQR)	47 (35-60)

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

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Introduction: Epidemiologic studies have suggested that overweight and obesity increase asthma incidence and are associated with a reduced asthma-related quality of life, more frequent exacerbations, and a decreased response to asthma medication such as corticosteroids, however, the possible mechanisms remain uncertain. We hypothesized that the poor response to glucocorticoid (GC) treatment in obese asthma patients is due to alterations in the normal functioning of the GC receptor, resulting from the metabolic syndrome and the abnormal systemic and/or pulmonary inflammatory process associated to obesity. Moreover, vitamin D deficiency has been associated with obesity and poor asthma control. Furthermore, reduced response to GC could be reversed by vitamin D intake in vivo and in vitro studies.
Objectives: *1)* To determine the clinical, inflammatory and functional characteristics of severe obese subjects with asthma before and after bariatric surgery. *2)* To demonstrate that the limited response to GC of obese asthmatic subjects is detected in vitro by the study of the suppression of PHA-induced T-cell proliferation by dexamethasone.
Methods and results: We evaluated severe (BMI ≥ 40 kg/m^2) and moderate (BMI ≥ 35 kg/m^2) obese asthmatics patients (OA), before bariatric surgery (n = 18 [14 female]; age: 57 median [41-66 interquartilic range] yr; FEV_1: 77,5 [47-107] %; BMI: 38,5 [31,8-52,7] kg/m^2). We also evaluated a group of non-obese asthmatics patients (A) (n = 3 [2 female]; age: 45 [23-70] yr; FEV_1: 86 [79-101] %; BMI: 23,6 [21,8-28,5] kg/m^2), a group of obese non-asthmatics patients (O) (n = 6 [6 female]; age: 48 [47-68] yr; FEV_1: 96 [87-105] %; BMI: 43,6 [39,5-51,1] kg/m^2) and a group of healthy subjects (C) (n = 9 [6 female]; age: 41 [24-67] yr; FEV_1: 96 [79-123] %; BMI: 24,4 [20,8-28] kg/m^2). We observed that T-cell proliferation was suppressed in vitro by dexamethasone treatment in a dose-dependent manner in all studied groups. OA group shown a trend toward a lower GC sensitivity compared to healthy subjects (IC50 OA: 33,51 nM; IC50 C: 24,98 nM, respectively). Moreover, when we added vitamin D to GC treatment we found a significant reduction in the IC50 in the OA group (IC50 VitD OA: 21,4 nM) (P < 0,007) and in healthy subjects (IC50 VitD C: 12,61 nM) (P < 0,007).
Conclusions: Obese asthma patients differ from healthy subjects in pulmonary characteristics and inflammatory profile. The OA group presented GC insensitivity in vitro, this could be due to some alterations in the normal functioning of the GC receptor, and suggest a possible mechanism of the poor response to GC treatment in these patients.

HETEROGENEITY IN LUNG 18F-FDG UPTAKE IN PRECAPILLARY PULMONARY HYPERTENSION

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Introduction: Proliferative changes in pulmonary vessels may contribute to the development of pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH). Previous studies using [18F]-fluoro-D-glucose positron emission tomography (PET-FDG) imaging in PAH have shown increased cellular metabolism in right ventricle (RV), while the results of uptake in pulmonary artery (PA) and lung parenchyma (LP) are contradictory. Little is known on these metabolic changes in CTEPH and their relationship with the distribution of thrombotic lesions. The present study aimed to evaluate cellular metabolism in LP, PA and RV by means of PET-FDG in patients with PAH or CTEPH, with proximal or distal lesions, as compared with healthy subjects.
Objectives: The main objectives are: *a)* To analyze the differences in FDG uptake, observed with PET scanning, in lung parenchyma, pulmonary arteries and right ventricle between patients with idiopathic PAH, CTEPH, and healthy controls; *b)* To assess the hypothesis that CTEPH-distal has a pulmonary uptake pattern different from CTEPH-proximal.
Methods: Four groups were evaluated: Healthy controls, n = 18; idiopathic PAH (iPAH), n = 18; proximal CTEPH (CTEPH-p), n = 13; and distal CTEPH (CTEPH-d), n = 10. The mean values of standardized 18F-FDG uptake of predefined regions of interest in LP (7 planes per lung in defined anatomical points), PA, RV and left ventricle (LV), were. SUV ratios in patients were related to functional class (FC), exercise tolerance, hemodynamic parameters and serum BNP.
Results and conclusions: The results are shown in Table. The VD/VI ratio and AP uptake were significantly correlated with the BNP. AP uptake was correlated with the walking test. We conclude that patients with precapillary pulmonary hypertension show increased FDG uptake in the right ventricle, particularly those with iPAH. The FDG uptake in both parenchyma and pulmonary arteries were significantly higher in the group with distal CTEPH.

Table. Patient characteristics and 18F-FDG uptake

	Control n = 18	iHAP n = 18	CTEPH-p n = 13	CTEPH-d n = 10
Age	35 ± 10	50 ± 14	62 ± 11	65 ± 7
Gender M/F	3/5	12/6	4/9	7/3
BMI (kg/m^2)	25 ± 5	27 ± 5	28 ± 5	35 ± 9*
FC, I-II/III-IV (%)	100/0	83/17	31/69	70/30
6MWD (m)	648 ± 82	519 ± 119*	411 ± 117*	416 ± 78*
mPAP (mmHg)	–	44 ± 11	48 ± 11	41 ± 14
CI (L/min/m^2)	–	2.32 ± 0.5	2.06 ± 0.4#	2.56 ± 0.4
PVR (dyn·s·cm–5)	–	751 ± 327	789 ± 206	515 ± 221
BNP (mg/L)	14 ± 12	97 ± 153	215 ± 211*	38 ± 26
LP SUVmean	0.53 ± 0.13#	0.55 ± 0.09	0.49 ± 0.12#	0.65 ± 0.09
PA SUVmean	1.45 ± 0.28	1.52 ± 0.21	1.53 ± 0.21	1.73 ± 0.36*
VD/VI Suv	0.50 ± 0.2	1.23 ± 0.9*	1.20 ± 0.4*#	0.73 ± 0.3

*P < 0.05 compared with control subjects. #P < 0.05 compared with CTEPH-d.

CHARACTERIZATION OF LUNG RESIDENT MESENCHYMAL STEM CELLS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Introduction: Smoking is the main environmental risk factor for COPD, but not all smokers develop thedisease. Lung resident mesenchymal stem cells (LR-MSC) have been described in healthylungs. We hypothesized that smoking may reduce the number and/or immunomodulatorycapacity of LR-MSC in susceptible smokers with COPD, thus contributing to the inflammatoryresponse that characterizes the disease.
Objectives: To isolate, characterize and compare numerically and functionally LR-MSC from: neversmokers (NS) and smokers (current (CS) and former (FS)) with and without COPD.
Methods: LR-MSC were isolated and expanded from fresh lung tissue samples from individuals (13 NSand 8 CS with normal spirometry, and 12 FS and 20 CS with COPD) using a sphere basedculture technique. They were characterized by flow cytometry and affymetrix arrays. Theimmunomodulatory capacity was assessed in vitro using cocultures with T and B cells, andafter pre-incubation with 2.5% and 5% of cigarette smoke extracts (CSE).
Conclusions: LR-MSC sharing phenotypic, transcriptional and functional characteristics with bone marrowderived MSC could be isolated from all groups. Their capacity to modulate CD8+ Tlymphocytes proliferation in co-culture was impaired in current smokers with COPD only.This deficit is reversible after quitting and reproducible in vitro.

APPNEA-QUESTIONS: A MOBILE APPLICATION TO PROMOTE CONTINUOUS POSITIVE AIRWAY PRESSURE IN OBSTRUCTIVE SLEEP APNEA PATIENTS

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Introduction: Obstructive sleep apnea (OSA) is highly prevalent chronic illness, with serious morbidity associations that represent a major burden over the health system. This makes it imperative to explore new management strategies, such as sleep telemedicine (TM), which encompasses the use of sleep-related medical information exchanged from one site to another via electronic communications to improve a patient's health. Based in our previous experiences with TM it is imperative that the technology applied for health purposes is fundamentally simple, easy to use, reliable and transparent[1]. OSA treatment with continuous positive airway pressure (CPAP) represents a major challenge for sleep specialists. Therefore, inspired in our former mobile health approaches for OSA management[2] we aim to design a new App called APPnea-Questions (APPnea-Q) which delivers a self-monitoring tool for OSA patients treated with CPAP. Here we described the app features, its feasibility and acceptance by current CPAP users and sleep specialized professionals.

Objective: To evaluate the feasibility and acceptance of APPnea-Q, in OSA patients treated with CPAP and sleep specialized professionals.

Methods and results: *Mobile application:* Patient interface: APPnea-Q incorporates new features in comparison to the previous version: *1)* A simple questionnaire (10 questions) with automated responses regarding compliance and symptoms, possible side-effects, and health sleep recommendations; *2)* Contact information to a Voice Mail Service working 24/7, were patients can contact if trouble continues, and receive assistance from a sleep specialized nurse in a 24-48 hour period; *3)* Frequent problems section, summarizes the most common side-effects (i.e., dry mouth, air leaks, nasal congestion) and possible solutions; *4)* General information on: CPAP use, healthy sleep, diet and exercise to favor an integral and more effective treatment approach to OSA. *Web-platform: Sleep professional tool:* Sleep professionals can review the information gathered from the questionnaires. All patients are filtered by non-satisfactory responses to appear at the top to identify patients in need of early assistance. *Feasibility and Acceptance of APPnea-Q: patients and sleep professionals:* Patients from 2 tertiary hospitals in Barcelona, Spain tested the APP: Hospital Clinic and Hospital Universitari de Bellvitge. All patients received a personal user name and password, to sign in. Sixty-one patients: male 48/61, mean (± SD) age 62 ± 11 years, body mass index: 32 ± 7 kg/m^2 and apnea-hypopnea index: 54 ± 18 events/h with middle to superior studies (80%) and referred as smartphones frequent users (82%). CPAP compliance was 5.6 ± 2.2 h. A System Usability Score applied to assess APPnea-Q usability perception by patients, rated as acceptable with a 77 ± 20 score and a 50th percentile of 80; and on a linear scale, the utility of the App was rated as 7,6/10. The majority of the patients rated the App content as useful: possible side-effects (87%); diet, healthy sleep and CPAP (80%); and Questionnaire automatic answers (75%). Most patients would recommend the App to other users (87%). Regarding Appnea-Q capacity to reduce hospital visits, 64% agreed, the rest were not sure or disagreed. Finally, only 41% were willing to pay 0.49 € to download the App, while 33% were not. There were 5 patients from our sample who had difficulties completing the questionnaire, and remarkably they were all over 75 years old. Sleep professionals (physicians, nurses and technicians) recruited from different sleep units all over Spain also tested the App and answer a short-inquest (n = 22). All sleep specialized physicians (n = 14) agreed that APPnea-Q can be useful to follow up OSA patients; particularly during the first month of CPAP therapy, or routine follow-up, only a few considered it for non-compliant patients. They also agreed that APPnea-Q could reduce consultations. Regarding the possibility to reduce work places, half disagree or did not know; only a few considered it a possibility. In the same way, all sleep specialized nurses and technicians (n = 8) agreed that APPnea-Q can be useful to follow up OSA patients; mainly during CPAP therapy 1st month, while half considered its use on routine patients. They also agreed with physicians that it could reduce consultations. About the possibility that this tool might reduce work places, most disagree or did not know.

Conclusions: We conclude that our mobile application (APPnea-Q) can be a feasible and well-received tool for OSA management according to patients and sleep professionals' perspectives, particularly during the first month of CPAP therapy, and could reduce visit consultations. However, the feasibly of the APPnea-Q among elderly patients should be assessed carefully.

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SELECTIVE ABERRANT CROSSTALK BETWEEN FIBROBLASTS AND CANCER CELLS THROUGH TISSUE INHIBITOR OF METALLOPROTEINASES 1-CD63 IN LUNG ADENOCARCINOMA

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Introduction: Tumour associated fibroblasts (TAFs) are important regulators of tumour growthand resistance to anti-tumoral treatments. We have recently shown that TAFs from the twomost frequent histologic lung cancer subtypes, adenocarcinoma (ADC) and squamous cellcarcinoma (SCC), respond positively to the antifibrotic drug nintedanib in the former only,thereby mimicking the selective therapeutic effects of nintedanib in ADC (but not SCC) reportedin a recent clinical trial. We also provided in vitro evidence that the tumour-promoting effects of TAFs are driven by different mechanisms in ADC and SCC. However, it remains to beelucidated which molecules are involved in the subtype-specific fibroblast-carcinoma crosstalk. Tissue inhibitor of metalloproteinases 1 (TIMP-1) is a multifunctional protein that has beenassociated with aggressive cancers and poor prognosis in lung cancer and other solid tumors,and is downregulated by nintedanib in a bleomycin model of pulmonary fibrosis. Intriguingly,our preliminary analysis has revealed that the known TIMP-1 epithelial receptor CD63 isoverexpressed in ADC compared to SCC.

Objectives: To study whether the selective tumour-promoting effects of ADC-TAFs aremediated by the binding of stromal TIMP1 to epithelial CD63.

Methods: ADC-TAFs and SCC-TAFs were stimulated with the potent fibroblast activator TGF-β1 in the presence or absence of nintedanib. We determined the TIMP-1 expression andsecretion into the conditioned medium from TAFs with qRT-PCR and ELISA, respectively. Theconditioned medium was also used to stimulate the growth and invasion of the ADC cell lineH1437 exhibiting either basal or reduced levels of CD63 by siRNA. In addition we performedimmunohistochemical analyses of CD63 in tissue sections from lung cancer patients (20 ADC,21 SCC).

Results and conclusions: Our results showed that TIMP-1 mRNA expression and secretionis stimulated by TGF-β1 in a significantly larger extent in ADC-TAFs compared to SCC-TAFs.Likewise, nintedanib elicited a larger downregulation of both TIMP-1 expression and secretionin ADC-TAFs compared to SCC-TAFs. We also confirmed that CD63 expression is higher inADC patients than SCC, and revealed that knocking-down CD63 in H1437 ADC cells issufficient to reduce the growth and invasion elicited by the conditioned medium of activatedADC-TAFs. Collectively these results unveil a novel stroma-carcinoma interaction driven byTIMP-1 and CD63 selectively in lung ADC, and support that such crosstalk is a major regulatorof the aberrant tumor-promoting effects of ADC-TAFs.

SUSTAINED AND INTERMITTENT HYPOXIA INCREASES SQUAMOUS LUNG CANCER CELL MALIGNANCY

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Introduction: Chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA)

have a great incidence in the population. OSA is characterized by recurrent obstructions of the upper airway leading to oscillations in the oxygen partial pressure. COPD, on the other hand, is characterized by the occlusion of the airways and sustained low oxygen partial pressure in lungs. An increasing number of patients have both respiratory disorders; this "overlap syndrome" causes more severe nocturnal hypoxemia than either disease alone. Thus, the intermittent (IH) and chronic hypoxia (CH) as a consequence of these respiratory diseases could modulate the malignant properties of lung cancer as previously suggested.

Objectives: In this work, the aim was to investigate whether IH or CH similar to that experienced in OSA, COPD and in patients with both diseases could increase the malignant properties of lung cancer.

Methods: An in vitro device able to subject cells to different oxygen pressures was employed. Squamous cancer cells were subjected to: 13% O_2 (healthy patients), 7% O_2 (mimicking CH in COPD), oscillations from 13% to 7% (IH 13-7) —as occurs in OSA—, and oscillations from 7% to 4% (IH 7-4) —simulating the overlap syndrome—. The proliferation and the expression of stemness markers were measured by flow cytometry and the migration ability was measured by wound healing assays.

Results and conclusions: The results showed that CH, IH 13-7 and IH 7-4 produce an increase of 69 %, $P < 0.05$, 69%, $P < 0.05$ and 73%, $P < 0.05$, respectively in the proliferation of squamous cancer cells but did not produce any change in cancer stem cell expression. In terms of cell migration, IH with an oxygen partial pressure oscillating from 7% to 4% produces a decrease (23.5%, $P < 0.05$) of their migration ability. Taken together, our findings suggest that patients with squamous cancer and suffering other respiratory diseases affecting to the availability of oxygen in the lungs could be associated with a more aggressive lung cancer.

ROLE OF MUC1 IN IDIOPATHIC PULMONARY FIBROSIS: MECHANISTIC INSIGHTS

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and irreversible form of fibrotic interstitial lung disease. MUC1, a membrane-bound O-glycoprotein, is considered as oncogenic molecule by altering signaling pathways involved in cellular processes related to IPF. In previous studies we have observed an upregulation of MUC1 and its phosphorylated forms in IPF lung tissue. However the exact participation of MUC1 in IPF is currently unknown.

Objectives: To analyze the mechanism of MUC1-induced lung fibrosis in different cellular and animal models of IPF.

Methods: The intracellular mechanism of MUC1 was evaluated by western blot, immunoprecipation and immunofluorescence in alveolar type II A549 and fibroblast MRC5, and IPF primary alveolar type II epithelial cells and lung fibroblasts. Cells were stimulated with TGFβ1. Lung tissue from human healthy/IPF and bleomycin-induced IPF mice wild type/ knockout MUC1 (KO-MUC1) was analyzed to explore MUC1 intracellular interactions by immunofluorescence.

Conclusions: Western blot indicated that TGFβ1 activated β-catenin and p-Smad2/3, which phosphorylated and activated MUC1 cytoplasmic tail (CT) at 1224 and 1229 treonin and tirosin residues. Immunoprecipitation and immunofluorescence studies showed that the multi-protein complex among pSmad2/3, β-catenin and MUC1-CT migrated into the nucleus to activate fibrotic genes in human cells and human and animal lung tissue. Unlike wild type mice, KO-MUC1 mice were protected against IPF, improving lung function, survival and fibrotic lung tissue remodeling. Therefore, pharmacologic targeting of MUC1-CT may be a promising option for the treatment of IPF.

CHRONIC INFECTION WITH *STAPHYLOCOCCUS AUREUS* AND RESPIRATORY MICROBIOME IN PATIENTS WITH CYSTIC FIBROSIS: THE IMPACT ON LUNG FUNCTION

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Introduction: Cystic fibrosis (CF) is an inherited multisystem disease characterized by abnormally thick airway secretions and persistent bacterial infection. Lung disease is the most important cause of morbidity and mortality and *Staphylococcus aureus* (SA) is a major cause of chest infection in these patients. Chronic infection with SA is associated with a tendency to a worst lung function in patients with CF and significantly higher level of RA in the microbiome.

Objectives: The aims of our pilot study were: *1)* to compare bacterial microbiome of upper airways (nasopharyngeal washes (N) and oropharyngeal swabs (OP)) and lower airways (sputum samples (S)) in a cohort of CF patients; *2)* to evaluate the impact of a chronic infection with SA on lung function; *3)* to assessed the level of *Staphylococcus* RA.

Methods: We assessed 17 patients with CF in the period between March 2013 and November 2014 who presented a clinical stability the month before the baseline visit. Clinical stability was defined as absence of acute symptoms or functional impairments or antibiotic treatments. We collected clinical data, spirometry results and respiratory samples at the baseline and at 3, 6 and 9 months. Respiratory samples included nasal lavage (NL), oropharyngeal swab (OS) and sputum (S) at the baseline, sputum at all sequent visits and a OS at the 6th months of the follow up. In this abstract we presented the preliminary results of the study.

Results: We assessed 17 patients, 53% males, with a median age of 13 years old (IQR 11-20) and a median FEV_1% of 94% (IQR 77-108). Sputum cultures were positive for SA at baseline in 6 out of 17 patients and 7 out of 17 patients presented a chronic SA infection. The regional variability of the microbiome was statistically different between NL, OS and S. Particularly, the difference was driven by lower variability in S, compared with other sites. In patients with chronic SA infection, FEV_1% was lower compared with other patients (mean 82.5% ± 23 SD vs mean 96.4% ± 16 SD, $P = 0.176$) and they presented a significantly higher RA of *Staphylococcus* (median 0.66 [IQR 0.11-0.66] vs 0.00 [IQR 0.0-0.09], $P = 0.012$).

Conclusions: Chronic infection with SA is associated with a tendency to a worst lung function in patients with CF and significantly higher level of RA in the microbiome.

MICROPARTICLES PROFILES IN VENOUS THROMBOEMBOLIC DISEASE AND CANCER

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Introduction: Cancer patients often develop thrombotic events that significantly contribute to complicated treatments, worse prognosis and higher mortality. Recent reports are focused on finding biomarkers diagnosis/progonosis of thrombotic events in neoplastic patients. In this sense, microparticles (MPs, lipidic microvesicles considered potential cellular biovectors) are considered promising biomarkers. Most studies compare MPs levels between cancer patients and cancer patients that develop thrombosis but with inconclusive results.
Objectives: To identity MPs profiles specific of cancer and thrombosis that help us to differentiate between both diseases.
Methods: We compared MPs expression levels and other blood parameters related to coagulation between two groups of patients: 1) Diagnosed of venous thromboembolism (VTE) that did not developed cancer during one year follow-up. 2) Diagnosed of cancer at advanced stage that did not developed VTE during one year follow-up. For that, at the time of the inclusion venous blood were extracted from all patients and were processed by flow cytometry to determinate levels of total MPs, tissue factor bearing MPs (TF+MPs), P-selectin glycoprotein ligand-1 bearing MPs (MPs+PSGL1) and MPs cellular origin. Additionally, plasma concentrations of D-dimer and soluble P-selectin (sP-selectin) were carried out by immunoassay techniques. Results were expressed as median and interquartile range and were compared using U-Man Whitney test. We studied 138 VTE patients without cancer and 96 cancer patients without VTE (lung, colon and upper intestinal cancer) and we found significantly higher levels of TF+MPs (events/mL) in VTE compared to cancer (median [IR]; VTE: 15,021 [23,524]; cancer: 10,462 [17,043]; P = 0.027). We also observed patients with VTE presented higher levels of D-dimer (µg/L) and sP-selectin (ng/mL) that those with cancer (D-dimer median [IR]; VTE: 3,890 [5,842]; cancer: 687 [1,123]; P < 0.001), (sP-selectin median [IR]; VTE: 50.54 [32]; cancer: 33.74 [20]; P < 0.001).
Conclusions: Levels of TF+MPs, D-dimer and sP-selectin vary grandly between unprovoked VTE patients and VTE-no-associated cancer patients. These differences could help to identify those cancer patients at greatest risk of VTE. Further cohorts studies are required to confirm this hypothesis.

PREVALENCE OF SENSITIZATION TO AVIAN OR FUNGAL PROTEINS IN DIFFERENT WORK ENVIRONMENTS: BIRD CONTROL WORKERS AND PARK AND GARDEN STAFF

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Introduction: Hypersensitivity pneumonitis (HP) is usually caused by the inhalation of organic substances such as avian and fungal proteins. In large cities, individuals who work in pest control are a possible risk group for the development of HP.
Objectives: The present study assesses a cohort of Urban Pest Surveillance and Control Service workers with high exposure to avian and fungal antigens, in order to determine their degree of sensitization to these antigens and the potential risk of developing HP.
Methods: The study population comprised bird investigators and/or managers at Urban Pest Surveillance and Control Service of the Public Health Agency, Parks and Gardens staff in Barcelona and employees of private urban pest control firms. All individuals underwent the following examinations: a medical interview regarding exposure, pulmonary function tests and sensitization studies to determine specific IgG antibodies. 1D/2D electrophoresis and western blots of pigeon serum were also performed in some HP patients and exposed workers.
Results: In this ongoing study, 101 workers have been recruited to date (76 men, average age: 42 years). Twenty five (28%) were smokers and 30 (33%) ex-smokers. 42% of the individuals were positive for IgG antibodies specific for parrots, while 32% were positive for pigeons and 21% for parakeets. Regarding fungal antigens, 81% presented specific IgG antibodies for Aspergillus sp and 70% for *Penicillium* sp. Alterations were observed in pulmonary function tests of some individuals. The degree of exposure of these workers to avian and fungal antigens is estimated to be high. Since 2010 the parrot population in the city of Barcelona has risen from 2,071 to 5,078, while the pigeon population has been high for decades (the current estimate is 115,000). Western blots of exposed workers and HP patients seemed to be different, although common antigens could be found.
Conclusions: A high degree of sensitization to avian and fungal antigens was observed in the study population, probably due to the high exposure rate of these employees. There were workers with alterations in some pulmonary parameters and western blots, suggesting that common antigens could exist between exposed workers and NH patients.

DETECTION OF ACUTE PHASE PROTEINS IN PLASMA TO IMPROVE TUBERCULOSIS DIAGNOSIS

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Introduction: Tuberculosis (TB) diagnosis, especially discrimination between active TB phases and latent phases, remains a challenge. TB diagnosis is based on the detection of the causative agent, *Mycobacterium tuberculosis.* The gold standard method is microbiological culture, which takes 2-8 weeks due to the slow growth rate of *M. tuberculosis.* Latent TB infection (LTBI) diagnosis is based on the detection of the immune response of the host against *M. tuberculosis.* One of the most used methods is QuantiFERON Gold In-Tube (QFN-G-IT; Qiagen, Germany), an in vitro test based on the detection of the interferon-gamma that is released by the T-cells after stimulation with antigens specific of *M. tuberculosis.* However, a gold standard for LTBI diagnosis has not been established. Therefore, detection of new biomarkers could serve as a tool for improved TB and LTBI diagnosis and patient management.
Objectives: To determine whether selected acute phase proteins —C-reactive protein (CRP), interferon gamma-induced protein 10 (IP-10), α1-acid glycoprotein (AGP), and α1- antitrypsin (AAT)— could help in the differentiation between uninfected individuals, LTBI individuals, and active TB patients.
Methods: Twenty-nine individuals were retrospectively included in the study: 11 patients with active TB (microbiologically confirmed), 9 individuals with LTBI (with positive QFN-G-IT), and 9 uninfected individuals (with negative QFN-G-IT). Whole blood samples were previously collected and analyzed with QFN-G-IT, by measuring the interferon-gamma on plasma samples after stimulation with *M. tuberculosis*-specific antigens and after no stimulation. Plasma samples (both stimulated and unstimulated) were recovered, and CRP, IP-10, AGP, and AAT concentrations were measured by ELISA (R&D Systems, USA; and Abcam, United Kingdom). Concentration values were plotted for stimulated plasma, unstimulated plasma, and after subtracting the value of unstimulated from that of the stimulated plasma.

Conclusions: Regarding the concentration levels of the acute phase proteins, CRP levels were significantly higher in TB patients (median: 11,293 ng/mL, IQR: 6,384-22,312) than in LTBI individuals (median: 882.6 ng/mL, IQR: 533.9-7,290) ($P < 0.01$) and in uninfected individuals (median: 4,261 ng/mL, IQR: 1,373-6,109) ($P < 0.05$) in stimulated plasma, and also in unstimulated plasma. IP-10 levels were significantly lower in uninfected individuals (median: 299.9 ng/mL, IQR: 188.9-424.1) than in LTBI individuals (median: 18,818 ng/mL, IQR: 8,332-41,730) ($P < 0.01$) and in TB patients (median: 20,675 ng/mL, IQR: 2,667-48,339) ($P < 0.001$) in stimulated plasma, and also in unstimulated plasma, and after subtracting the value of unstimulated from that of the stimulated plasma. AGP levels were significantly lower in uninfected individuals (median: 455.8 ng/mL, IQR: 324.1-750.9) than in LTBI individuals (median: 823.2 ng/mL, IQR: 485.3-1,650) ($P < 0.05$) in unstimulated plasma, but there was substantial overlap in the concentrations. AAT levels were significantly higher in TB patients (median: 3,492 ng/mL, IQR: 2,846-4,222) than in LTBI individuals (median: 2,538 ng/mL, IQR: 2,122-3,383) ($P < 0.05$) in stimulated plasma, but there was substantial overlap in the concentrations. In summary, TB patients had higher CRP levels than LTBI and uninfected individuals both in stimulated and unstimulated plasma, and uninfected individuals had lower IP-10 levels than LTBI and TB both in stimulated and unstimulated plasma, and after subtraction. Therefore, CRP and IP-10 could be of use for discriminating between uninfected individuals, LTBI individuals, and active TB patients. These preliminary results are currently being validated in a larger study. Furthermore, a prospective study including patients with suspicion of active TB is ongoing to characterize better the utility of these markers.

PROCALCITININ'S ROLE FOR PNEUMONIA DIAGNOSIS INLUNG TRANSPLANT PATIENT READMITTED TO INTENSIVE CARE UNITS

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Introduction: Acute respiratory failure and sepsis followed by graft failure are the leadingcauses of ICU readmission (ICUr) and death after perioperative period following lungtransplant (LT). Pneumonia is the main aetiology of these scenarios. Thus, it is of paramountimportance to diagnose this entity with time to antibiotics start dependent outcomes, in thisespecially vulnerable subgroup of immunocompromised patients in whom procalcitonin (PCT) has not been tested.
Objectives: To assess the association of PCT with diagnosis of pneumonia during the first 72 hours after ICUr in LT patients.
Methods: Prospective cohort study of all LT adults requiring ICUr after initial posttransplantationICU discharge, between 2012 and 2016 in a tertiary hospital with a very welldeveloped LT program (first in Spain and second in Europe). PCT plasma levels weremeasured at 24 hours, 48 hours and 72 hours after ICUr in 76 LT patients. Median time afterLT was 6 months (interquartile range: 2-25). Univariate and multivariate logistic regressionmodels, as well as ROC curves were constructed.
Results: Median values of PCT at 24, 48 and 72 hours were significantly higher in patientswith final diagnose of pneumonia ($P < 0.005$). Odds ratio (OR) for PCT at 24 hours was 1.24 (confidence interval [CI] 95%: 1.08-1.54), OR for PCT at 48 hours was 1.11 (95%CI: 1.04-1.24) and OR for PCT at 72 hours was 1.41 (95%CI: 1.13-2.10). In the multivariate analysis,only PCT at 24 hours remained as statistically significant associated with pneumonia (OR:1.47; 95%CI: 1.12-2.19) ($P < 0.02$). For PCT at 24 hours, considering a cutoff of 4.89 g/L, sensitivity was 46.4%, specificity was 97.9% (AUC of 70.87%). False negative results were associated with acute respiratory distress syndrome (ARDS). Among patients with a falsenegative result, 53.3% had ARDS at the time of re-admission, while this percentage was14.9% among patients with a true negative result ($P < 0.02$).
Conclusions: A cut-off of PCT > 4.89 g/L within 24 hours is able to predict pneumonia in LTpatients re-admitted to ICU. Sensibility might be improved considering clinical information atthe time of re-admission.

ROLE OF SURVIVIN IN EXPERIMENTAL MODELS OF PULMONARY ARTERIAL HYPERTENSION

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Introduction: Imbalance between cell proliferation and apoptosis may underlie the development of PAH (pulmonary arterial hypertension). PAH is currently considered as an uncontrolled proliferative process in pulmonary arteries. Proteins involved in the apoptosis pathway may play a role in PAH and their inhibition may represent a potential therapeutic target. Survivin is a member of the apoptosis inhibitor gene family involved in cell proliferation. In this study we investigate the potential role of survivin in the pathogenesis of PAH.
Objectives: This study aimed to explore if the expression of survivin is increased in a mice model of PAH. We also investigated the effects of the survivin inhibitor (YM155) in the mice model.
Methods: C57/BL6 mice were exposed to hypoxia and SU5416 (injected s.c. weekly) for 3 weeks. YM155, a potential suppressor of survivin expression, was also administrated s.c. during 7 days of the third week after hypoxia and SU5416 injection. Right ventricular systolic pressure (RVSP) was measured by right heart catheterization. Right ventricle (RV) hypertrophy was evaluated by the Fulton index. Vascular remodeling and survivin expression were assessed in lung sections by immunohistochemistry for smooth muscle actin (SMA) and survivin respectively. Survivin gene and protein expression were evaluated by qPCR and western blot respectively.
Results: Mice model showed significant increase in the RVSP (22 ± 3 vs 36 ± 10 mmHg [$P = 0.000002$] in controls vs SU5416 + hypoxia treated, respectively). Right ventricle hypertrophy was increased in the mice exposed to SU5416 + hypoxia (RV/LV + septum ratio, 0.29 ± 0.02 and 0.37 ± 0.07 control vs SU5416 + hypoxia [$P = 0.0005$]). SU5416 + hypoxia-treated mice developed vascular remodeling (43,6% SMA+ vessels/mm^2 in controls and 64,13% in SU5416 + hypoxia [$P = 0.0002$]) and showed a trend for an increased expression of survivin in intrapulmonary arteries (8,5% survivin+ vessels/mm^2 in controls and 23,9% in SU5416 + hypoxia [$P = 0.0002$]). Survivin gene and protein expression was 5 fold higher in SU5416 + hypoxia mice ($P < 0.001$) compared to controls. Additionally, treatment with survivin inhibitor YM155 showed reduced levels of RVSP, right ventricular hypertrophy, vascular remodeling and protein-gene survivin expression in mice exposed to hypoxia and SU5416.
Conclusions: Survivin expression is increased in mice experimental model of PAH and treatment with YM155 reverses its effect, all these findings suggest that survivin might be involved in the pathogenesis of the disease.

EPIDEMIOLOGY OF ADULT INVASIVE PNEUMOCOCCAL DISEASE AFTER INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINE 13

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Introduction: *Streptococcus pneumoniae* is a major human pathogen causing important invasive and non-invasive diseases. In Spain, the 7-valent pneumococcal conjugate vaccine (PCV7) introduced in 2001 was replaced by PCV13 in 2010 for children vaccination.
Objectives: We aimed to analyze the impact of PCV13 introduction for children in adult invasive pneumococcal disease (IPD).
Methods: This is laboratory-based multicenter study involving six Spanish hospitals. All IPD isolates from adults (≥ 18 years) were prospectively collected for serotyping in the Spanish reference laboratory and genotyped by PFGE or/and MLST. The antimicrobial susceptibility was tested by microdilution following the CLSI recommendations. Three periods were analyzed: 2008-2009 (pre-PCV13), 2012-2013 (early-PCV13) and 2015-2016 (late-PCV13).
Conclusions: A total of 2,187 pneumococcal episodes were detected (949 in pre-PCV13, 609 in early-PCV13 and 639 in late-PCV13). The overall incidence of adult IPD decreased from 12.25/100,000 population in 2008-2009 to 8.1/100,000 in 2012-2013, and remained stable in the last period (2015-2016; 9.54/100,000 population). By serotype group, IPD due to PCV7 serotypes decreased (16.59 to 11.85 to 10.46/100,000 population) as well as the incidence of IPD due to PCV13 serotypes (62.95 to 43.41 to 27.41/100,000 population). The most frequent serotypes in late-PCV13 were: serotype 8 (5.97% to 5.9% to 15.02%), 12F (2.77% to 5.42% to 7.82%), 24F (1.55% to 3.38% to 4.08%), 22F (2.77% to 4.85% to 4.08%) and 11A (1.88% to 4.20% to 4.24%), none of them included in the PCV13. The 21.9% of isolates were penicillin-non-susceptible, and was mainly associated with serotypes: 11A (nonPCV13) and 14 and 19A (both included in PCV13). While 65% of erythromycin-resistant isolates were serotype 24F. In conclusion, the decrease of adult IPD observed after the introduction of PCV13 for children has been maintained in a late PCV13 period. The serotype replacement observed deserves further analysis. The presence of antibiotic-resistant non-PCV serotypes (11A and 24F) could be a threat for the treatment of serious pneumococcal diseases such as complicated pneumonia or meningitis. Broader conjugate vaccines or non-serotype- based vaccines are necessity for the prevention of pneumococcal disease.

SEPSIS AND MECHANICAL VENTILATION ALTER THE TIGHT JUNCTION PROTEINS OF THE ALVEOLAR WALLS IN RATS

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Introduction: The tight junction (TJ) complexes of the alveolar epithelium are intercellular structures that regulate lung permeability by preventing the passage of plasma molecules into the alveolar airspaces. They are composed of transmembrane (e.g. occludin) and cytoplasmic proteins (e.g. ZO1, –2, –3) that ultimately bind to the actin fibers of the cytoskeleton.
Objectives: To determine whether sepsis and a protective strategy of mechanical ventilation alter the tight junction complexes in the alveolar walls of rat lungs in vivo.
Methods: We performed an abdominal sepsis model induced by cecal ligation and puncture (CLP) followed by mechanical ventilation (MV) in adult rats (Sprague-Dawley, male, 300-350 g of b.w.) under general anesthesia (ketamine and midazolam ip). Rats with sham surgery (laparotomy only without CLP) were included as control group. After 24 h, subgroups of sham and CLP rats were subjected to MV with a protective strategy (Vt = 9 mL/kg + PEEP 5 cmH_2O) for 4 h. Therefore, four groups of rats were established (n = 10/group): Sham-without MV, CLP-without MV, Sham+MV and CLP+MV. After euthanasia (thiopental ip and exsanguination), we collected lung tissue, bronchoalveolar lavage fluid (BALF) and plasma. We measured the levels of the TJ proteins (occludin and ZO1) by ELISA and IF, cytokines (IL-1β, IL-6 by ELISA) and apoptosis (caspase 3 and TUNEL) in the rat samples. The values were compared by ANOVA with Bonferroni correction. All assays were carried out at least in triplicate.
Results: *1)* The levels of cytokines (IL-1β, IL-6) in lung homogenates, BALF and plasma were similar in all groups, except for a decrease in the levels of IL-1β in the BALF of the CLP+MV group compared with all the other groups. *2)* Compared with the control group (sham-without VM), CLP, MV and CLP+MV increased the activity of caspase 3 and the number of apoptotic cells in rat lungs. *3)* Compared with the sham-without MV group, the levels of occludin were increased in the lungs of rats with CLP (with or without MV) (sham-without MV: 1.3 ± 0.5, CLP-without MV: 3.5 ± 1.5, sham+MV: 1.5 ± 0.5, CLP+MV: 3.0 ± 1.4; pg/μg total protein [mean ± SD], $P < 0.05$). Only the combination of CLP and MV increased the levels of occludin in BALF and plasma. In contrast, ZO1 levels decreased in the lungs of rats after CLP, MV or CLP+MV (sham-without MV: 684 ± 126, CLP-without MV: 442 ± 148, sham+MV: 477 ± 124, CLP+MV: 303 ± 148; pg/μg total protein [mean ± SD], $P < 0.05$). In CLP-without MV rats, ZO1 levels increased in the BALF and decreased in plasma.
Conclusions: Abdominal sepsis and MV with a protective strategy cause apoptosis and alter the expression of TJ proteins in the lung without modifying the expression of cytokines. Changes in the levels of the TJ proteins in BALF and plasma could be used as diagnostic biomarkers in acute lung injury.

DIFFERENTIAL EXPRESSION OF PARP IN LUNG CANCER OF PATIENTS WITH COPD AND IN MICE WITH EXPERIMENTAL LUNG TUMORS

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Introduction: Lung cancer (LC) is one of the major leading causes of cancer death worldwide. Chronic Obstructive Pulmonary Disease (COPD) is an independent risk factor for LC. Poly-ADP ribose polymerase (PARP)-1 and PARP-2 are the most representative isoforms of the PARP family which play crucial roles in DNA repair and other cell functions. We hypothesized that the expression of PARP-1 and PARP-2 may differ in the tumors compare to non-tumor lungs of both of patients and mice. COPD may also influence PARP expression in the lungs of patients.
Objectives: To evaluate the selective expression of PARP-1 and PARP-2 in lung tumors from patients with and without COPD and in mice with experimental adenocarcinoma.

Methods: *1)* Patients: Human samples were obtained from LC patients with and without COPD (2 groups of patients were recruited, n = 9/group). From these patients, lung tumor and non-tumor lung specimens were obtained (thoracotomy for the treatment of their lung neoplasm). Samples were always obtained before patients had received any chemo or radiotherapy. The absence of non-tumor was confirmed by the expert pathologist in all the patients. *2)* Mice: Lung tumors (LP07 adenocarcinoma cells) were harvested from the lungs of wild-type BALB/C mice after one month. Two groups were established (n = 9/group): non-tumor control mice and the mice bearing the lung tumors. The expression of PARP-1 and PARP-2 were determined in lung tumors and non-tumor specimens from both human and mouse studies using immunoblotting with specific selective monoclonal antibodies.
Results: *1)* Human studies: While PARP-1 expression was significantly greater in the tumors of COPD patients than in tumors of non-COPD LC patients that of PARP-2 was significantly reduced in the tumors of the former patients compared to the non-tumor control samples. *2)* Mouse studies: Whereas PARP-1 expression was significantly lower in the lung tumors of the mice than in the non-tumor lung specimens; expression of PARP-2 was significantly higher in the former samples compared to the non-tumor control lungs.
Conclusions: COPD induces a differential expression profile of PARP-1 and PARP-2 isoforms in the lung tumors of the patients. In mice, PARP-1 and PARP-2 were also differentially regulated in the lung tumors. These results suggest that PARP-1 and PARP-2 play different roles in lung cancer in mice and humans, especially in those with COPD. Further studies are underway in order to elucidate the specific roles of these two isoforms and their potential mechanisms of action in LC in both patients and mouse models.

PHYSIOPATHOLOGICAL CARDIORESPIRATORY RESPONSES TO 2 CHRONIC HYPOXIC TREATMENTS IN RATS

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Introduction: Chronic sustained hypoxia (CSH) initiates physiological adaptations to maintain oxygen levels but can also have deleterious effects such as pulmonary hypertension (PH). Chronic intermittent hypoxia (CIH), the main hallmark in obstructive sleep apnea (OSA), is associated with the development of systemic arterial hypertension and cardiovascular pathologies. The pathogenesis of both hypoxic situations is related to oxidative stress and inflammation generating endothelial dysfunction.
Objectives: To compare systemic and pulmonary hypertension and endothelial dysfunction development in rats exposed to CSH vs CIH.
Methods: Male Wistar rats were exposed to sustained hypoxic atmosphere (12%O_2; pO2 ≈ 70 mmHg, 14 days) or intermittent hypoxia (5%O_2 40 s, 21%O_2 80 s, 8 h/day, 21 days). Pulmonary artery (PA) pressure by right heart catheterization, systemic blood pressure (SBP) by carotid artery catheterization, Fulton index, vasomotor responses in PA using small vessels wire myography and L-arginine-NO metabolism (HPLC-FD) were measured.
Conclusions: Systemic and pulmonary hypertension, besides left ventricular hypertrophy, occurs in response to CIH exposure in rats, preserving pulmonary artery endothelial integrity, but decreasing NO production and eNOS protein expression. Conversely, CSH exposure triggers CB mediated respiratory chemoreflex, PH and right ventricular hypertrophy, besides blunted pulmonary vascular relaxation to carbachol and reduced plasma L-arginine: asymmetric dimethyl arginine (ADMA) ratio. CSH and CIH point to different targets and different mechanisms to produce endothelial dysfunction and finally systemic or pulmonary hypertension in hypoxic rat models.

UTILITY OF MALDI-TOF MS AS A NEW TOOL FOR *STREPTOCOCCUS PNEUMONIAE* SEROTYPING

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Introduction: Nowadays, more than 95 different *Streptococcus pneumoniae* serotypes are known, being less than one third responsible for the majority of severe pneumococcal infections. After the introduction of conjugate vaccines, a change in the epidemiology of the serotypes causing invasive pneumococcal disease has been observed making the surveillance of circulating serotypes especially relevant. Some recent studies have used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology to identify the most frequent pneumococcal serotypes that cause invasive disease.
Objectives: The main aim of this work was to assess if the discriminatory peaks previously described in the literature for serotypes 6B, 19A, 19F, and 35B were present in the spectra of pneumococcal clinical isolates of the same serotypes, regardless of the origin of the sample or the genotype. Another aim was to analyze if the mass spectra obtained by MALDI-TOF MS showed specific discriminatory peaks using three different transformed pneumococci of serotypes 11A, 19F, 19A, and if those peaks were also present in the spectra of clinical isolates of the same serotypes.
Methods: *Clinical isolates:* All *S. pneumoniae* isolates included in this study were obtained from the frozen (–80C) strain collection available at the Microbiology Department of Donostia University Hospital (Donostia-San Sebastián, Spain). Prior to freezing, all isolates were identified using the optochin sensitivity and bile solubility tests. Pneumococci were serotyped using the Quellung reaction with sera provided by the Statens Serum Institute (Copenhagen, Denmark); serotyping by Quellung was performed in some isolates after determining the capsular type by an in-house designed multiplex PCR. Multi-locus sequence typing (MLST) was performed as previously described. *Isolates included in the analysis of reported serotype-defining peaks:* To evaluate the specificity of the previously described discriminatory peaks for serotypes 6B and 19A, 19F, 35B, the mass spectra of 60 clinical isolates previously serotyped 6B (n = 10), 19A (n = 26), 19F (n = 19), and 35B (n = 5), were studied. Twenty of these isolates had the same sequence type (ST) to those used for defining discriminatory peaks in other studies. Besides, 5 reference strains of serotype 6B (ATCC 700670, ATCC 700675 ATCC BAA-342, ATCC BAA-658, ATCC 700903), 3 of serotype 19A (ATCC 700678, ATCC 700904, ATCC 700674), and one each of serotypes 19F (ATCC 700905) and of serotype 35B (Statens Serum Institute serotype 35B) were also included. S. pneumoniae *transformants used in defining serotype-specific peaks:* Three encapsulated and one unencapsulated isogenic *S. pneumoniae* transformants were tested. The strains employed in this study were M11 (unencapsulated derivative of the common laboratory strain R6) and P181, P191 and P242 constructed by transformation of strain M11 with DNA of strains 5086 (serotype 19A), 1064 (serotype 19F) and 2963/13(serotype 11A) respectively. Also five

serotype 11A clinical isolates and the above mentioned 19F and 19A clinical isolates were studied to define other serotype-specific peaks. *MALDI-TOF MS studies:* Frozen pneumococcal isolates were thawed and cultured onto Trypticase-soy agar with 5% sheep blood plates and incubated at 37C for 20–24 h in a 5% CO2 atmosphere. Bacterial extracts for MALDI-TOF MS identification (Bruker, Daltonics, Germany) were obtained by an ethanol–formic acid extraction method to generate high-quality spectra, according to the manufacturer's recommendations. Mass spectra acquisition was performed using a Microflex LT mass spectrometer (Bruker Daltonics, Germany) and the Flex Control software (version 3.4) with default parameter settings. Evaluation of the mass spectra was carried out using the Flex Analysis and MALDI Biotyper 3.1 software and library (version 3.1.66, Bruker Daltonics Germany, with 7.421 entries, last updated March 2018). Each isolate was analyzed in triplicate and the resulting average measure of each peak (m/z) was exported to a spreadsheet where a Pearson matrix correlation index was calculated using GraphPad Instat version 3.05 software (GraphPad Software Inc., La Jolla, CA, USA). After analysis, the set of discriminatory peaks obtained from mass spectra reported in the literature were compared to those obtained from our clinical and transformed isolates.

Conclusions: Most of the proposed peaks defined in the literature for the identification of serotypes 6B, 19F, 19A, 35B were not found in the spectra of the 10 reference isolates nor in those of the 60 clinical isolates tested corresponding to these four serotypes. The analysis and comparison of the mass spectra of genetically modified pneumococci (transformed strains) did not allow the establishment of new discriminatory peaks for serotypes 11A, 19F, and 19A. MALDI-TOF MS did not proved to be a valid technique for direct *S. pneumoniae* serotyping.

QUALITY CONTROL OF HOME NON-INVASIVE MECHANICAL VENTILATORS

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Introduction: Home non-invasive ventilation (H-NIV) is broadly used. Previously, we found considerable differences when comparing prescribed H-NIV settings, the actual settings in the ventilator and actual performance of ventilators[1]. Given that technologies have considerably advanced, we currently carried out a quality control study to assess the performance of new-generation home mechanical ventilators.

Objectives: To evaluate the performance of new-generation home mechanical ventilators.

Methods: H-NIV pressure-set devices from 20 patients were tested during outpatient visits by recording the difference between the prescribed ventilator variables and the values set on the ventilator panel control. Also, ventilators performance was tested using a portable device (CITREX H4, Imtmedical, Switzerland) when the ventilator was connected to a resistance (R)-compliance (C) lung model with different values of R and C and an unintended leak of 30 L/min (at a pressure of 10 cmH_2O).

Results: The H-NIV devices of 20 patients were tested. Pressure preset ventilators were used in all patients. No significant differences were found in the values provided by the ventilator and the parameters measured by the ventilator tester under baseline conditions (R = 5 $cmH_2O/(L/s)$, C = 30 mL/cmH_2O, intentional leak): IPAP: 16.94 ± 3.98 and 16.35 ± 4.59 (P = 0.89), EPAP: 5.87 ± 2.07 and 6.44 ± 2.04 (P = 0.41), respectively (m ± SD, in cmH_2O). The differences did not change when severe leaks were applied (Leak 30 L/m) (P = 0.66 and 0.25, respectively). Moreover, the agreement between the data provided by the ventilator and the data measured by the testing device was optimal for a wide range of R and C values in the simulated lung.

Conclusions: This study shows an adequate agreement between the values provided by the ventilators and the values actually measured by the ventilator tester.

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REVISITING NOCARDIOSIS AT A TERTIARY CARE INSTITUTION: ANY CHANGE IN RECENT YEARS?

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Introduction: Nocardiosis is an uncommon but severe infection, affecting both immunocompetent and immunosuppressed patients.

Objectives: The aim of our study was to analyze relevant changes in incidence, clinical and microbiological characteristics of nocardiosis over the last 8 years at our institution.

Methods: We reviewed the clinical records of patients with *Nocardia* species (2010-2017) and compared them to our previous cohort (1995-2006). *Nocardia* isolates were identified by 5' end 16S rRNA gene PCR targeting the first 500bp of the gene and sequencing. Susceptibility tests were determined by broth microdilution (CLSI guidelines).

Results: In the recent cohort (2010-2017), 35 adult patients (57.1% male, median age: 75.9 years) were identified. Nine cases were only colonized and were excluded. The underlying conditions were: COPD (40.0%), asthma (12.5%), cancer 25% (hematologic 4.2%, other 20.8%), HSCT (8.3%), HIV (3.8%) and corticosteroids (46.2%). Comparing both cohorts, the incidence of Nocardia infection increased from 6.6/100,000 admissions to 8.9/100,000. There was a decrease in the rate of HIV patients (27% vs. 3.8%, P = 0.02) and SOT recipients (18.9% vs. 0%, P = 0.03). As for clinical presentation, pulmonary nocardiosis increased (70.3% vs. 92.3%, P = 0.03), while there was no difference in skin and soft tissue (8.1% vs. 3.8%, P = 0.49) and CNS infections (5.4% vs. 3.8%, P = 0.47). *Nocardia* species did not change and the most common were *N. cyriacigeorgica* (32.4% vs. 42.2%, P = 0.27), *N. farcinica* (24.3% vs. 15.4%, P = 0.39), *N. otitidiscaviarum* (10.8% vs. 3.8%, P = 0.31), *N. veterana* (8.1% vs. 3.8%, P = 0.49) and *N. abscessus* (5.4% vs. 15.4%, P = 0.18). Resistance to antibiotics remained stable: cotrimoxazole (10.8% vs. 3.2%, P = 0.34), imipenem (5.4% vs. 9.1%, P = 0.52) and amikacin and linezolid remained 100% active. No differences were found in breakthrough nocardiosis (24.3% vs. 7.7%, P = 0.09), overall mortality (35.1% vs. 26.9%, P = 0.49) or related mortality (21.6% vs. 15.4%, P = 0.53).

Conclusions: Nocardiosis has increased in the last decade, affecting mainly patients with chronic respiratory conditions and those under corticosteroid treatment. Infections in HIV and SOT patients have practically disappeared due to the improvements in antiretroviral

therapy and cotrimoxazole prophylaxis. Pulmonary affection remains the most common localization. Nocardia species, antimicrobial susceptibility and outcome have not changed in recent years.

β-LACTAM PENETRATION INTO EPITHELIAL LINING FLUID BASED ON MULTIPLE BRONCHOALVEOLAR LAVAGE SAMPLING IN SWINE PNEUMONIA MODEL

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Introduction: Defining epithelial lining fluid (ELF) concentrations is desired for antibiotics developed for pneumonia. For ethical reasons, bronchoalveolar lavage (BAL) sampling in humans is routinely done at a single time point, thereby creating ambiguity in the precise ELF profile. It is unknown if additional sampling of the ELF would lead to more accurate exposure estimates. The swine pneumonia model was used to characterize the robust ELF profiles (5-BAL) of two β-lactams for comparison with models employing 1-BAL and 2-BAL sampling time points only.
Objectives: Describe the influence of collecting only one BAL sample from each subject in the population pharmacokinetic profile. Compare the penetration ratios and the probability of target attainment achieved by different BAL sampling approaches.
Methods: 15 ventilated swine were infected with *Pseudomonas aeruginosa* to establish pneumonia and then treated for 72 hours with ceftolozane/tazobactam (C/T) 50 mg/kg q8h (n = 7) or piperacillin/tazobactam (TZP) 200 mg/kg q8h (n = 8). Plasma and BAL concentrations were measured in each swine at 1, 2, 4, 6, and 8 hours after the first dose. Urea correction was used to calculate ELF values. Ceftolozane and piperacillin plasma and ELF data were fitted to a two-compartment model using the nonparametric adaptive grid program in Pmetrics. Hypothetical models were refitted after randomly selecting either 1-BAL or 2-BAL sampling time points from each swine. A 5,000 subject Monte Carlo simulation was performed for each model to define the probability of target attainment (PTA) (60% free time above the minimum inhibitory concentration [MIC]) and ELF penetration (area under the curve in ELF [AUCELF] vs. free AUCplasma). The KS-test was used to analyze distribution differences, reporting maximum vertical deviation (D) as percent difference; D < 20% was defined as negligible.
Results: 29 C/T and 34 TZP plasma samples and 29 and 33 BAL samples were available for the full model, respectively. 1-BAL and 2-BAL sampling models used 7-8 plasma and 15-16 BAL samples, respectively. All models adequately fitted the data. C/T PTA at 4 mg/L was 93.7, 92.9, and 95.3%, for the full, 1-BAL and 2-BAL models. TZP PTA at 16 mg/L was 55.8, 46.8, and 46.7%, respectively. C/T median (interquartile range) penetration differences were negligible between the full (94% [62 – 134]) and 1-BAL (82% [60-136], D = 10%) or 2-BAL models (97% [62-144], D = 9%). TZP penetration differences were also minimal between the full (32% [9 – 67]) and 1-BAL (17% [5-49], D = 17%) or 2-BAL models (27% [9-44], D = 14%).
Conclusions: No remarkable differences in plasma and ELF AUC nor in penetration ratios between sampling approaches were found when the 5,000-subject simulations were run and consistent PTA in ELF were displayed across all MICs for both drugs. These data suggest that antibiotic ELF models constructed from a single BAL time point result in similar exposure estimates to full ELF profiles.

EFFECT OF HYPERCAPNIA IN IN VITRO HUMAN PRIMARY CULTURE OF ALVEOLAR CELLS

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Introduction: Recent insights have emerged regarding the impact of hypercapnia on cellular and molecular function. Hypercapnia may have potentially beneficial effects in patients with ARDS, which are independent of the benefits from ventilation with low tidal volumes. However, other studies suggest that CO_2 can act as a signaling molecule via pH independent mechanisms resulting in deleterious effects in the lung.
Objectives: Determine the effects and mechanism of action of different CO_2 and pH levels in human alveolar type II cells (hATII).
Methods: After a signed informed patient consent, hATII were isolated from lung biopsies of patients that underwent lobectomy, and were incubated at 37 ºC with 5% CO_2 concentration (normocapnia) or 15% CO_2 concentration (pCO_2 = 80 mmHg). The media in both groups were buffered with 15 mM THAM to maintain pH at 7.4. Analysis of pro (IL-6, IL-1β) and anti-inflammatory markers (IL-10, IL-4), as well as apoptosis (caspase 3, 8 and 9) and permeability markers (CCL2) were done. Results were analyzed by one way ANOVA using the Turkey's range test.
Results: hATII at 15% CO_2 produced and increased IL-6 and IL-1β. IL-1β peak was reversed with THAM. Hypercapnia was associated with a decreased in CCL2 levels (monocyte recruitment) and an increase in apoptosis independent of pH (caspase 3, 8 and 9).
Conclusions: Human alveolar cells exposed to hypercapnia produced an inflammatory response, with a decrease in monocyte recruitment and a significant increase in apoptosis, all of them independent of pH. Considering the biological effects of hypercapnia, there is a need for more evidence from well-powered RCTs in order to determine its global effects in the lung.

DEVELOPMENT OF A NEW MICROARRAY SET-UP FOR HIGH THROUGH-PUT SCREENING OF EXOSOME GLYCOSYLATION

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Introduction: Extracellular vesicles are membrane-enclosed vesicles released from cells, whose composition may change under different physiological and pathological conditions. They are involved in intercellular communication and regulation of cellular functions. An elevated concentration of extracellular vesicles in blood and altered composition can be a sign of a pathological state. Host cell-derived vesicles include apoptotic bodies, microvesicles, and exosomes, which

vary in size, composition, and biosynthesis. Exosomes, the smallest vesicles (30-100 nm), contain host-derived proteins, carbohydrates, lipids, and nucleic acids. Numerous studies have examined the lipid, protein and RNA/DNA content of exosomes. However, very scarce information on their carbohydrate composition is available. Exosomes are expected to share glycosylation patterns with their parental cell, which could change in response to different pathologies. In particular, evidence for a correlation between surface glycosylation and properties of tumour cells, as e.g. tumour-immune escape, is emerging. Recently, a cell surface proteoglycan, glypican-1 (GPC1) has been identified as a potential non-invasive diagnostic and screening tool to detect early stages of pancreatic cancer, as it is specifically enriched on cancer cell-derived exosomes. Thus, isolation and characterization of exosomes in body fluids could enable the identification of specific markers that distinguish cancer exosomes from normal exosomes, aiding in the diagnosis and management of cancer.

Objectives: To set-up a novel microarray method enabling the high-throughput characterization of the glycosylation patterns of normal and tumour cell-derived exosomes in serum, in comparison with total serum.

Methods: Exosomes were isolated from 500 µl serum samples of 4 healthy people and 8 COPD patients using the Total Exosome Isolation reagent (Invitrogen, Thermofisher) following the procedure recommended by the manufacturer. Total clarified sera, supernatants of exosome isolation and isolated exosomes were printed in microarrays onto 16 pad nitrocellulose coated glass slides using a Sprint arrayer (Arrayjet Ltd.) and tested for the binding of a panel of 3 biotinylated antibodies and 28 biotinylated lectins in the presence or absence of their respective inhibitors. FITC-Annexin V and AF647-Streptavidin were used as negative controls.

Conclusions: *a)* Positive binding of anti-CD63 antibody to all exosome samples confirmed the integrity of isolated exosomes while no binding of Annexin-V was detected. *b)* Neither the low intrinsic fluorescence of the samples nor binding of streptavidin alone were significant enough to interfere with the results. *c)* The lectin binding patterns observed for exosome samples were clearly different from those observed for total serum, indicating that exosomes display distinctive glycosylation patterns. *d)* Thus, the method can be used for the screening of exosomes and sera samples in the search of new biomarkers.

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PRIMARY CARE PHYSICIANS CAN COMPREHENSIVELY MANAGE SLEEP APNEA PATIENTS: A NON-INFERIORITY RANDOMIZED CONTROLLED TRIAL WITH SEMI-AUTOMATIC ALGORITHM FOR OBSTRUCTIVE SLEEP APNEA MANAGEMENT

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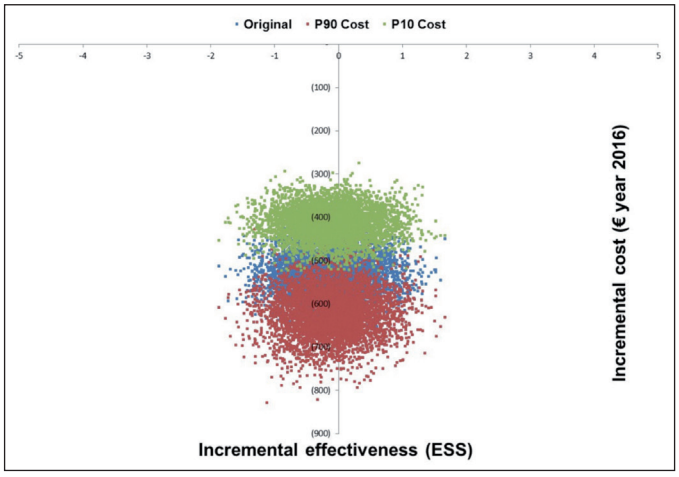
Introduction: General practitioners play a passive role in obstructive sleep apnea (OSA) management. Simplification of the diagnosis and use of a semiautomatic algorithm for treatment can facilitate the integration of general practitioners, which has cost advantages.



Objectives and methods: To determine differences in effectiveness between primary healthcare area (PHA) and in-hospital specialized management protocols during six months of followup. A multicenter, non-inferiority, randomized, controlled trial with two open parallel arms and a cost-effectiveness analysis was performed in six tertiary hospitals in Spain. Sequentially screened patients with an intermediate to high OSA probability (50% of patients with a low to high OSA suspicion) were randomized to PHA or in-hospital managements. The PHA arm received a portable monitor (PM) with automatic scoring and semiautomatic therapeutic decision-making. The in-hospital arm underwent polysomnography (PSG) and specialized therapeutic decision-making. Both arms received continuous positive airway pressure (CPAP) treatment or only sleep hygiene and dietary treatment. The primary outcome measure was the Epworth sleepiness scale (ESS). Secondary outcomes were the health-related quality of life, blood pressure, incidence of cardiovascular events, hospital resource utilization, CPAP adherence and within-trial costs.

Results: In total, 307 patients were randomized and 303 were included in the intention-to-treat analysis. Based on the ESS, the PHA protocol was non-inferior to the in-hospital protocol. Secondary outcome variables were similar between protocols. The cost-effectiveness relationship favored the PHA arm, with a lower cost of 537.8 € per patient and similar effectiveness (ESS and QALYS difference, –0,13 and –0.03, respectively). The sensibility analysis according to percentiles of cost among centers showed a mínimum savings of 430.9 € and a maximum savings of 620.9 €. Figure shows Bayesian approach for some cost percentiles.

Conclusions: In-hospital management is not necessary for approximately half of patients with a low to high OSA suspicion. Given the clear economic advantage of outpatient management, this finding could change established clinical practice.

HYPOXIA-INDUCED PD-1/PD-1 IN SLEEP APNEA PATIENTS

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Introduction: Obstructive sleep apnea (OSA) is associated with higher cancer incidence, tumor aggressiveness and cancer mortality as well as greater severity of infections, which have been attributed to an immune deregulation.
Objectives: To assess the expression of programmed cell death-1 (PD-1) receptor and its ligand (PD-L1) on the immune cells from OSA patients, and to explore its consequences on immune-suppressing activity
Methods: Monocytes and T-cells from severe (n = 14) and mild-moderate OSA patients (n = 32) and healthy subjects (n = 29) were characterized by qRT-PCR, flow cytometry and in vitro assays. We also studied the immune response on in vitro and murine models which reproduced intermittent hypoxia (IH) conditions mimicking OSA.
Conclusions: IH up-regulated the PD-1/PD-L1 crosstalk in OSA patients resulting in a reduction of CD8+ T-cells activation and cytotoxicity, providing biological plausibility to the increased incidence and aggressiveness of cancer and the higher risk of infections described in these patients.

D-DIMER AND HIGH-SENSITIVITY C-REACTIVE PROTEIN LEVELS TO PREDICT VENOUS THROMBOEMBOLISM RECURRENCE AFTER DISCONTINUATION OF ANTICOAGULATION FOR CANCER-ASSOCIATED-THROMBOSIS

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Introduction: Optimal duration of anticoagulation for cancer-associated thrombosis (CAT) remains unclear.
Objectives: This study assessed D-dimer (DD) and high-sensitivity C-reactive protein (hs-CRP) levels after the withdrawal of anticoagulation treatment to predict the risk of venous thromboembolism (VTE) recurrence among patients with CAT.
Methods: Prospective, multicenter study to evaluate CAT with ≥ 3 months of anticoagulation that was subsequently discontinued. Blood samples were taken when patients stopped the anticoagulation and 21 days later to determine DD and hs-CRP levels. All patients were followed up 6 months to detect VTE recurrence.
Results: Between 2013 and 2015, 325 patients were evaluated and 114 patients were ultimately enrolled in the study. The mean age was 62 ± 14 years and nearly 40% had metastasis. Ten patients developed VTE recurrence within 6 months (8.8%, 95% confidence interval [CI]: 4.3-15.5%). The DD and hs-CRP levels after 21 days were associated with VTE recurrence. The subdistribution hazard ratios were 9.82 for hs-CRP (95%CI: 19-52) and 5.81 for DD (95%CI: 1.1-31.7).
Conclusions: This study identified that hs-CRP and DD were potential biomarkers of VTE recurrence after discontinuation of anticoagulation in CAT. A risk-adapted strategy could identify low-risk patients who may benefit from discontinuation of anticoagulation.

EFFECT OF LONG-TERM STORAGE TIME ON ANTIGENICITY AND INTEGRITY IN TISSUE SAMPLES

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Introduction: Quality of tissue samples is essential for reproducibility of biomedical research results. Emerging biospecimen science is focused on clarifying the impact of pre-analytical factors on the sensitivity and specificity of biomarkers under development.
Objectives: OPTIMARK project, a multi-center initiative carried out by 13 biobanks within the R&D working group of Spanish National Biobank Network, aims to select and validate the pre-analytical factors relevant on tissue samples. Both antigenicity and integrity in relation with long-term storage were the first factors to be assessed, using snap frozen and FFPE tissue samples.
Methods: A total of 374 retrospective non-tumor tissue samples (colon, brain, lung, breast, stomach and endometrium) preserved from less than 1 year to more than 20 years were tested. Eight different cellular markers with ubiquitous distribution among tissues were selected, according to Human Protein Atlas database, to evaluate quality of antigenicity. RNA integrity number (RIN) was also evaluated for paired frozen samples using Agilent 2100 Bioanalyzer.
Results and conclusions: Ki-67 protein was identified as a potential quality biomarker for proliferative tissues when long term storage effect was evaluated. However, vimentin and CD31 proteins showed no differences on immunostaining quality between groups. Additional markers recently tested in lung tissue samples that showed a slight correlation between staining intensity and sample age were TTF-1, BCL-2 and beta-catenin. Except on gastric samples, optimal RIN values were obtained, although with high variability, maximum in brain samples. No correlation was observed between the RIN and long term storage. Only in colon, breast and endometrium sample groups show a slight reduction of RIN values among time. Correlations have been found on loss of antigenicity according to sample age depending on the marker used. However, the effect of long term storage on RNA integrity of frozen samples seems to be affected by other factors.

DETECTION OF *STREPTOCOCCUS PNEUMONIAE* INVASIVE DISEASE DIRECTLY FROM BLOOD SAMPLES WITH THE HELIX POMATIA AGGLUTININ (HPA) BY FLUORESCENCE IMAGING

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Introduction: *Streptococcus pneumoniae* is one of the most common human pathogens, remaining as one of the leading causes of pneumonia, bacteremia and meningitis. *S. pneumoniae* meningitis is the most frequent among adults where the early development of complications is frequent; so the importance of fast detection techniques to pneumococcal diagnosis is essential for early antibiotic therapy to avoid death and poor outcomes. However, culturing the strain remains as the gold standard technique for diagnosis. So Do-

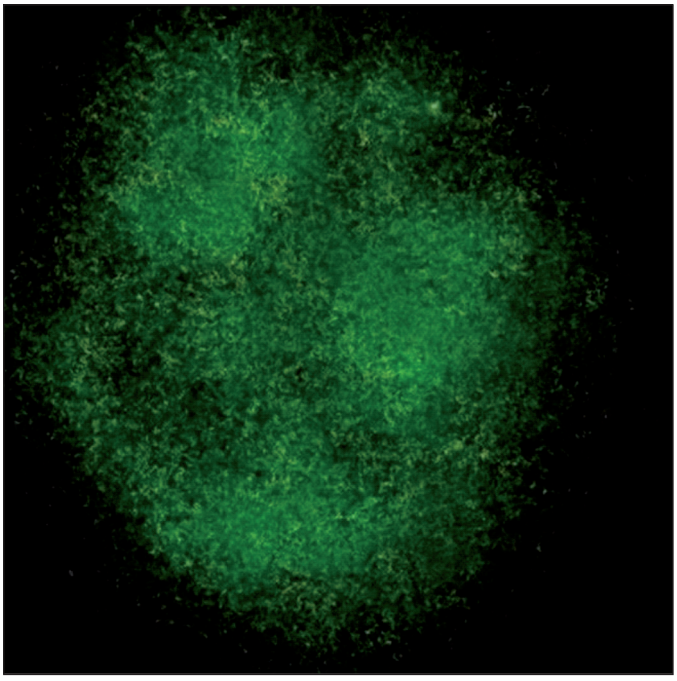

Figure. Positive sample under fluorescence microscope 100×.

menech et al[1] presented that HPA with fluorescence imaging was a potential, rapid diagnostic tool to detect *S. pneumoniae* directly from blood samples. They were able to demonstrate in their experiments in vitro that HPA bound and detected pneumococci regardless of the presence of capsule. This identification was based on the junction of the cell wall teichoic and lipoteichoic acids terminal residues (αGalNac) with HPA.

Objectives: The aims of our work were: to prove that HPA with a fluorescence microscope was as good as blood culture or spinal fluid culture to detect *S. pneumoniae* in cases of bacteremia and meningitis. To serotype the pneumococci identified in these samples.

Methods: The study was approved by the ethics committee of the Donostia University Hospital. All patients or legal representatives voluntarily signed consent forms for further analysis of the haemogram tube which had been previously collected for biochemical analysis. HPA is a 9 month-study (November 2017-July 2018). During the period of study 23 hemogram tubes from 23 different patients who had bacteremia or meningitis and whose blood or spinal fluid cultures had been confirmed as positive were collected. An aliquot of 90 µl of blood from a haemogram tube was mixed with 10 µl of HPA. Ten microliters of this mixture were examined under a fluorescent microscope between the glass slide and cover. If the blood type was A, centrifugation of the sample (90 µl of blood sample plus 10 µl HPA) at 2,000 r.p.m for thirty seconds was required prior to visualization under microscope. Of the 23 hemogram tubes analyzed 17 (73, 9%) were positive to *S. pneumoniae.* The detection of pneumococci on blood samples type A showed a poorly performing with only a 28.6% percentage of success (5/7) in the samples analyzed. Overall 14 different serotypes were detected :(3, 4, 7F, 8, 9L, 11A, 12F, 15B, 19A, 22F, 23F, 24F, 35F, 36). The most frequent one was the 8 serotype which gathered the 34.8% (8/23) of all the samples, following far behind by the serotype 3 (3/23) 13%.

Results and conclusions: HPA with fluorescence microscope showed to be a good technique to detect S.pneumoniae directly from haemogram tube, lowering significantly its effectiveness in those samples which were blood type A. The HPA with fluorescence had no influence whatsoever on the serotypes detected.

References

1. Domenech M, García E. Fluorescence Imaging of *Streptococcus pneumoniae* with the *Helix pomatia* agglutinin (HPA) As a Potential, Rapid Diagnostic Tool. Front Microbiol. 2017;8:1333.