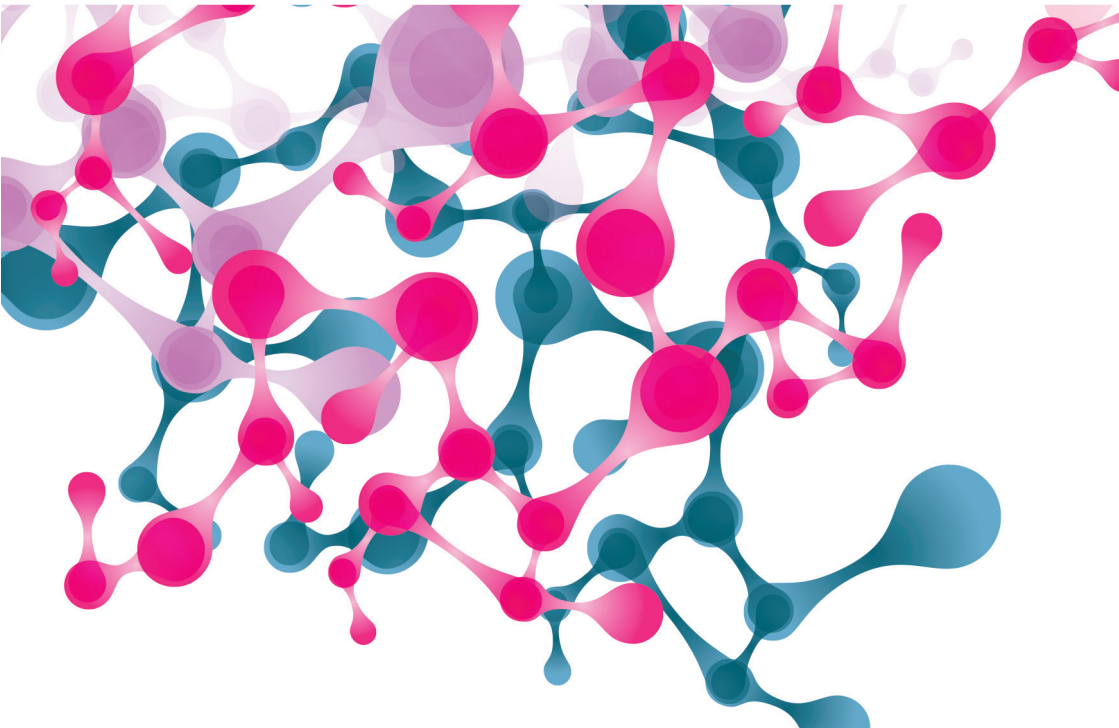
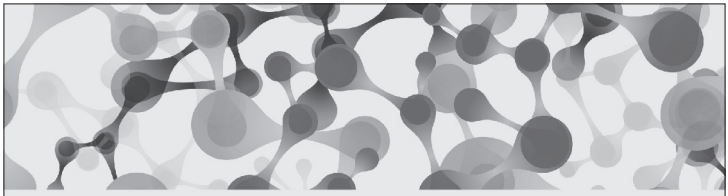
8th IMIBIC YOUNG INVESTIGATORS MEETING

IMIBIC Building • Conference Room • Córdoba, 30-31 May, 2017

Abstract Book





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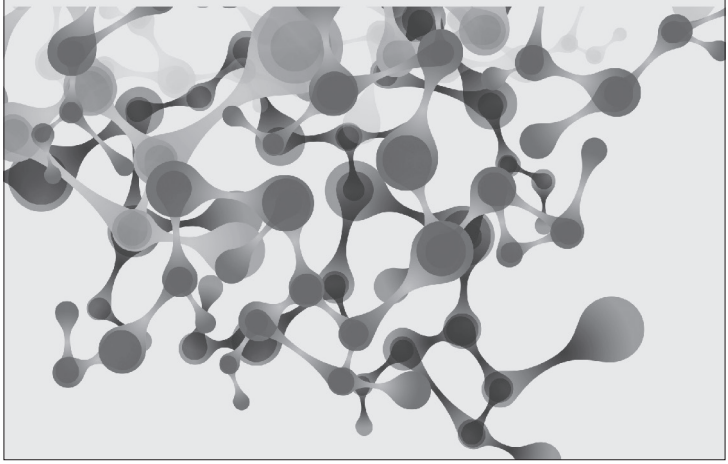

Acknowledgements
We thank the External Reviewers and the members of the Scientific Committee for their kind collaboration. We greatly acknowledge the Colegio de Médicos de Córdoba for its support and commitment to promote research among residents.

8th IMIBIC YOUNG INVESTIGATORS MEETING

PROGRAMME

Day 1 (30th MAY)	
08:30-09:00	**Registration and Poster display**
09:00-09:30	**Opening ceremony**
09:30-11:00	**SESSION I. Nutrition, Endocrine and metabolic diseases**
Ia 09:30-9:45	Atrial fibrillation, genetic and biomarkers in the Atherosclerosis Risk in Communities Study. **Antonio Pablo Arenas de Larriva**
Ib 09:45-10:00	Adipokines and their receptors are widely expressed in mouse prostate and are distinctly regulated therein by the metabolic environment. **André Sarmento Cabral**
Ic 10:00-10:15	Perturbed central ceramide signaling as novel mechanism for obesity-induced precocious puberty: Evidence from preclinical studies. **Violeta Heras Domínguez**
Id 10:15-10:30	Unbalanced proteostasis in preadipocytes in obesity-related metabolic disease. **Julia Sánchez Ceinos**
Ie 10:30-10:45	Chronic consumption of a Mediterranean diet improves endothelial dysfunction in patients with diabetes mellitus. **José David Torres Peña**
If 10:45-11:00	Involvement of a novel AMPK-Kisspeptin pathway in the metabolic control of puberty. **Alexia Barroso Romero**
11:00-11:30	**Coffee Break**
11:30-12:15	**Poster Showcase (SESSION I Nutrition, Endocrine and metabolic) & SESSION I Active ageing and fragility. Infectious and Immunological diseases. Organ transplantation)**
12:15-13:45	**SESSION II. Active ageing and fragility. Infectious and Immunological diseases. Organ transplantation**.
IIa 12:15-12:30	A prospective study to evaluate the impact of early everolimus on the recurrence of hepatocellular carcinoma after liver transplantation. **Beatriz Gros Alcalde**
IIb 12:30-12:45	Quality of Life of Informal Caregivers of People with Alzheimer's Disease. Conductual Intervention. **Patricia Luque Carrillo**

IIc 12:45-13:00	External validation of a risk score for carbapenm-resistant Klebsiella pneumoniae bloodstream infection in a prospective cohort of rectal carriers. **Ángela Cano Yuste**
IId 13:00-13:15	Vitamin E from food sources can prevent cellular aging: CORDIOPREV study. **Andreea Corina Baba**
IIe 13:15-13:30	Effect of hypoxic preconditioning on rat Adipose Derived Mesenchymal Stem Cells (ASCs) functionality. Study in a diabetic rat model. **Ana María López Díaz**
IIf 13:30-13:45	Diagnosis of acute hepatitis E genotype 3 infection by viral isolation from saliva. **Pedro López López**
13:45-14:15	**In Memory of Enrique Aguilar Benítez de Lugo**
14:15-15:45	**Lunch**
15:45-17:15	**SESSION III. Chronic and Inflammatory diseases**
IIIa 15:45-16:00	Assessment of short-term effectiveness of five local treatment modalities in patients with symptomatic knee osteoarthritis. **Mª Lourdes Ladehesa Pineda**
IIIb 16:00-16:15	Regulation of Renal Klotho and FGFR1 Expression. **Ma Victoria Pendón Ruiz de Mier**
IIIc 16:15-16:30	Diagnostic potential of NETosis-derived products for disease activity, atherosclerosis and therapeutic effectiveness in Rheumatoid Arthritis patients. **Patricia Ruiz Limón**
IIId 16:30-16:45	Lithium recovers altered mechanosensory and locomotory behaviors in neuroligin-and neurexin-deficient mutants of Caenorhabditis elegans. **Ángel Rodríguez Ramos**
IIIe 16:45-17:00	Defective glucose and lipid metabolism in rheumatoid arthritis is determined by chronic inflammation in metabolic tissues. **Iván Arias de la Rosa**
IIIf 17:00-17:15	Consumption of alcoholic beverages in nursing students at the University of Cordoba. **Pedro Manuel Rodriguez Muñoz**
17:15-17:45	**Posters**

17:45-19:15	**SESSION IV. Cancer (Oncology and Oncohematology)**
IVa 17:45-18:00	Components of the splicing machinery are drastically dysregulated in neuroendocrine tumors and associated with malignancy and aggressiveness. **Sergio Pedraza Arévalo**
IVb 18:00-18:15	Development of a CRISPR-based system to reactivate epigenetically silenced genes. **Iván Devesa Guerra**
IVc 18:15-18:30	Use of MALDI imaging technology to predict the response to antiangiogenic therapy in colorectal cancer. **Francisco Manuel Conde Pérez**
IVd 18:30-18:45	Diagnostic accuracy of MRI-Transrectal Ultrasound Fusion prostate Biopsy in men with previous prostate biopsies: The FUPROS16 project. **Enrique Gómez Gómez**
IVe 18:45-19:00	Metabolomics analysis of human sweat by gas chromatography–time of flight/mass spectrometry. **María del Mar Delgado Povedano**
IVf 19:00-19:15	Alteration of nitric oxide production targets cancer stem cells in breast cancer and augments the efficacy of anti-hormonal therapy. **Laura M. López Sánchez**

Day 2 (31th MAY)

08:30-09:00	**Registration and Poster display**
09:00-10:30	**SESSION V. Nutrition, Endocrine and metabolic diseases**
Va 09:00-09:15	Identification of candidate serum biomarkers of pediatric Growth Hormone deficiency using SWATH-MS next-generation proteomics and feature selection algorithms. **Ignacio Ortea García**
Vb 09:15-09:30	Gender interacts with metabolic abnormalities to influence carotid atherosclerosis in elderly patients. **Mª Magdalena Pérez Cardelo**
Vc 09:30-09:45	Mechanisms and consequences of obesity-induced hypogonadism: Role of a novel hypothalamic miR-137/Kisspeptin pathway. **María Soledad Avendaño Herrador**

Vd 09:45-10:00	Evaluation of the metabolic and inflammatory status in prepuberal children with a history of Extrauterine Growth Restriction or Prematurity. **Mª Dolores Ordoñez Díaz**
Ve 10:00-10:15	Significance of circulating miRNAs as predictive biomarkers in pre-diabetes and new diagnosed type 2 diabetes mellitus. **Rosa Jiménez Lucena**
Vf 10:15-10:30	Plasma profile of cytokines and adhesion molecules in children with autism spectrum disorder. **Cristina Pérez García**
10:30-11:00	**Coffee Break**
11:00-11:45	**Poster Showcase (SESSION II Chronic and Inflammatory diseases & SESSION II Cancer (Oncology and Oncohematology)**
11:45-13:00	**SESSION VI. Cancer (Oncology and Oncohematology)**
VIa 11:45-12:00	The Splicing Machinery is Profoundly Deregulated in Prostate Cancer: Pathological and Clinical Implications. **Juan Manuel Jiménez Vacas**
VIb 12:00-12:15	Targeted DNA demethylation in human cells by fusion of a plant 5-methylcytosine DNA glycosylase to a sequence-specific DNA binding domain. **Jara Teresa Parrilla Doblas**
VIc 12:15-12:30	Regulation of Notch1 expression and activity by DYRK2: New Insights of Carcinogenesis Signaling Pathways. **Rosario Morrugares Carmona**
VId 12:30-12:45	Neutrophil-to-Lymphocyte Ratio as prognostic factor in SBRT for Lung Cancer. **Fabiola Romero Ruperto**
VIe 12:45-13:00	Plasmatic Levels of miRNAs as Reliable Diagnostic Tool for Prostate Cancer Patients. **Vicente Herrero Aguayo**
13:00-14:00	**Plenary Lecture. Dr. Roger Gomis, IRB Barcelona**
14:00-14:30	**Awards and Closing ceremony**

ORAL COMMUNICATIONS
Abstracts

SESSION I

Nutrition, Endocrine and metabolic diseases

Ia. Atrial fibrillation, genetic and biomarkers in the Atherosclerosis Risk in Communities Study

Authors: *Antonio P. Arenas de Larriva, MD[1,2] Javier Delgado-Lista[2], Faye L. Norby, MPH,[1] Lin Y. Chen, MD, MS,[3] Elsayed Z. Soliman, MD, MSc, MS,[4] Ron C. Hoogeveen, PhD,[5] Dan E. Arking, PhD,[6] Laura R. Loehr, MD, PhD,[7] Alvaro Alonso, MD, PhD[8]*

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Background. Ceruloplasmin (CP) may promote structural changes in the atrium making it more arrhythmogenic. We assessed the associations between CP, CP-associated genetic variants, and incident atrial fibrillation (AF) in the Atherosclerosis Risk in Communities (ARIC) study.

Methods and results. We studied 10,059 men and women without prevalent AF aged 53 to 75 years in 1996-1998 and followed through 2012. Circulating CP was measured in stored blood samples obtained in 1996-1998. Polymorphisms rs11708215 and rs13072552, previously associated with CP concentrations, were measured in 10,059 and 8,829 participants respectively. AF was ascertained from study electrocardiograms, hospital discharge codes, and death certificates. Multivariable Cox models were run to study the association between circulating CP, CP-associated polymorphisms, and the incidence of AF. Over 10.5 years of mean follow-up, 1357 cases of AF were identified. After adjusting for traditional risk factors and biomarkers, higher levels of circulating CP were associated with incident AF (hazard ratio [HR] 1.33, 95% confidence interval [CI] 1.11, 1.61 comparing top to bottom quartiles). Both rs11708215 and rs13072552 were significantly associated with CP levels. Presence of the CP-increasing alleles in rs11708215 and rs13072552, however, were significantly associated with lower risk of AF in whites (HR 0.84, 95%CI 0.76, 0.94, p = 0.002 and HR 0.83; 95%CI 0.69, 0.99, p = 0.043 respectively per CP-increasing allele in the final adjusted model) but not in African Americans.

Conclusions. Even though higher CP concentrations were associated with increased AF risk, genetic variants associated with higher CP decreased the risk of AF in whites. Our results suggest that circulating CP levels may not be causally related to risk of incident AF.

Ib. Adipokines and their receptors are widely expressed in mouse prostate and are distinctly regulated therein by the metabolic environment

Authors: *André Sarmento-Cabral*[1,2,3,4,5], *Raúl M. Luque*[1,2,3,4,5]

Affiliations: [1]Maimonides Institute of Biomedical Research of Cordoba (IMIBIC), Cordoba; [2]Reina Sofia University Hospital (HURS), Cordoba; [3]Department of Cell Biology, Physiology and Immunology, University of Cordoba (UCO); [4]CIBER Physiopathology of Obesity and Nutrition (CIBERobn); [5]Agrifood Campus of International Excellence (CeiA3), Cordoba, Spain.

Adipose tissue-derived adipokines (i.e. leptin, adiponectin and resistin) play important roles in the regulation of several patho-physiological processes (e.g. glucose/lipid metabolism, food-intake, etc.) through the activation of specific receptors. However, although adipokines and their receptors are widely distributed in many tissues and exhibit a clear modulation by the particular metabolic conditions (e.g. obesity and/or fasting), their expression, regulation, and putative action on normal prostate glands (PGs; a hormone dependent organ tightly regulated by the endocrine-metabolic milieu) is still to be defined. Whole mice PGs from different in vivo models under extreme metabolic conditions (e.g. fasting and obesity) and primary mouse PGs cell cultures were used to comprehensively characterize, for the first time, the expression pattern and actions of different adipokine systems (i.e. leptin, adiponectin and resistin and its receptors) in mouse PGs. Our results revealed that adiponectin, resistin, adiponectin receptors (1 and 2) and leptin receptor are co-expressed at different levels in PG-cells, wherein they are finely regulated under fasting and obese conditions. Furthermore, treatment with different adipokines exerted both homologous and heterologous regulation of specific adipokines/receptor synthesis, and altered the expression of key proliferation/oncogenesis markers (i.e. Ki67, c-Myc and p53) in mouse PG-cell cultures. Moreover, we found that some of these actions might be elicited through the activation of ERK-signaling. Altogether, our data show for the first time that various adipokines/receptors-systems are differentially expressed in normal PG-cells; that their expression is under a complex ligand/receptor-selective regulation under extreme metabolic conditions, and that they mediate distinctive and common direct actions in PG-cells (i.e. homologous/heterologous regulation of ligands/receptors synthesis, modulation of proliferation markers, and activation of ERK-signaling pathway), suggesting a relevant role of these systems in the regulation of PG patho-physiology.

Ic. Perturbed central ceramide signaling as novel mechanism for obesity-induced precocious puberty: Evidence from preclinical studies

Authors: *Violeta Heras[1], Juan M. Castellano[1], Daniela Fernandois[3], Inmaculada Velasco[1], Juan Roa[1], María J. Vazquez[1,2], Francisco Ruiz-Pino[1], Rafael Pineda[1], Encarnación Torres[1], Maria Soledad Avendaño[1], Miguel López[4], Nuria Casals[5], Francisco Gaytán[1,2], Leonor Pinilla[1,2], and Manuel Tena-Sempere[1,2].*

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Recent studies suggest a link between childhood obesity and alterations in the timing of puberty; a phenomenon that has been associated with increased risk of metabolic diseases. While strong evidence supports the involvement of perturbed hypothalamic signaling pathways in this phenomenon, the targets and underlying mechanisms are still unknown. Ceramides are a family of sphingolipids that have recently emerged as putative mediators of metabolic disorders and transmitters for the central actions of leptin and ghrelin, two key hormones for the regulation of metabolism and puberty. Yet, the eventual role of central ceramide signaling in the control of puberty, especially in metabolically compromised conditions, remains unexplored.

Here, we evaluated the hypothalamic ceramide levels and the impact on pubertal timing of manipulations of central ceramide signaling in female rat models of normal and/or obesity-induced advanced puberty. Furthermore, we explored the potential interplay of ceramide signaling with the puberty-induced neuropeptide, kisspeptin, in the control of puberty.

Early-onset obesity increased hypothalamic ceramide levels and caused precocious puberty, while blockade of central ceramide signaling by administration of myriocin (MYR) partially reverted such phenotype. In turn, activation of central ceramide synthesis in control rats resulted in advanced puberty, whereas its blockade by MYR delayed pubertal timing. In both cases, gonadotropin levels were not affected. The partial rescue of puberty induced by kisspeptin treatment in prepubertal undernourished rats was prevented by co-administration of MYR. However, MYR did not attenuate kisspeptin-induced GnRH/LH responses, nor did it alter hypothalamic Kiss1 mRNA expression in prepubertal normal rats.

Our data document a novel role of central ceramide signaling in the control of pubertal timing, its alteration due to early-onset obesity and its interplay with kisspeptin under subnutrition conditions; yet, the lack of impact of the central manipulation of ceramide signalling on GnRH/LH secretion suggests the involvement of alternative effector pathways, which are presently under investigation.

Id. Unbalanced proteostasis in preadipocytes in obesity-related metabolic disease

Authors: *Sánchez-Ceinos J.[1,2], Ovelleiro D.[3], del Río-Moreno M.[1,2], Pedraza-Arévalo S.[1,2], Luque R.[1,2], Castaño JP.[1,2], Membrives A.[4], López-Miranda J.[2,5], Vázquez-Martínez R.[1,2], Guzmán-Ruiz R.[1,2], Malagón MM[1,2].*

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In obesity, adipocytes undergo a constellation of stress processes that compromise their function and contribute to the development of insulin resistance (IR), which is a major risk factor for type 2 diabetes (T2D), dyslipidemia, or cardiovascular disease. Adipocyte oxidative and endoplasmic reticulum (ER) stress and impaired protein homeostasis (i.e., proteostasis) have been linked to the pathogenesis of obesity. It has been also shown that adipocyte precursors, i.e., preadipocytes, are dysfunctional in obese individuals, yet the molecular challenges undergone by preadipocytes of the two distinct fat depots, subcutaneous (SAT) and visceral (VAT), in obesity and IR/T2D remain not fully elucidated. To investigate the molecular pathways and factors that are altered in the obese preadipocytes and might contribute to metabolic disease, we carried out a multicomparative proteomic analysis by iTRAQ-coupled LC-MS/MS of preadipocytes isolated from SAT and VAT of normoglycaemic (NG) and T2D obese patients. Differentially expressed proteins were functionally annotated using databases and significant pathways were further investigated by gene expression and immunoblot analyses. Proteomic data analysis showed that the human preadipocyte proteome is enriched in proteins involved in signal transduction and cell cycle, metabolism of proteins and nucleic acids, chromatin organization and intracellular traffic. Comparative proteomics of human preadipocytes revealed significant depot-specific differences in several key pathways between NG and T2D obese patients, namely mRNA splicing in SAT preadipocytes and protein folding in VAT preadipocytes. Further RT-PCR analyses confirmed the dysregulation of spliceosome components and splicing factors in T2D SAT preadipocytes, while changes in members of the unfolded protein response, including ER-associated protein degradation (ERAD), were observed in T2D VAT preadipocytes. Our data suggest that T2D in obesity is associated with the dysregulation of the cellular machinery involved in protein biogenesis, folding and degradation in the cells responsible for the renewal and maintenance of the adipose tissue, the preadipocytes.

Ie. Chronic consumption of a Mediterranean diet improves endothelial dysfunction in patients with diabetes mellitus

Authors: *Jose David Torres-Peña, Juan Francisco Alcala-Diaz ,Francisco Gomez-Delgado, Carolina Fernández-Gandara, Oriol A. Rangel-Zúñiga, Javier Delgado-Lista, Jose Lopez-Miranda*

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Background and Aims: Previous evidences support the fact that endothelial dysfunction plays a key role in the development of atherosclerosis cardiovascular disease (ASCVD). Our aim was to explore whether the chronic consumption of a healthy dietary pattern [Mediterranean diet (MedDiet) rich in olive oil or low-fat diet (LF Diet)] is associated with an improvement of ED according to their diabetic status in patients from the CORDIOPREV clinical trial (NCT00924937).
Materials and Methods: Endothelial function was explored in 805 participants (those with completed follow-up ultrasound image studies) using ultrasonography of brachial artery and measurements were used to calculate the brachial artery flow mediated vasodilatation (BAFMD) before and after 1.5 y of intervention with a MedDiet [35% of calories from fat (22% monounsaturated) and 50% from carbohydrates] and LF diet [28% fat (12% monounsaturated) and 55% of calories from carbohydrates]. We categorized participants as patients with prediabetes, diabetes, and without diabetes according to the American Diabetes Association (ADA) criteria.
Results: MedDiet improved BAFMD in patients with diabetes (5.2±0.4 vs. 3.7±0.4; p=0.044) and prediabetes (4.9±0.4 vs. 3.8±0.4; p=0.04) compared to baseline and induced and improvement in flow mediated vasodilation(FMD) compared to LF diet in patients with diabetes (5.2±0.4 vs.3.7±0.4; p=0.01). Finally, both diets maintained FMD stable in patients without diabetes.
Conclusions: Our findings demonstrate that the chronic consumption of a MedDiet rich in virgin olive oil improves endothelial function in patients with prediabetes and diabetes. This takes great importance given that diet must be the cornerstone of the treatment of patients with diabetes at high cardiovascular risk.

If. Involvement of a novel AMPK-Kisspeptin pathway in the metabolic control of puberty

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Puberty is gated by nutritional and metabolic signals. Conditions of metabolic stress, including malnutrition and obesity, are linked to perturbed pubertal timing through mechanisms that remain ill defined. AMP activated protein kinase (AMPK), a master cellular energy sensor that becomes activated in conditions of energy insufficiency, has a major central role in whole-body energy homeostasis. However, the role of brain AMPK in puberty onset, as metabolically sensitive phenomenon, is unknown. We report here a novel pathway for the control of puberty, involving central AMPK and kisspeptin, the puberty-activating neuropeptide encoded by Kiss1. Activation of brain AMPK in immature female rats substantially delayed puberty. Conversely, inhibition of central AMPK in conditions of negative energy balance partially rescued pubertal onset. Virogenetic over-expression of a constitutive-active form of AMPK, selectively in the hypothalamic arcuate nucleus (ARC), an area that holds a key population of kisspeptin neurons, partially delayed puberty onset and reduced LH levels. Co-labeling experiments demonstrated expression of p-AMPK in ARC kisspeptin neurons, while activation of AMPK reduced expression of Kiss1 at this site. Finally, conditional ablation of 1-AMPK subunit selectively in Kiss1 cells did not perturb the timing of puberty in female mice fed ad lib, but largely prevented the delay in puberty onset caused by chronic subnutrition. All in all, our data demonstrate that brain AMPK signaling plays a key role in the metabolic control of puberty, acting at least partially, via a repressive modulation of ARC Kiss1 neurons.

SESSION II

Active ageing and fragility. Infectious and Inmunological disesases. Organ transplantation

IIa. A prospective study to evaluate the impact of early everolimus on the recurrence of hepatocellular carcinoma after liver transplantation

Authors: *Beatriz Gros[1], Marta Guerrero[1], Manuel Rodríguez-Perálvarez[1,2,3], Gustavo Ferrín[2,3], Lydia Barrera[4], José María Álamo[4], [Ma] Dolores Ayllón[2,5], José Luis Montero[1,2,3], Javier Briceño[2,5], Marina Sánchez[6], Javier Padillo[4], Enrique Fraga[1,2,3], Miguel Ángel Gómez-Bravo[4], Manuel de la Mata[1,2,3].*

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BACKGROUND: Everolimus-based immunosuppression is frequently prescribed to prevent the recurrence of hepatocellular carcinoma (HCC) after liver transplantation (LT), in absence of supporting clinical evidence. We aimed to evaluate the impact of early initiated everolimus on the recurrence of HCC after LT.

METHODS: Prospective study including a consecutive cohort of patients with HCC who underwent LT in two LT institutions (2012-2015). Exclusion criteria were: re-transplantation, multiorgan transplantation and HIV-positive. The immunosuppression protocol consisted in tacrolimus, steroids and early introduction of everolimus. A historical control group -without everolimus- matched by HCC histological features (ratio 2:1) was used for analysis. The primary outcome was tumor recurrence after LT.

RESULTS: 207 patients were included (88.4% male, mean age 55.6), comprising 69 prospectively enrolled patients who received early everolimus (cases) and 138 matched historical controls. Cases and controls were comparable in terms of aetiology of liver disease, pre-LT AFP (p=0.98), locoregional bridging therapy (71.7% vs 61.9%; p=0.20), stage beyond Milan criteria (23.5% vs 27.5%; p=0.54), and moderate-poor histological differentiation (61.5% vs 63.6%; p=0.80). However, the baseline rates of mVI were increased in cases as compared with controls (27.9% vs 13.2%; p=0.01). The use of everolimus had no significant impact on HCC recurrence, neither in the overall cohort (12.8% with everolimus vs 12.4% without everolimus at 36 months; p=0.89), nor in subgroups with mVI or exceeding Milan criteria (p=0.26 and p=0.23 respectively). In the multivariate Cox regression analysis, the number of nodules and mVI status were independent predictors of HCC recurrence (RR=1.01; p=0.025 and RR=5.47; p<0.001 respectively), whereas the use of everolimus was not (RR=0.78; p=0.68). Overall mortality rates were similar between cases and controls: 28.6% vs 27.5% at 36 months respectively (p=0.74).

CONCLUSIONS: A universal prescription of everolimus may not be justified in LT patients with HCC. Randomized trials focusing on high-risk subpopulations are warranted.

IIb. Quality of Life of Informal Caregivers of People with Alzheimer's Disease. Conductual Intervention

Authors: *Patricia Luque-Carrillo*, Ignacio Morales-Cane*, María Aurora Rodríguez-Borrego*.*

Affiliations: *Maimonides Institute of Biomedical Research of Cordoba. University of Cordoba. University Hospital Reina Sofia of Cordoba.

Introduction: Quality of life of informal caregivers of people with Alzheimer's disease is mainly affected by factors related to the disease itself, such as functional impairment (Dauphinot et al., 2015), cognitive impairment (Garcia-Alberca et al., 2011), presence of behavioral problems (Huang et al., 2012), etc.
Therefore, it is important to work on the acceptance of the disease through coping strategies that facilitate caregivers´work and that improve their quality of life (Iavarone et al., 2014).
Methods: Quasi-experimental study "pretest-posttest" during which two interventions are carried out: a motor intervention to patients and a group therapy intervention based on problem solving to caregivers. Before and after the implementation of the interventions, an assessment of the functional state of patients and an assessment to caregivers in terms of burden, depression, anxiety and quality of life were carried out. Random sample of 69 patients with their caregivers, other 69 people.
Results: In those caregivers who did not attend group therapy, deterioration in their quality of life was observed in the SF-36 items: body pain (p=0.025), vitality (p=0.003), and health transition (p=0.042). In those caregivers who attended the group terapy, there was an improvement in their quality of life in the items: body pain (p=0.004), mental health (p=0.002) and general health (p=0.003).
In those caregivers whose relative with Alzheimer´s disease entered into a Nursing Home, it was observed a decrease in the level of burden (p=0.001), a decrease in the level of depression (p=0.023), and an improvement in their quality of life expressed in: body pain (p=0.034), general health (p=0.016), vitality (p=0.039), mental health (p=0.014) and health transition (p=0.004).
Discussion/Conclusions: Practice of problem-solving techniques to improve the quality of life of informal caregivers can be considered positive. Likewise, it has been seen that the admission of patients with Alzheimer´disease into a nursing home implies a work reduction for their family caregivers, causing an improvement in their quality of life.

IIc. External validation of a risk score for carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection in a prospective cohort of rectal carriers

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We performed a prospective longitudinal observational cohort study of all adult (≥18 years) patients with a carbapenem resistant *Klebsiella pneumoniae (CR)* in the culture of rectal swab, from July 2012 to November 2015 in the context of an outbreak that started in July 2012 and later endemia. During the study period, we identified 94 CR-Kp rectal carriers. Of them, 22 (23,4%) developed a CR-Kp bloodstream infection after the diagnosis of colonization. The principal aim of the study was to provide an external validation of the risk score (previously published by Giannella et al. CMI 2014;20:1357-1362) for CR-Kp bloodstream infection. A secondary aim was to evaluate the ability of the score to predict the risk that the rectal carriers develop CR-Kp bloodstream infection when we collected blood samples due to suspected bacteremia. Logistic regression was used to quantify the risk factors for Cr-Kp bloodstream infection, revealing that admission to the Intensive Care Unit was independent risk factor (OR, 5,76; 95% CI, 1,49-22,1). Area under curve (AUC) was calculated to quantify the discriminative ability of the score [0.90 (0,83-0.96), p <0,0001], suggesting a high prognostic ability to predict the risk for bloodstream infection. The AUC was 0.88 (0,80-0,96), p< 0,0001, when we evaluate the ability of the score to predict the risk of CR-Kp bloodstream when clinically suspected. This may be useful for making decisions on the clinical management of patients. In this cohort, a score ≥5 exhibited a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 100%, 37,5%, 32,8%, 100%, 52,1%, respectively, in the primary aim. For the second aim the values were respectively for a score ≥ 4: 100%, 31,4%, 37,2%, 100%, 51,3%.

Patients with intestinal colonization by CR-Kp who develop sepsis and have a score ≥ 4 should receive empirical treatment that covers these multiresistant bacteria.

IId. Vitamin E from food sources can prevent cellular aging: CORDIOPREV study

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Objective: Diet, and especially vitamins, are potential nutrients that can influence in telomere shortening modulating the oxidation and the inflammation processes. Our objective was to explore whether the intake of different vitamins are involved in the length of telomeres in patients with established cardiovascular disease.

Subjects and Methods: This study was conducted with the framework of CORDIOPREV study (NCT00924937), in which 1002 patients with coronary artery disease where included. Information about dietary habits was obtained using a semi quantitative 146-items food frequency questionnaire. The intake of macro and micronutrients was calculated using Food composition table by Morreira et al (2013) DNA was isolated from peripheral blood samples using "Salting Out" method. Relative telomeres length (RTL) was measured by real time PCR.

Results: A total of 131 patients (13% of the total) had a vitamin E intake below the recommended daily intake of this vitamin in Spanish adults. These patients had shorter RTL than those with adequate vitamin E intake (1.13 ±0.048 vs. 1.31 ±0.023, respectively, p=0.005). Gradually, subjects in the first tertile of vitamin E intake, above 21.32 mg / day, showed a higher RTL (1.36 ±0.040), than those in the middle tertile of vitamin E intake (RTL 1.27 ±0.037) and in the lower tertile (RTL 1.28 ±0.021). There were no significant differences between RTL and the intake of other fat-soluble vitamins, such as vitamin A, vitamin D and vitamin K, and also with water-soluble vitamins.

Conclusion: These findings suggest the importance of adequate consumption of vitamin E with potent antioxidant capacity and the importance of diet as a biomodulating tool of the senescence process.

IIe. Effect of hypoxic preconditioning on rat Adipose Derived Mesenchymal Stem Cells (ASCs) functionality. Study in a diabetic rat model

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Background: Accumulated reports have shown therapeutic efficacy of Adipose Derived Mesenchymal Stem Cells (ASCs) in the treatment of diabetes induced metabolic abnormalities, including diabetic retinopathy and diabetic nephropathy. ASCs are usually cultured under normoxic conditions corresponding to atmospheric oxygen (21%). Nevertheless, physiological oxygen tension in stem cell environment is lower (<7%). The main objective of this study was to evaluate the effect of hypoxia on the functionality of ASCs from streptozotocin-induced diabetic rats.

Methods: This study was developed in both Healthy-ASCs and Diabetic-ASCs. Healthy-ASCs or Diabetic-ASCs were culture expanded under standard conditions or pre-conditioned under hypoxia for 48h before performing functionality analysis. ASCs from each experimental group were analyzed. Phenotype was determined by flow cytometry. Proliferation capacity and differentiation potential towards mesoderm were also analyzed. Growth-factors secretion (VEGF, IGF, IL-6, EGF and IDO) was quantified by ELISA. Angiogenic capacity was analyzed using an endothelial tube formation assay. Moreover, hypoxia-induced gene expression profile was determined by microarray analysis.

Results: Diabetic-ASCs showed a normal phenotype but were less proliferative and angiogenic than healthy-ASCs. Osteogenic differentiation potential was impaired in diabetic-ASCs, while adipogenic differentiation potential showed no changes compared to healthy-ACSs. Gene expression analysis revealed that a group of genes showed altered expression levels in diabetic-ASCs. The effect of hypoxia on both groups did not result in phenotype or differentiation potential changes compared to ASCs cultured under normoxia. However, in both groups, healthy and diabetics-ASCs, hypoxic pre-conditioning: 1) significantly enhanced angiogenic capacity; 2) Slightly increased cell proliferation; 3) Resulted in increased levels of VEGF, IGF, HGF and EGF secretion; 4) Modified the expression pattern of certain genes.

Conclusions: Hypoxia pre-conditioning enhances ACSs therapeutic functions. This fact could improve autologous cell therapy efficacy, but further studies are still needed.

IIf. Diagnosis of acute hepatitis E genotype 3 infection by viral isolation from saliva

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Objective: To evaluate the diagnostic value of testing HEV RNA in saliva

Methods: Prospective pilot study including patients with suspected acute HEV hepatitis infection. A whole blood sample was obtained from all patients. Serum was obtained from whole blood. Additionally, a neat saliva sample was collected by passive drool using the Saliva Collection Aid. Serum and saliva samples were processed and analyzed up to two hours after collection for HEV RNA by RT-PCR. An epidemiological survey of all patients was carried out at the time of inclusion. Positive predictive value (PPV) for diagnosis of HEV infection by detecting the presence of virus in saliva was calculated.

Results: A total of 34 patients with suspected acute HEV infection were included in the study. HEV RNA in serum was confirmed by RT-PCR in 8 of these patients (23.5%; 95% CI: 12.2%-40.2%). HEV was isolated in the saliva of 8 of 34 patients (23.5%; 95% CI: 12.2%-40.2%). All patients with HEV RNA amplified in saliva had detectable HEV RNA in serum. The PPV for diagnosis of acute HEV infection in saliva using RT-PCR was 100% (95% CI: 62.8%-100%). HEV was not isolated in the saliva of any of the 26 patients without detectable HEV RNA in serum.

Conclusions: Our study shows the first evidence that acute HEV infection can be diagnosed by assessing viral load in saliva.

SESSION III

Chronic and Inflamatory diseases

IIIa. Assessment of short-term effectiveness of five local treatment modalities in patients with symptomatic knee osteoarthritis

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Background: Inside the therapeutic algorithm of knee osteoarthritis (OA) it is included the Non-Arthroscopic Joint Lavage (NAJL) since around 1934 Burman reported that arthroscopies improved the symptoms. The large variety of potentially interventions available has raised the need to assess their effectiveness.

Objectives: To compare the short-term effectiveness among five treatment strategies in patients with symptomatic knee OA.

Methods: An open, controlled, randomized prospective study involving 150 patients of whom 76.7% were females. Patients had knee OA according to American College of Rheumatology criteria, with Kellgren-Lawrence radiographic grades II-III. They were randomly assigned to five groups of treatment, 1)NAJL (n=30), 2)NAJL+hyaluronic acid (HA) (n=32), 3)NAJL+ corticosteroid (CS) (n=32), 4)HA (n=31), and 5)CS (n=25). Evaluations took place at baseline, one and three months. Western Ontario and McMaster University Osteoarthritis Index (WOMAC) and Lequesne scores were recorded. Statistical analysis included mixed analysis of variance, with post-hoc comparisons with Sidak's adjustment, and multiple linear regression (MLR) to identify those possible factors associated to WOMAC total at 3 months.

Results: Regarding WOMAC total, significative differences were found in NAJL at one month ($p<0.001$) and at 3 months ($p<0.001$); and in NAJL+CS at one month ($p=0.018$). For Lequesne, significative differences were found in HA at one month ($p=0.003$) and at 3 months ($p=0.019$) versus baseline; and in CS, at one month ($p<0.001$). The WOMAC function at baseline, NAJL+HA, and infiltration with CS are the variables that show a significant association with WOMAC total at 3 months. The group that received NAJL+HA had poorer outcomes.

Conclusions: One month after treatment, best outcomes were obtained with HA due to results found in Lequesne scale when comparing HA versus NAJL+HA, and NAJL+CS. Three months after treatment, the most effective modality treatment was NAJL; since we did find significant differences regarding articular stiffness, physical function and Lequesne scale.

IIIb. Regulation of Renal Klotho and FGFR1 Expression

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In renal failure hyperphosphatemia occurs despite a marked elevation in serum Fibroblast Growth Factor 23 (FGF23). Abnormal regulation of FGFR1-Klotho receptor complex could cause a resistance to the phosphaturic action of FGF23. This study investigates the regulation of renal Klotho and FGFR1 in experimental animals and HEK293 cells. In normal rats, rat recombinant FGF23 infusion enhanced phosphaturia and increased FGFR1, whereas Klotho expression is reduced. Uremic rats on a high phosphate (P) diet, presented hyperphosphatemia with marked elevation of FGF23 and increased fractional excretion of P; this was associated with a striking reduction of Klotho expression and an increase in FGFR1. After neutralization of FGF23 using anti-FGF23 administration, phosphaturia was still abundant and the Klotho expression remained low and FGFR1 was also reduced. These results suggest that renal Klotho is modulated by phosphaturia and FGFR1 by FGF23. Calcitriol administration prevented the drop of renal Klotho expression. In HEK293 cells, high P produced nuclear translocation of β-catenin together with a reduction of Klotho. Wnt/β-catenin inhibition with Dkk-1 prevented the P-induced down-regulation of Klotho. The addition of calcitriol to high P medium was able to recover Klotho expression. In conclusion, high FGF23 levels increase FGFR1, whereas phosphaturia decreases Klotho expression through the activation of Wnt/β-catenin pathway.

IIIc. Diagnostic potential of NETosis-derived products for disease activity, atherosclerosis and therapeutic effectiveness in Rheumatoid Arthritis patients

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Objectives: 1) To assess the association of NETosis and NETosis-derived products with the activity of the disease and the development of cardiovascular disease in RA; 2) To evaluate the involvement of NETosis on the effects of biologic therapies such as anti-TNF alpha (Infliximab) and anti-IL6 drugs (Tocilizumab). Methods: One hundred and six RA patients and 40 healthy donors were evaluated for the occurrence of NETosis. Carotid-intimae media thickness was analyzed as early atherosclerosis marker. Inflammatory and oxidative stress mediators were quantified in plasma and neutrophils. Two additional cohorts of 75 RA patients, treated either with Infliximab (n=55) or Tocilizumab (n=20) for six months, were evaluated. Results: NETosis was found increased in RA patients, beside myeloperoxidase and neutrophil elastase protein levels. Cell-free nucleosomes plasma levels were elevated, and strongly correlated with the activity of the disease and the positivity for autoantibodies, alongside inflammatory and oxidative profiles in plasma and neutrophils. Moreover, ROC analyses showed that cell-free nucleosomes levels could identify RA patients showing early atherosclerosis with high specificity. RA patients treated either with IFX or TCZ for six months exhibited decreased generation of NETs. Concomitantly, clinical parameters and serum markers of inflammation were found reduced. Mechanistic in vitro analyses showed that inhibition of NETs extrusion by IFX and TCZ further abridged the endothelial dysfunction and the activation of immune cells, thus influencing the global activity of the vascular system. Conclusions: NETosis-derived products may have diagnostic potential for disease activity and atherosclerosis, as well as for the assessment of therapeutic effectiveness in RA.

IIId. Lithium recovers altered mechanosensory and locomotory behaviors in neuroligin- and neurexin-deficient mutants of *Caenorhabditis elegans*

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Neurexins and neurologins are cell adhesion proteins that contribute to differentiation, plasticity and specificity of synapses. Mutations in these genes have been associated with autism and other mental disorders. The human neurexin and neuroligin genes are orthologous to *nrx-1* and *nlg-1* from *C. elegans*, respectively. The *nrx-1* and *nlg-1*-deficient mutant have impaired mechanosensory and locomotory behaviors. In fact, previously we have shown that the human genes are functional in the nematode.

Lithium cation is a mood stabilizer. Although it is the main medicament to treat bipolar disorder, its mechanism of action is still unknown. Recent studies carried out in *C. elegans* have shown that lithium can inhibit the enzyme biphosphate-3'-nucleotidase (BPNT-1). The inhibition of BPNT-1 results in a selective dysfunction of ASJ chemosensory neurons which triggers reversible physiological changes. Additionally, it has been shown that *C. elegans* exposure to lithium affects its development, maturation and reproduction, as well as regulate survival through modulation of histone methylation and chromatin structure.

In the present study, we have observed that *nrx-1* and *nlg-1*-deficient mutants of *C. elegans* recover mechanosensory and locomotory behaviors when are exposure to different concentrations of lithium. However, at the same concentrations, lithium has an impairing effect in wild type strain on these behaviors. In order to further understand the lithium mechanism of action, we carried out experiments silencing the expression of *bnpt-1* by RNAi. Our study also shows that *bnpt-1* (*RNAi*) in *nrx-1* or *nlg-1*-deficient mutants and *nrx-1;nlg-1*-deficient double mutant, recovers mechanosensory and locomotory in absence of lithium. The results linked lithium, BNPT-1 expression and the synaptic adhesion molecules, neurexin and neuroligin. These observations might facilitate the comprehension of the molecular mechanisms by which lithium may interact with the nervous system.

IIIe. Defective glucose and lipid metabolism in rheumatoid arthritis is determined by chronic inflammation in metabolic tissues

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Aim: To investigate whether rheumatoid arthritis (RA) inflammatory activity accounts for the defects observed in glucose and lipid metabolism in these patients, by using two main strategies: 1) extensive profiling of a cohort of RA patients; and 2) through the development of mechanistic studies in both, collagen induced arthritis (CIA) mouse model and 3T3L1 adipocytes treated with RA serum.

150 RA patients and 50 healthy donors were included. IR was quantified using the homeostatic model assessment of IR (HOMA-IR). Mice: 20 CB57J/BL mice were used in CIA modelling. Plasma, leukocytes, muscle, liver and adipose tissue (AT) were collected. 3T3L1 adipocytes were treated with serum from RA patients and healthy donors. Levels of HOMA-IR significantly correlated with the RA disease activity, C-reactive protein levels and erythrocyte sedimentation rate, suggesting a link between the degree of systemic inflammation and the development of IR in these patients.

These results were strengthened by observing that the induction of arthritis in mice resulted in a global inflammatory state characterized by defective carbohydrate and lipid metabolism in leukocytes, liver, muscle and AT, consistent with defects in insulin signaling. AT was the organ more susceptible to the RA-induced metabolic alterations.

These metabolic effects were recapitulated by treatment of adipocytes with serum from RA patients.

Conclusions: 1) IR was closely associated with an increase in disease activity and systemic inflammation in RA patients. 2) Induction of arthritis in mice promoted an increase in inflammation markers and a reduction of genes involved in lipid uptake and storage, generating an IR state. 3) The inflammatory components in RA serum induced lypolisis, reduced adipogenesis, and increase inflammation and IR in adipocytes 3T3L1.

In sum, our results suggest that chronic inflammation associated with RA might directly impact relevant metabolic tissues, altering glucose and lipid homeostasis and favouring the development of IR.

IIIf. Consumption of alcoholic beverages in nursing students at the University of Cordoba

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Background: Alcoholic beverages are the most consumed psychoactive substances amongst the population aged 15-64 years in Spain (1). This consumption implies important death and disease rates (2). In the period of the university there are high levels of consumption and problems due to abuse (3). Objective: To find out the consumption characteristics of alcohol in undergraduate nursing students. Methods: Observational descriptive study. Subjects and scope of study: University Nursing students between 18-30 years old, University of Cordoba (Spain). Sample: 84 subjects, 21 per course. Variable: consumption of alcoholic beverages. Instrument: questionnaire for the health information areas (4). Report from the Research Ethics Committee of Córdoba. Information and Informed Consent Form. Data analysis: χ^2 test with qualitative data, analysis of variance with quantitative. A $p < 0.05$ was considered. Results: 83.3% consume alcohol, 21.4% a day or less a month, 48.8% from 2-4 days a month and 11.9% from 2-3 days a week. All students say they consume more alcohol on weekends (including Thursday). 72.2% of men and 86.4% women. Most frequently beer (40.5%). The onset of consumption between the ages of 16 and 18 is 51.2%, between 13-15 years of age of 22.6%, and after the age of 18, 10.7%. By the end of their studies, 16.7% of the students consume less alcohol whereas 6% drink more. Regarding the course, it is observed that 9.5% of the students in the first course consume less compared to 28.6% in the fourth year. Conclusions: Nursing students at the University of Cordoba have a high alcohol consumption rate, slightly higher than the consumption of young people between 14 and 18 years in general population. Women drink more. Students on their last year drink more alcohol than during their first year. Being Nursing student isn't a protective factor face to consumption.

SESSION IV

Cancer (Oncology and Oncohematology)

IVa. Components of the splicing machinery are drastically dysregulated in neuroendocrine tumors and associated with malignancy and aggressiveness

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Dysregulation of the splicing machinery is emerging as a novel cancer hallmark, due to its association with multiple dysfunctions in tumor cells. An inappropriate function of the components of this machinery (spliceosome and splicing factors) could be linked with the aberrant generation of tumor-associated splicing variants. Indeed, our group have previously reported that overexpression of alternative splicing variants of somatostatin receptor 5 (sst5TMD4) and ghrelin (In1-ghrelin) is directly associated to malignancy features in pancreatic neuroendocrine tumors (pNETs). Therefore, in this study we aimed to characterize, for the first time, the pattern of expression of a selected set of components of the splicing machinery in pNETs samples, compared to adjacent non-tumoral control tissues, and to determine their relationship with the aggressiveness of these tumors. Accordingly, we designed a PCR-based array to determine the expression levels of components of the major (n=13) and minor spliceosome (n=4) and associated splicing factors (n=28) using a microfluidic technology in 20 pNET-samples (47% G1, 47% G2 and 6% G3) and adjacent control-tissues. The results revealed that the expression of several splicing machinery components was altered in pNETs compared to non-tumoral tissues. Remarkably, important splicing factors and components of spliceosome were clearly overexpressed in pNET-samples, and their expression correlated with relevant malignancy features. Furthermore, *in vitro* assays using NETs cellular models (i.e. BON-1/QGP-1 cells) demonstrated that overexpression of some of this factors could increase cell proliferation, while their silencing (using specific siRNAs) markedly decreased cell proliferation, suggesting a role in the aggressiveness of NETs and their putative suitability as therapeutic targets. Altogether, our results demonstrate that the splicing machinery is dysregulated in pNETs, which could be associated with NETs development/progression, and therefore could provide novel diagnostic biomarkers and therapeutic tools for this pathology.

IVb. Development of a CRISPR-based system to reactivate epigenetically silenced genes

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DNA methylation (5-methylcytosine, 5-mC) is a crucial epigenetic mark associated to gene silencing, and its targeted removal is a major goal of epigenetic editing. In animal cells, DNA demethylation involves iterative 5mC oxidation by TET enzymes followed by replication-dependent dilution and/or replication-independent DNA repair of its oxidized derivatives. In contrast, plants use specific DNA glycosylases that directly excise 5mC and initiate its substitution for unmethylated C in a base excision repair process.

The CRISPR methodology derives from a bacterial adaptive immune system that uses an RNA-guided nuclease (Cas9) to target and destroy invading DNA. The use of a catalytically inactive nuclease (dCas9) co-expressed with a short guide RNA (sgRNA) allows using CRISPR/dCas9 as a general platform for RNA-guided targeting of different effector proteins to specific genomic regions. Fusion of dCas9 to effector domains with epigenetic functions can be used for targeted transcriptional regulation in human cells.

In this work, we have fused dCas9 to the catalytic domain of Arabidopsis ROS1 5mC DNA glycosylase (ROS1_CD), and we have explored the possibility of directing ROS1_CD glycosylase activity to a specific target sequence in human cells. As positive control, TET1 human protein has been also fused to dCas9. The targeted activity of both fusion proteins, co-expressed with different sgRNAs in human HEK293 cells, was tested on a luciferase reporter gene previously silenced by *in vitro* methylation.

Luciferase reporter assays and expression analysis by qRT-PCR showed that both single or combined sgRNAs efficiently targeted dCas9-ROS1_CD for reactivation of the silenced luciferase gene. In contrast, no reactivation was detected when those same gRNAs were used to target dCas9-TET1. Bisulfite pyrosequencing revealed that reactivation induced by dCas9-ROS1_CD correlates with a decrease in DNA methylation levels.

Together, these results show that plant 5mC DNA glycosylases can be used for targeted active DNA demethylation and gene reactivation in human cells.

IVc. Use of MALDI imaging technology to predict the response to antiangiogenic therapy in colorectal cancer

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Angiogenesis, the process by which new blood vessels are produced from pre-existing ones, is a key step in the tumor progression and metastatic spread of tumor cells. Although antiangiogenic therapy has demonstrated efficacy, not all patients respond in the same way and biomarkers that distinguish between groups of responders and non-responders patients are needed. More specifically there are a number of long term responders with an extended period of treatment before progression of disease. Mass spectrometry imaging (MSI) is a very promising analytical technique in oncology and particularly in the discovery of biomarkers that predict treatment response.

We analyzed 29 formalin-fixed paraffin-embedded tumor tissues from 22 patients with metastatic colorectal cancer to predict the response to the antiangiogenic drug bevacizumab based on the proteomic information obtained with matrix-assisted laser desorption/ionization (MALDI) MSI. First, starting only from proteomic hyper-spectral data, spatially-aware shrunken centroid unsupervised analysis was used to virtually micro-dissect tumor tissue away from whole specimen. Second, based on Random Forest Algorithm a classification was realized according to time to progression. Patients were classified into two groups corresponding to < 12 months of treatment or ≥ 12 months of treatment. Both methodologies were performed under R environment (Cardinal and Caret packages respectively).

We were able to precisely distinguish tumor from healthy tissue and to virtually dissect the former for subsequent classification analysis. The best classifier generated showed an AUC of 0.83 (specificity 0.760, sensitivity 0.745) as determined by cross-validation. An *in situ* MALDI TOF/TOF analysis determined the m/z 1198.7 and 1325.7, which characterize the responding class, as an actin domain and histone H4 respectively. Interestingly, these proteins have been previously related with the angiogenic process.

This study highlights the usefulness of MALDI-MSI both for the dissection of tumor regions without any other device and the prediction of response to antiangiogenic treatment.

IVd. Diagnostic accuracy of MRI-Transrectal Ultrasound Fusion prostate Biopsy in men with previous prostate biopsies: The FUPROS16 project

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Objectives: To compare the detection rates (CDRs) of MRI-transrectal ultrasonography fusion-guided biopsy with the standard 12-core biopsy methods (fusion- vs. standard-biopsy, respectively) and to evaluate the performance of multiparametric magnetic resonance imaging (mpMRI) in predicting significant prostate cancer (SigPCa) in men with at least one previous prostate biopsy.

Methods: A prospective study in patients with elevated serum PSA levels and/or abnormal digital rectal examination from various Andalusian Hospitals was performed. Participants underwent a 1.5 T mp-MRI without an endorectal coil. In all cases, one radiologist graded all suspicious lesions according to PIRADS score v.2. Moreover, fusion-biopsy of suspicious prostate lesions and standard-biopsy methods were performed in all patients. We calculated and compared CDRs of both biopsy-techniques and evaluated the area under the receiver operating characteristic curves (AUCs) of mpMRI in predicting any cancer and SigPCa.

Results: A total of 102 patients were analyzed (n=60 with PCa and n= 46 with SigPCa). The median PSA was 8.7ng/ml with a PSA density of 0.13ng/ml/cc. 64.4% of patients had at least two previous biopsies. Standard-biopsy method did not detect significantly more number of cancers [n=45 (44.1%) vs. n=50(49.1%)], but fusion-biopsy detected more numbers of SigPCa [n=39 (38.2%) vs. n=29 (28.5%)] and less insignificant-PCa [n=6 (5%) vs. n=21(21%); p<0.05]. Interestingly, when both methods were considered together, more SigPCa were diagnosed [n=46 (45%)].The rate of SigPCa and Gleason grade increased in correlation with PIRADS score (r=0.56; P<0.001). PSA density and a concordant lesion in ultrasonography with positive elastography were associated with SigPCa in the fusion-biopsy (p<0.05). The AUC for the diagnosis of SigPCa using mpMRI was 0.75 (0.66-0.85).

Conclusions: Altogether, our data indicate that using mpMRI and subsequent MRI/TRUS fusion-biopsy, instead of standard-biopsy, method might improve the detection of clinically SigPCa in men with previous negative biopsies. However, application of both biopsy-methods together may improve the diagnosis of SigPCa.

IVe. Metabolomics analysis of human sweat by gas chromatography–time of flight/mass spectrometry

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Human sweat is a promising biofluid to be implemented in clinical studies by its composition and non-invasive sampling. In fact, sweat has been used in metabolomics studies to discriminate lung cancer patients versus risk factor individuals. Elucidation of sweat metabolome has so far been done using three analytical platforms (NMR, LC–MS and GC–MS). A total of 103 metabolites have been detected by combination of the three platforms, being GC–MS the approach providing the highest coverage, with detection of more than 60% of them. However, these studies did not pay attention to non-polar metabolites, presumably a minor fraction. The detection of non-polar compounds would increase the interest of sweat for clinical studies. With these premises, a method for metabolomics analysis of human sweat by gas chromatography–time of flight/mass spectrometry (GC–TOF/MS) in high resolution mode has been developed to expand the detection of compounds, with special emphasis on non-polar compounds. Different sample preparation strategies were compared to check their effect on the profile of sweat metabolites. The performance of three extractants with different polarity and a deproteinization protocol were compared by using a dual approach: (1) application of a derivatization protocol prior to GC–MS and, (2) direct injection. One hundred forty-three compounds were tentatively identified by high resolution MS. Mainly lipids, but also other interesting metabolites were identified. It is worthy to distinguishing the ability of the GC–MS protocol to detect lipids, family that constituted around 32% of the compounds detected. Among the tested protocols, methyoximation plus silylation after liquid–liquid extraction with dichloromethane was the most suited option to obtain a representative snapshot of sweat metabolome. As most of the identified metabolites are involved in key biochemical pathways, this study opens new possibilities to the use of sweat in searching for lung cancer biomarkers.

IVf. Alteration of nitric oxide production targets cancer stem cells in breast cancer and augments the efficacy of anti-hormonal therapy

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Cancer stem cells (CSCs) are a cell subpopulation in tumors involved in processes such as recurrence, tumor dormancy, metastasis and resistance to anti-tumoral treatments. There are studies suggesting that CSCs may be involved in the resistance to hormone therapy with tamoxifen in estrogen receptor (ER)-positive breast tumors. CSCs have cellular signaling mechanisms related to nitric oxide (NO) that are involved in the generation and maintenance of this cellular subpopulation. Therefore, the main objective of the present study was to analyze the effects of the alteration of NO levels in ER^+ breast cancer stem cells and to study whether the depletion of NO in combination with hormone therapy could increase the efficacy of antitumor treatment.

The treatment of breast cancer cells with the NO scavenger C-PTIO exerted an anti-CSC effect that was suppressed with the NO donor DETANONOATE. NO depletion decreased the expression of both Notch1, which participates in pathways for self-renewal of CSCs in breast cancer, and ALDH, which is a CSC-specific marker in breast cancer, and this effect was significantly attenuated with the addition of the NO donor. Specific silencing of the NOS2 gene, which codes for inducible NO synthase (iNOS), impaired the ability of MCF-7 cells to form mammospheres and caused a significant decrease in the expression of CSCs markers such as CD44, Notch1, and specific stem cell markers, including OCT-4 and Nanog. Notably, NO depletion with C-PTIO or iNOS silencing significantly increased the sensitivity of MCF-7 cells to the anti-hormonal treatment with tamoxifen.

In conclusion, NO plays an important role in the biology of CSCs in ER^+ breast cancer and the alteration of NO production in these tumors may contribute to increase the efficacy of hormone therapy avoiding the development of resistance to these treatments in clinic.

SESSION V

Nutrition, Endocrine and metabolic diseases

Va. Identification of candidate serum biomarkers of pediatric Growth Hormone deficiency using SWATH-MS next-generation proteomics and feature selection algorithms

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Background: Currently there is no single biochemical parameter available for the accurate diagnosis of growth hormone (GH) deficiency in children. There is a need for non-invasive biomarkers.

Methods: Serum samples from 15 patients with GH deficiency and 15 healthy controls were obtained. Samples were depleted from the seven highest abundance proteins (Hu7 MARS kit, Agilent) and 50 ug of protein were digested with trypsin. An ***ad-hoc*** spectral library was built from the samples using LC-MS/MS top65 DDA runs with a 2 hour 5% to 30% ACN gradient in a nanoHPLC nLC415 (Eksigent) coupled to a Q-TOF mass spectrometer (Sciex TripleTOF 5600+). Samples were then run using a variable SWATH method using the same LC-MS/MS platform, and the quantitative data for each protein in the library was extracted using the MS/MS^{ALL} with SWATH Acquisition MicroApp v2.0 (Sciex).

For selecting those proteins that best discriminate between individuals with GH deficiency and controls, a feature selection workflow including three different algorithms for classification (RF-Boruta, SCAD-SVM, PAM) was applied using the bootfs R package. In order to obtain a final biomarker set with the minimum number of features producing the best classifying performance, ROC analyses were performed on the top-ranked proteins and their combinations as obtained from bootfs. The significantly affected pathways and gene ontology over-represented components were analysed using iPathwayGuide (Advaita).

Results: 263 proteins could be confidently detected and quantified on each sample. The top 10 biomarker candidates are FIBA, APOA4, FHR4, SAA2, FINC, CXCL7, C4BPA, APOC4, F13A and APOF (Fig.1). The combination of three proteins, APOA4, FHR4 and CXCL7, showed the best classification performance.

Conclusions: The analysis of serum proteins by SWATH-MS is a useful method for discovering potential biomarkers of GH deficiency in children. Three proteins classified GH deficiency patients and controls with best performance.

Vb. Gender interacts with metabolic abnormalities to influence carotid atherosclerosis in elderly patients

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Aging plays an essential role in the progression of atherosclerosis. Patients with obesity and metabolic abnormalities are at high-risk of recurrent cardiovascular disease (CVD). It is unknown the influence of gender on the association between metabolic phenotypes of obesity and carotid atherosclerosis. Our aim was to analyze whether gender interacts with metabolic abnormalities determining changes in carotid intima media thickness (IMT-CC) in elderly patients.

In 236 men and 62 women from the CORDIOPREV study (NCT00924937), aged ≥ 65 years, we explored the IMT-CC according to body size phenotypes: (i) normal weight, metabolically healthy; (ii) normal weight, metabolically unhealthy; (iii) overweight, metabolically healthy; (iv) overweight, metabolically unhealthy; (v) obese, metabolically healthy; and (vi) obese, metabolically unhealthy. Metabolically unhealthy was defined by the presence of two or more metabolic abnormalities.

Normal and over-weight metabolically unhealthy men displayed greater IMT-CC compared with those metabolically healthy (0.82±0.03mm vs 0.72±0.01mm, p=0.001; 0.79±0.02mm vs 0.73±0.01mm, p=0.008, respectively). No differences were observed in the subgroup of obese men. Women exhibited no differences on IMT-CC according metabolic abnormalities, independently whether or not they were obese.

Our results showed an interaction between gender and metabolic phenotypes of obesity by determining differences in carotid atherosclerosis, which may be contributing to the higher CVD risk in elderly women compared to elderly men.

Vc. Mechanisms and consequences of obesity-induced hypogonadism: Role of a novel hypothalamic miR-137/Kisspeptin pathway

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Obesity is a life threatening condition associated with numerous comorbidities. Among them, central hypogonadism, i.e., low circulating levels of testosterone, has been recently suggested a putative contributor to the metabolic complications of obesity, especially in men. Yet, the mechanisms for kisspeptins obesity-induced hypogonadism (OIH), and its contribution to the generation/evolution of the metabolic alterations of obesity remain ill defined. Yet, recent data has suggested that OIH may be caused by suppression of hypothalamic Kiss1 system; kisspeptins being potent activators of the reproductive axis that ultimately stimulate testosterone secretion. However, the mechanisms for Kiss1 suppression in obesity remains unknown.

Our recent evidence suggests that microRNAs (miRNAs) are putative regulators of the Kiss1 system. In this work, we aimed to identify novel miRNAs capable of regulating kisspeptin expression and to evaluate their potential contribution to OIH. Bioinformatic analyses on KISS1 gene were conducted with different algorithms (http://www.targetscan.org/; http://www.ebi.Ac.uk/enright-srv/microcosm /htdocs/targets/v5/; http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/; http://www.microrna. org/microrna/home.do) to seek for potential miRNA regulators. Selection of miRNA candidate(s) was based on the following criteria: 1) to be identified in at least two different databases; 2) to show an evolutionary conserved seed region (rat, mouse, human); and 3) to be deregulated by metabolic alterations, associated with changes in hypothalamic kisspeptin expression. Using these criteria, miR-137 was selected as a robust putative modulator of KISS1. This was tested biochemically using a luciferase reporter assay, which documented a repressive interaction of miR-137 at the 3'UTR of KISS1. Further confirmation was obtained in vivo, using a Target Site Blocker (TSB) approach. TSB, tailored to block the repressive interaction of miR-137 selectively at Kiss1 3'UTR, were injected centrally in a male rat model of OIH, with severe suppression of testosterone (T) and gonadotropin (LH) levels, and marked metabolic alterations: increased systolic blood pressure (SBP), glucose intolerance and insulin resistance. TSB administration not only restored T and LH levels and increased hypothalamic kisspeptin, but also ameliorated insulin resistance, glucose intolerance, SBP and cardiac hypertrophy, without detectable changes in body weight. Our results are the first to provide conclusive evidence about the relevant role (and eventual therapeutic value) of a novel central miR-137/kisspeptin pathway in OIH, and strongly suggest that central hypogonadism is a major contributor for the metabolic complications of obesity.

Vd. Evaluation of the metabolic and inflammatory status in prepuberal children with a history of Extrauterine Growth Restriction or Prematurity

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Introduction: Extrauterine Growth Restriction (EUGR) is defined as a growth values lower than the 10th-percentile at 36 weeks corrected gestational age or at discharge. The aim was to study metabolic risk variables in prepubertal EUGR or prematurity children.

Material-methods: A total of 211 were enrolled including 38 EUGR children, 50 children who were preterm (Prem) and 123 healthy term infants. The anthropometric parameters, blood pressure (BP) levels and general biochemical parameters were calculated. Plasma levels of relevant interleukins (IL), C-reactive protein (CRP), hepatocyte growth factor (HGF), chemoattractant factor of macrophages type 1 (MCP-1), tumor necrosis factor alpha (TNF-α), inhibitor plasminogen activating factor 1 (PAI1t), adipokines and antioxidant enzyme were analized.

Results: The mean age was 8.5y. Lower BMI-Zscore and higher BP values were observed in the EUGR children compared with the other two groups. Although the biochemical concentrations were normal, the EUGR group showed higher glycemia, HGF, MCP-1, TNF-α and resistin levels, and lower adiponectin levels. In the Prem group, the highest levels of PAI1t, insulin, HOMA and leptin were detected. In both groups the concentrations of CRP and IL-8 were higher and the values of HDLc and glutation peroxidase (GPox) were significantly lower compared with controls.

Various significant correlations were observed. In the EUGR group, leptin exhibited the strongest association with BMI Z-score, insulin, HOMA and HGF; and adiponectin with HDLc. In the logistic regression analysis, the predictors of metabolic status of the EUGR children were BP levels, glucose, resistin and TNFα, as well as lower values of BMI-Zscore, adiponectin, HDLc and GPox.

Conclusions: In prepubertal children with antecedents for EUGR or prematurity, an alteration in metabolic programming conditioing a higher risk of inflammation and oxidative status can be present. These findings could condition a greater risk of metabolic syndrome and cardiovascular disease in later stages.

Ve. Significance of circulating miRNAs as predictive biomarkers in pre-diabetes and new diagnosed type 2 diabetes mellitus

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Objective: To study four circulating miRNAs associated with insulin signaling and beta-cell function and their potential use as biomarkers of developmental risk and diagnosis of T2DM.

Methods: 462 non-T2DM patients in baseline were selected from the CORDIOPREV study. Levels of four miRNAs related with insulin signaling and beta-cell function were measured by real-time RT-PCR in: 106 T2DM-incident subjects, 30 PreDM-incident subjects, 223 PreDM-prevalent subjects and 78 subjects disease-free; after a follow-up median of 55 months. Plasma levels and the insulin signaling and beta cell function indexes evolution during the follow-up period were analyzed. The predictive value of miRNAs in T2DM-development risk was evaluated by Cox regression analysis.

Results: We observed that plasma levels of *miR-150* and *miR-30a-5p* were higher and *miR-15a* and *miR-375* lower in T2DM - Incident subjects with intermediate levels in PreDM- Prevalent subjects and PreDM - Incident subjects than disease-free subjects. Moreover, we also observed that patients with high *miR-150* and *miR-30a-5p* levels at baseline are related with decreased levels of disposition index after the follow-up period. Our study showed the predictive value of these four miRNAs plasma levels in the risk of T2DM-development, this is supported with HR from 1.640 and 3.002 associated to low plasma levels of *miR-375* and *miR-15a*, respectively, and HR from 4.014 and 2.532 associated to high plasma levels of *miR-150* and *miR- 30a-5p*, respectively.

Conclusion: Changes in the expression profiles of *miR-150, miR-30a-5p, miR-15a*, and *miR-375* in plasma may be used as predictive biomarkers allowing an early diagnosis and risk stratification of T2DM.

Vf. Plasma profile of cytokines and adhesion molecules in children with autism spectrum disorder

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Introduction: In the etiopathogenesis of autism spectrum disorder (ASD) multiples assumptions have been described. Pro-inflammatory status as an alteration in cellular adhesion molecules in neurodevelopment could influence the pathophysiology of ASD.
Objective: To assess plasma levels of inflammatory cytokines and cellular adhesion molecules in a sample of children with ASD and in a control group of healthy children.
Material and methods: 54 children from 2-6y diagnosed with ASD were selected. A healthy control group of 59 children with similar age were also included. The ASD diagnosis was made in accordance with diagnostic criteria of DSM-V according to data obtained by clinical interview (protocol ADI.R and ADOS test). Patients with ASD were subdivided in 2 groups according to neurodevelopmental regression (AMR) and without regression (ANMR). The analysis of cytokines: myeloperoxidase (MPO), type 1 plasminogen activator inhibitor (PAI-1); regulated on activation normal T cell expressed and secreted (RANTES); interleukins (IL1B, IL6, IL8), the chemo-attractant protein of monocyte (MCP); cathepsin, brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and cellular adhesion molecules (NCAM, SICAM, SVCAM) was realized by xMAP™ Luminex®.
Results: The average age in ASD group was 43.7±11.2 months and in the control group was 89,9±21.9 months ($p<0.001$). The distribution by gender in ASD group was 45 boys and 9 girls compared with 30 boys and 24 girls in the control group ($P=0.002$). In relation with cytokines, NGF was higher in children with ASD. However, by subgroups related with regression, ANMR children showed lower NCAM and higher NGF plasma levels compared with healthy children.
Conclusion: There are no marked differences between cytokines levels in children with ASD. However, variations in NCAM and NGF could be involved in the neurodevelopment in ANMR children. These results point to the possibility of different biological pathways implicated and heterogeneity of pathologies that converge in the ASD.

SESSION VI

Cancer (Oncology and Oncohematology) Active ageing an fragility

VIa. The Splicing Machinery is Profoundly Deregulated in Prostate Cancer: Pathological and Clinical Implications

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Prostate cancer (PCa) is the most common cancer type and the second leading cause of cancer-related death among men in developed countries. Recently, the deregulation of alternative splicing (AS), a process catalysed by the spliceosome in coordination with numerous splicing factors (SFs) that produces different proteins from a single gene, has emerged as a hallmark of cancer. Indeed, dysregulation of AS is especially relevant in PCa inasmuch the generation of oncogenic variants (e.g., AR-v7, sst5TMD4, ln1-ghrelin) from certain relevant genes has been associated with its development and/or aggressiveness. Thus, we hypothesized herein that the splicing machinery could be dysregulated in PCa and therefore related to its development and/or progression. To test this idea, we used a microfluidic-based PCR array to determine the expression of key components of the major (n=13) and minor spliceosome (n=4) and associated SFs (n=28) in PCa-samples (n=42) compared to normal-control prostate tissues (n=9). This analysis revealed a severe dysregulation of multiple components of the splicing machinery in PCa, wherein the expression of some of these factors was associated with important clinical parameters (e.g. metastasis, castration resistance). Indeed, non-supervised clustering analysis suggested the existence of two subpopulations of PCa patients according to the expression pattern of the splicing machinery components, which presented different clinical characteristics. Particularly, the group of PCa patients characterized by higher expression of certain splicing machinery components exhibited significantly higher aggressiveness features (e.g. metastases). Finally, *in vitro* studies using PCa cell-lines demonstrated that the experimental modulation (overexpression and/or silencing) of some of the splicing machinery components mentioned above influenced various functional parameters (i.e. proliferation, migration, etc.). Altogether these results indicate that the splicing machinery is drastically dysregulated in PCa and may provide novel tools to develop diagnostic markers and/or therapeutic targets.

VIb. Targeted DNA demethylation in human cells by fusion of a plant 5-methylcytosine DNA glycosylase to a sequence-specific DNA binding domain

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DNA methylation at carbon 5 of cytosine (5-methylcytosine, 5mC) is a stable but reversible epigenetic mark associated to gene silencing. DNA demethylation involves either dilution of 5mC by DNA replication in the absence of methylation, designated passive DNA demethylation, or enzymatic processes for replication-independent removal of 5mC termed active DNA demethylation. Targeted active DNA demethylation is a major goal of epigenetic editing, a new discipline aimed to modulate gene expression by overwriting of epigenetic marks at specific genome regions, which can be very useful in basic research and might also have important applications in the study of diseases that show epigenetic alterations, such as cancer. In mammalian cells, DNA demethylation involves iterative oxidation of 5mC by TET dioxygenases, followed by passive replication-dependent dilution and/or active replication-independent removal of oxidized 5mC derivatives through DNA repair. In contrast, plants use specific DNA glycosylases that directly excise 5mC without prior modification and initiate its substitution for unmethylated C through a base excision repair (BER) pathway. *Arabidopsis thaliana* REPRESSOR OF SILENCING 1 (ROS1) is a representative member of these plant-specific 5mC DNA glycosylases. In this work, we have explored the possibility of directing *Arabidopsis* ROS1 DNA demethylase activity to a specific target sequence in human cells by fusing its catalytic domain (ROS1_CD) to the DNA binding domain of yeast GAL4 (GBD). We show that the resultant GBD-ROS1_CD fusion protein binds specifically a GBD-targeted DNA sequence *in vitro*. We also found that transient *in vivo* expression of GBD-ROS1_CD in human cells specifically reactivates transcription of a methylation-silenced reporter gene, and that such reactivation is accompanied by decreased methylation levels at several CpG sites of the targeted promoter. All together, these results show that plant 5mC DNA glycosylases can be used for targeted active DNA demethylation in human cells.

VIc. Regulation of Notch1 expression and activity by DYRK2: New Insights of Carcinogenesis Signaling Pathways

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Notch1 is a highly conserved transmembrane protein with a crucial role in cancer development, cancer cell survival, proliferation and differentiation. The expression of Notch1 intracellular domain (Notch1-IC) is associated with poor survival in cancer patients and its regulation represents a potential strategy for cancer treatment. Although Notch1 activation mechanisms have been amply described, very little is known about degradation control processes.

In the present study, we first identify the dual specificity tyrosine-regulated kinase 2 (DYRK2) as a new regulator of Notch1, which mediates its phosphorylation and proteasomal degradation, probably through its interaction with Fbw7 ubiquitin ligase. DYRK2 is a Ser/Thr kinase involved in the regulation of cell processes such as cell proliferation and differentiation, and which has been attributed a key role in tumour development and/or progression. Our results evaluated the crosstalk between DYRK2 and Notch1 signalling during tumorigenesis, showing that Notch1 degradation is dependent of both its activation and its kinase activity. We show that DYRK2 depletion cells by siRNA present Notch1 accumulation together with lower phosphorylation levels. We identified that, in response to DNA damage by chemotherapeutic drug, DYRK2 promotes its stabilization, thus facilitating Notch1-IC degradation. Consequently, the transcriptional activity of Notch1-IC was inhibited in response to chemotherapeutic agents. Furthermore, we found inversely proportional levels of DYRK2 and Notch1 in diverse lung cancer cell lines (A549, MOR and H727). We also found that tissues of patients with cancer showed increased levels of Notch1 and decreased levels of DYRK2 and Fbw7. Taken together, these results propose Notch1 as a novel target of DYRK2, displaying a new pathway involved in carcinogenesis that has been unexplored until date. In addition, they suggest that DYRK2-induced phosphorylation of Notch1-IC plays an important role in cancer prevention and could be a potential biomarker for diagnosing cancer treatment.

VId. Neutrophil-to-Lymphocyte Ratio as prognostic factor in SBRT for Lung Cancer

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Introduction and Objectives: Inflammation plays an important role in cancer. Neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte ratios (PLR) are inflammatory markers, correlated with clinical outcomes after stereotactic body radiotherapy (SBRT).
Our aims are to evaluate the prognostic value of NLR and PLR in patients treated with SBRT for early stage non-small-cell lung cancer (NSCLC) and to identify the health outcomes in terms of local control (LC), metastases free survival (MFS) and overall survival (OS).
Material and Methods: Sixty patients diagnosed with NSCLC (61 lesions) were treated by SBRT between july 2012-september 2016. The treatment schedule was 5x12Gy (67,2%) for peripheral lesions and 8x7,5Gy (32,8%) for central lesions. Neutrophil, platelet and lymphocyte pretreatment levels were determined retrospectively. Local disease and pattern of progression were evaluated by CT and PET/CT. Receiver Operating Characteristics (ROC) analysis was performed to examine cutoff values for OS and LC. The Kaplan-Meier analysis was used to estimate patient survival
Results: Fifty-five men and five women with a median follow-up period of 17.3 months (4.4-142.4) were treated. The mean age was 74 years (51-87). The distribution by stages was: IA stage: 49 (81.7%); IB stage: 11 (18,3%) (TNM 7th Ed).
NLR and PLR cutoff values for further survival analysis were determined based on the ROC analysis, and they were 2.76 and 130.5. There were statistically significant differences in LC and OS (p=0,01 and p=0,03 respectively) depending on the NLR, but not on PLR (p=0,2 and p=0,25 respectively).
LC at 36 months was 92.8%. MFS at 12 and 36 months were 89% and 74.1% respectively. OS at 12 and 36 months were 89% and 60.7%.
Conclusions: A pretreatment NLR>2.76 is associated with worse outcomes in patients with NSCLC undergoing SBRT. This marker could be used as prognostic factor.
SBRT for early NSCLC provides good results in LC, MFS and OS.

VIe. Plasmatic Levels of miRNAs as Reliable Diagnostic Tool for Prostate Cancer Patients

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Prostate cancer (PCa) is the most common cancer type in men and one of the leading causes of cancer-associated death. Currently, serum prostate-specific antigen (PSA) levels is the only non-invasive biomarker routinely used in screening of PCa; however this method presents severe limitations. In this scenario, miRNAs, which are small non-coding RNAs that control post-transcriptional gene expression, have become promising diagnostic tool in several pathologies. However, although initial studies have been implemented in PCa with limited number of miRNAs, their diagnostic potential in PCa patients is still to be defined. Therefore, to more comprehensively explore the putative role of miRNAs as non-invasive diagnostic tool in PCa, we employed an Affymetrix miRNA 4.1 Array on plasma samples from healthy (n=20) and PCa (n=20) patients, which screened the presence and abundance of all known human miRNAs hitherto. Moreover, as PCa is strongly influenced by obesity, a major health problem that courses with severe comorbidities and can modulate the expression of certain miRNAs, both groups were stratified in normoweight and obese groups according to the BMI. This analysis indicated that 185 miRNAs were statistically altered ($p<0.01$) in the plasma of PCa patients compared to controls (108 were increased and 77 reduced) and that their plasmatic profile could accurately discriminate between PCa and control patients. Furthermore, individual ROC analysis revealed that 28 miRNAs presented AUC>0.88 when comparing PCa patients vs controls and that some exhibited even better diagnostic capacity when the patients were stratified by BMI. Additionally, some of these changes were confirmed by qPCR and are being validated in an ampler population (n=384) of controls and PCa patients. Therefore, these data indicate the existence of a panel of miRNAs that could distinguish between healthy and PCa patients and could represent more reliable tools for PCa diagnosis.

POSTER
Abstracts

POSTER SESSION I

Nutrition, Endocrine and metabolic diseases

P1. Development of type 2 diabetes is associated with early, predictive changes in the expression of certain splicing machinery components

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Metabolic syndrome (MetS) and type-2 diabetes (T2D) development is critically affected by the loss of phenotypic flexibility (i.e. the difficulty to cope with stressors to maintain metabolic homeostasis). Thus, it is essential to identify key modifiers of phenotypic plasticity that define individual susceptibility to develop these metabolic-pathologies. Particularly, there is emerging evidence that aberrant splicing processes are dysregulated in several endocrine-tissues under adverse metabolic-conditions (i.e. T2D). We hypothesized that changes in splicing machinery components could be altered in different tissues, including PBMCs (whose gene expression pattern commonly reflects disease characteristic), and therefore may serve as early indicator of MetS/T2D-development. Thus, the expression of selected relevant components of the major/minor spliceosome (n=17), and associated splicing factors (SFs; n=28) was evaluated in PBMCs of individuals with high risk to develop T2D due to a previously occurred cardiovascular event (CORDIOPREV study). Specifically, all patients (n=105) that developed T2D during the first 55 months of follow-up and non-T2D matched-controls (n=109) were included in the analysis. PBMCs were isolated from basal and post-prandial blood at the inclusion in the study. Results revealed that the basal expression of certain spliceosome components and SFs was altered in PBMCs from patients who developed T2D compared to controls, especially on those patients that developed T2D during the first three years of follow-up. However, the most remarkable differences between the two-group of patients were observed during the post-prandial phase (i.e. the expression of several spliceosome-components/SFs was increased in control-patients during the post-prandial phase, while this induction was blunted in T2D-developing individuals), which might suggest that the dysregulation in the splicing machinery precedes the development of T2D. Altogether, our results reveal the existence of T2D development-associated spliceosome alterations, which could be related to the loss of phenotypic flexibility, and could help to predict development of T2D in high-risk patients.

P2. Utility of spot urine sample to study tubular function: validation and agreement

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Backround: The 24 hour collection is the gold standard to study basic renal function, but it is cumbersome and unreliable in many cases. We analyze the validity and agreement of different timed urine samples that could replace this method.

Methods: Prospective observational study in 52 children from our Pediatric Nephrology outpatient office aged 5 to 9 years. We analyzed 4 serial timed urine specimens collected during 24 hours and along with a blood sample. ROC curve assessed validity. For agreement we tested Pearson Correlation, Interclass Coefficient Correlation and Bland Altman plot.

Results: For the glomerular function, protein and albumin creatinine ratios had good agreement and validation in all samples with 24 hour collection that improved for low values.

Morning sample had the better agreement and validation for calcium/creatinine ratio. Cut off point of 0,2 mg/mg had excellent sensitivity and specificity in morning and first morning void samples.

First morning void showed the best agreement for the rest of parameters, although some of them showed great dispersion.

Conclusions

First morning void may be useful for screening in the study of basic renal function in children. Any sample could be used as screening for Pr/Cr and Alb/Cr. It is a challenge to define the better timed urine sample depending on the goal of our study.

P3. Hepatic Steatosis in Obese Women is Associated with Alterations in Splicing Machinery Components

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Hepatic steatosis is a common obesity-associated pathology characterized by the accumulation of fat within the liver, which can progress to liver fibrosis, cirrhosis and hepatocellular carcinoma. There is emerging evidence that alternative mRNA splicing is dysregulated in many tissues under adverse metabolic conditions such as obesity. Moreover, the aberrant expression of splicing variants could contribute to the comorbidities of this pathology. Since the appearance of alternative splicing variants might be linked to a dysregulation of the cellular machinery responsible for this process [(spliceosome components and splicing factors (SFs)], the objective of the present study was to determine the association between the expression pattern of the components of this machinery and hepatic steatosis. To this end, we collected liver biopsies samples from obese women (IMC>30) with (n=32) and without (n=9) hepatic steatosis to determine the expression levels of selected components of the major (n=13) and minor (n=4) spliceosomes and SFs (n=28) using a qPCR-based array. The results revealed that the liver of steatotic patients exhibit a severe dysregulation of certain spliceosome components and SFs compared to non-steatotic patients. Although these alterations were not associated with the level of hepatic steatosis, non-supervised clustering analysis revealed the existence of groups of steatotic patients with specific alterations in spliceosome components and SFs, which also presented distinctive hepatic and clinical-metabolic alterations (e.g. ALT, hyperglycemia, hyperinsulinemia, etc.). Finally, *in vitro* approaches with liver cell lines demonstrated that fat accumulation altered the expression of certain spliceosome components and SFs and that the modulation (silencing) of certain splicing machinery components altered fat accumulation, indicating a bidirectional crosstalk. Therefore, these results suggest a close bidirectional relationship between the development of hepatic steatosis and its associated comorbidities with the dysregulation of splicing machinery, which may provide novel diagnostic/therapeutic tools for this pathology.

P4. The consumption of two models of healthy diet produces a differential effect on endothelial damage and regenerative capacity according to the degree of endothelial dysfunction: CORDIOPREV study

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Our aim was to determine whether long-term consumption of two models of healthy diet produces a differential effect in endothelium damage and regenerative capacity processes in patients with cardiovascular heart disease (CHD), classified according to the degree of endothelial dysfunction. 806 patients from the CORDIOPREV study (NCT00924937), 253 patients with severe endothelial dysfunction [flow-mediated dilation (FMD) of the brachial artery <2%] and 553 patients with mild endothelial dysfunction / normal endothelial function (FMD>2%), were randomized to receive one of the following dietary models for a period of one year: A) Mediterranean diet, B) Low-fat and rich in complex carbohydrate diet. Endothelial damage [endothelial microparticles levels (EMPs), reactive oxygen species (ROS), apoptosis and cell senescence] and endothelial regenerative capacity [endothelial progenitor cells (EPCs), cell proliferation and angiogenesis] were evaluated at baseline and after the dietary intervention. Our results shows that Mediterranean diet decreases activated EMP levels and apoptotic EMPs:EPCs ratio and increases EPC levels, as well as decreases endothelial damage and increases endothelial regenerative capacity processes, independently of the degree of endothelial dysfunction. However, a low-fat diet increases EPC levels in patients with FMD>2% and increases apoptotic EMPs:EPCs ratio in patients with FMD<2%, while increases endothelial damage and decreases endothelial regenerative capacity processes independently of the degree of endothelial dysfunction (all $p<0.05$). In conclusion the degree of endothelial dysfunction, in CHD patients, determines a differential response to consumption during 1-year of two models of healthy diet. A Mediterranean diet reduced endothelial damage, which was associated with an improvement of endothelial regenerative capacity, independently of endothelial dysfunction degree. However, a low-fat diet produced higher levels of endothelial damage compared to Mediterranean diet, independently of endothelial dysfunction degree but was only able to activate endothelial repair mechanisms in patients with FMD>2%. These results highlight the possibility to establish new and personalize strategies, through different dietary patterns that improve endothelial dysfunction and regenerative capacity, to reduce cardiovascular risk in CHD patients.

P5. Sphingolipids and ether-phospholipids biosynthesis and its regulation in obesity and insulin resistance

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Adipose tissue (AT) governs lipid homeostasis in the organism and plays a critical role in the progression of insulin resistance (IR) and other obesity-associated diseases. Although much is known about the clinical pathology of metabolic disorders, the molecular mechanisms underlying the dysregulation of AT lipid metabolism in obesity remain unclear. In this context, sphingolipids (such as sphingomyelins) and ether-phospholipids, two lipid classes with both signaling and structural properties, have recently emerged as key players and potential biomarkers in metabolic disorders. Thus, regulation of these lipids and/or their biosynthetic enzymes (Sphingomyelin Synthase 1,2 - SMS1, SMS2; Alkylglycerone-phosphate synthase - DHAP and Choline/Ethanolamine Phosphotransferase 1 - CEPT1) may represent valuable candidates for the development of novel therapeutic strategies to tackle obesity-related diseases. In this study, we evaluated the expression levels of the enzymes involved in the biosynthesis of sphingolipids and ether-phospholipids in adipocytes, based on lipidomic data of AT from obese individuals with different degrees of insulin sensitivity. Specifically, we exposed adipocytes to different obesogenic insults mimicking various pathophysiological conditions that have been linked to the progression of metabolic disease including exposure to high glucose and high insulin levels (HGHI), lipid overload (hypertrophy) using palmitate or oleate treatments, and to an inflammatory environment using TNF-a treatment. We observed that prolonged exposure of adipocytes to HGHI altered the expression of all the enzymes tested, suggesting that the hyperglycaemia/hyperinsulinemia in obesity greatly contribute to the dysregulation of both sphingolipids and ether-phospholipids. TNF-a increased the expression of ether-phospholipid-related enzymes and caused dissimilar changes in sphingomyelin-related enzymes. Finally, oleate-induced adipocyte hypertrophy only seemed to dysregulate sphingolipid production, while palmitate had no effect. Our results show that diverse obesogenic insults do impact, yet differently, in the expression of sphingolipid and ether-phospholipid biosynthetic enzymes, which, in all, may contribute to the development of metabolic disease in obesity.

P6. Mediterranean diet improve the postprandial hypertriglyceridemia and remnant cholesterol concentrations in patients with type 2 diabetes mellitus

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Objective: Patients with type 2 diabetes mellitus (T2DM) have an exaggerated postprandial lipemia response, which has been associated with increased cardiovascular risk. Although the initial treatment of T2DM is based on diet and physical exercise, no evidence currently exists about the effect of diet on postprandial hyperlipidemia. Our aim was to analyze whether the chronic consumption of two healthy dietary patterns is associated with an improvement of postprandial hypertriglyceridemia and remnant cholesterol concentrations in patients with T2DM.

Material and methods: Those patients from the CORDIOPREV study who underwent an oral fat load test meal (FLT) at baseline and three years after follow up were selected (n=241 patients with T2DM and 316 non-diabetics). Subjects were randomized to a Mediterranean diet rich in olive oil (MedDiet) [35% of calories from fat (22% monounsaturated) and 50% from carbohydrates] and Low fat diet (LF diet) [28% fat (12% monounsaturated) and 55% of calories from carbohydrates]. Serial blood test analysing lipid fractions were drawn at 0, 1, 2, 3 and 4 hours during postprandial state. We also determined remnant cholesterol (RC) as a marker associated with cardiovascular disease.

Results: After three years of follow-up, patients with T2DM had an improvement in the postprandial hypertriglyceridemia, measured as the triglyceride-rich lipoproteins (TRLs)-TG ($p = 0.001$) and the area under the curve (AUC) of TG ($p = 0.001$), compared with baseline. Specifically, patients with T2DM showed a greater reduction of the AUC of TG after the MedDiet (17.3% ($p = 0.003$)) compared with the LF diet (6.8% ($p = 0.1$)). No significant differences were found in the non-diabetic patients subgroup. Additionally, the MedDiet induced a significant improvement of the AUC of RC concentrations in patients with T2DM ($p = 0.04$). There was no significant improvement after the LF diet.

Conclusions: Our findings demonstrate that the chronic consumption of a MedDiet rich in olive oil improve the postprandial hypertriglyceridemia and RC concentrations in patients with T2DM. It should be interesting to identify these patients to intervene them more aggressively and reduce cardiovascular risk.

P7. Hypoxia-Inducible Factor (HIF)-1 Regulatory Pathway and its Potential for Therapeutic Intervention in Metabolic Disease

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The survival of multicellular organisms depends on good oxygen homeostasis, where the hypoxia-inducible factor (HIF-1α) is the key protein to regulate this pathway. Recent research shows that this protein plays an important role in the most relevant stages of metabolic disease: cell dysfunction, insulin resistance, adipocyte dysfunction and inflammation. These data have pointed out the potential interest of the hypoxia response pathway as a therapeutic target. Prolyl-hydroxylases proteins (PHDs) and one of the ubiquitin-ligases responsible for its regulation, SIAH2 protein, stand out for their importance. Although the specific manipulation of this pathway is complex, some molecules with this capacity have been described.

In this report we first demonstrate how the hypoxia response pathway is altered during beta cell differentiation due to the degradation of PHD2, which is accompanied by a stabilization of the transcription factors HIF-1α and HIF-2α. Furthermore, we show how some endolipids (N-acyl-dopamines) present the ability to induce Beta-cell differentiation in AR42J and Human islet-derived precursor cells (hIPCs) through the alteration of this pathway. We present relevant data underlining the ability of N-palmitoyl-dopamine (PALDA) and N-stearoyl-dopamine (STEARDA) to induce insulin production in hIPCs. In parallel to this relevant finding, we have analyzed the effect of these two endolipids in the context of T2D-associated vascular disease. Initial data set indicates that both are able to induce angiogenesis in *in vitro* models. Besides, we have analyzed the impact of mutations in key components in the hypoxia response pathway in the CORDIOPREV cohort (950 patients). We have identified four SNPs related to the hypoxia pathway associated with the risk of developing diabetes. Two of these SNPs have been found to be related to metabolic syndrome. In summary, this work identifies new key biomarkers associated/related to T2D and metabolic disease, which could be significant to develop new strategies of prevention and prediction of this relevant global health problem.

P8. A role for the small GTPase Rab34 in lipid accumulation in hepatocytes

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Rab small monomeric GTPases, a family that comprises more than 60 members in humans, coordinate the interaction between the intracellular compartments in eukaryotic cells. Recently, our group identified one of this GTPases, Rab34, in a proteomic screening of the lipid-storage organelles, the lipid droplets (LDs), in adipocytes. Specifically, we observed that Rab34 associated with the surface of LDs as soon as these appeared in differentiating adipocytes, suggesting a role of this GTPase in lipid management. Indeed, functional studies indicated that Rab34 favors triglyceride accumulation in adipocytes under physiological conditions. Herein, we aimed at characterizing the contribution of Rab34 in the lipid metabolism dysregulation that occurs in situations of fatty acid overload, such as that associated to obesity. To do so, we carried out *in vitro* experiments using hepatocytes, a cell type that ectopically accumulates the excess of free fatty acids, often leading to obesity-related hepatic steatosis. Our confocal microscopy studies demonstrate that Rab34 relocates from its original distribution in resting HepG2 hepatocytes, the Golgi membranes, to the LD surface, likely via a retrograde transport pathway through the endoplasmic reticulum-Golgi intermediate compartment (ERGIC), in cells exposed to long-term (18 h) oleate treatment. These results suggested a role for Rab34 in fatty acid uptake and intracellular transport in hepatocytes, which is consistent with our proteomic data on oleate-treated hepatocytes showing that the Rab34 interacts with components of the retrograde Golgi-to-ER transport machinery, including Arf1/COPI, a system that has been shown to mediate the transfer of proteins such as perilipin2 (PLIN2) or the lipid biosynthetic enzyme GPAT4 to the LD surface. These results, together with our observation that Rab34 protein levels increase in the liver of either genetically- or diet-induced obese animals, strongly support a role for Rab34 in the formation and maintenance of LDs in hepatocytes under both physiological and pathological conditions.

P9. Analytical platforms for qualitative/quantitative analysis of microbiota related metabolites in nutritional studies

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The human gastrointestinal tract is colonized by ≈10^{14} microorganisms collectively termed as the microbiota [1]. It is well-known that the microbiota plays a major role in health and certain types of diseases [2]. Thus, qualitative and/or quantitative disruptions of the microbiota composition are increasingly correlated to gut diseases, but also to obesity, insulin resistance and type 2 diabetes [3]. Metabolomics analysis of biofluid samples is being increasingly used to explore the metabolites related with the gastrointestinal tract and to compare their profiles under different physiological or disease conditions [4]. In this research, we propose platforms for the analysis of metabolites related to the microbiota in human plasma, using two analytical approaches: LC–MS/MS and GC–MS in high resolution mode.

Human plasma samples were analyzed by LC–QTOF and GC–TOF, using a targeted approach in both cases. GC–MS analysis was focused on short chain fatty acids (SCFAs) and branched-chain amino acids (BAAs) by application of a deproteination protocol with propanol. The deproteinated sample was derivatized with propyl chloroformate and the derivatives extracted with hexane. Samples subjected to LC–MS/MS were previously deproteinated with a methanol–acetonitrile solution. The identification by LC–MS/MS was supported on MS and MS/MS information by searching in the METLIN and the Human Metabolome Databases.

The combination of the two platforms allowed determining 46 microbiota related metabolites. The main families of identified metabolites were bile acids, bilirubin metabolites, BAAs, other amino acids metabolites and SCFAs. These platforms can be used in any nutritional or clinical study dealing with the impact of diet on the microbiota or with the interaction between pathological conditions and microbiota regulation.

P10. Effect of dietary fat on postprandial endotoxemia in the elderly people

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Objective: The postprandial inflammatory response may be caused, at least partially by increased intestinal absorption of bacterial endotoxins. Our objective was to evaluate the effect of the amount and type of fat in the diet on the levels of LPS (lipopolysaccharide) and LBP (LPS binding protein) in elderly men and women

Material and methods: This study was carried out on 20 elderly people (10 men/10 women) who consumed of 3 dietary models with different amount and type of fat for 4 weeks each by a randomized crossover design: 1. Mediterranean diet enriched in MUFA with virgin olive oil. 2. SFA-rich diet. 3. Low-fat high-carbohydrate diet enriched in n-3 PUFA (**α**-linolenic acid from plant origin) (CHO-PUFA diet). At the end of each period, after a 12-h fast, the subjects received a breakfast with a fat composition similar to the dietary period ended. The levels of LPS and LBP were determined in fasting state after each dietary intervention period and 4 hours after the administration of the fat meal with the same fat composition that the diet previously consumed

Results: Our results showed a postprandial increase in LPS levels and a decrease in LBP after consumption of the fat meal administered after the LF n-3 diet ($P = 0.001$ and $P = 0.002$, respectively), whereas no postprandial changes were observed after the fat meal administered after the MED and SFA diets. Likewise, we observed a statistically significant trend to lower levels of fasting LPS after consumption of the LF n-3 diet ($P = 0.083$)

Conclusion: Our results suggest that postprandial endotoxemia is modulated by diet in elderly people

P11. Stability study for analysis of Vitamin D and its principal metabolites in serum samples

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The role of vitamin D to prevent non-skeletal diseases such as cancer, autoimmune, cardiovascular or infectious disorders has been related to abnormal concentrations or ratios of vitamin D metabolites in humans. This connection has launched the interest in the clinical effects of vitamin D and its metabolites and also the development of new analytical methods with extra levels of sensitivity and selectivity. In this context, the stability of vitamin D metabolites and their lifetime in clinical samples have an essential relevance to ensure the quality of results. For this reason, a stability study of vitamin D3 and its metabolites - 25(OH)D_3, 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ - in human serum has been developed by testing four different ways of samples storage (at room temperature, in a refrigerator at 4 ºC, and freezer at -20 ºC and -80 ºC) for two months. Additionally, the influence of freeze-thaw cycles on the concentration of the target analytes was evaluated. Serum samples were analyzed by an automated SPE-LC-MS/MS method that is participating in an external validation program termed DEQAS.

The four analytes experienced some differences in stability according to the storage conditions. As example, the circulating form, 25(OH)D_3, only provided a decrease of concentration under room temperature storage, while 24,25$(OH)_2D_3$ was only stable in the studied period after freezing at -80 ºC. Concerning freeze-thaw cycles, the two dihydroxymetabolites decreased their concentration after the third cycle; while the rest of analytes were stable after five freeze-thaw cycles. An additional test was based on the stability in lyophilized samples stored for two months, which revealed that the four analytes were stable under lyophilisation of the serum sample. This strategy could open a promising way to store serum/plasma for long periods prior to vitamin D analysis

P12. Characterization of human adipose tissue acetylome in obesity-associated insulin resistance. Identification of new targets related to metabolic disease

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Post-translational modifications (PTMs) play an important role in multiple diseases. Acetylation is one of the most frequent PTM associated with pathologies and is closely related to inflammation and energy homeostasis, key processes leading to adipocyte dysfunction in obesity. The identification of acetylated targets and their interactions with other proteins may help further elucidate their potential role in obesity-related adipose deregulation. Herein, we aimed at identifying the acetylome of subcutaneous (SAT) and visceral (VAT) adipose tissue (AT) from lean and morbidly obese subjects with different degrees of insulin sensitivity (normoglycaemia –NG-, or insulin resistance -IR) by means of immunoprecipitation and HPLC-MS/MS analysis. A total of 539 acetylated proteins were identified corresponding to 20% of the AT proteome. VAT presented a higher content in acetylated proteins as compared to SAT. However, the number of acetylated proteins in VAT decreased in IR *vs.* NG obese individuals and lean subjects. Furthermore, pathway analysis revealed changes in acetylation of proteins related to mitochondrial dysfunction, fatty acid β-oxidation, and mTOR signaling, among others. To get further insights on the effect of protein acetylation, we characterized one of the proteins identified by proteomics, the lipid chaperone and adipokine, FABP4, whose acetylated form was only detected in the cytoplasmic fraction of AT extracts but not in nuclear extracts. We generated a triple mutant (TM-FABP4; K22I, K32I, K59I) FABP4 variant by site-directed mutagenesis and examined its distribution in overexpression experiments using 3T3-L1 adipocytes. Confocal studies revealed that, in contrast to wild-type FABP4, TM-FABP4 remained mainly cytosolic upon exposure of 3T3-L1 adipocytes to oleate. Taken together, our results suggest a role for protein acetylation in the (de)regulation of AT function which, among other processes, may affect intracellular lipid traffic and lipid-regulated transcriptional responses mediated by FABP4.

P13. POWER2DM study. Rationale and Methodology: Development of a technology platform to improve empowerment and reduce cardiovascular complications in diabetic patients

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Objective: To improve the self-control of Diabetes Mellitus by the patient and to provide the responsible physician with both the collection of data from the disease control status and the decision making on cardiovascular risk control.
Material and methods: Power to Diabetes Mellitus (POWER2DM) is a multicentric, multidisciplinary study of the European H2020 (http://power2dm.org). Based on information from several wearable technology sensors (FSL Abbott, Spire, and Fitbit Charge, and in combination with the KADIS system © predictor of pancreatic function by AI), this project aims to establish a personalized "glycemic profile" that helps us to anticipate periods of hyper and hypoglycemia in diabetic patients. Through a software for patients (App) and another for physicians (PC) and supported by decision trees made by a team of psychologists and based on the theory of behavior change, the system will give personalized recommendations for lifestyle, treatment , predict cardiovascular risk, and simulate how treatment change and lifestyle adherence may improve glycemic control and improve cardiovascular risk. The study is carried out in three clinic centers (In Spain, Germany and the Netherlands) and four technological centers.
Results and conclusions:
POWER2DM intends to provide an intuitive and manageable technological support so that the diabetic patient and his doctor, through Smartphone and desktop application can instantly access a system that provides support for decision making on diabetes control and the cardiovascular risk factors improving the quality of life and the prognosis of the disease.

P14. Autism spectrum disorder is not associated with neurotoxicity by mercury

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Introduction: The autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by disruptions in communication and social interactions, and the presence of repetitive or limited behavioural patterns, activities and interests. One of the etiopathogenic theories is the possibility of neurotoxicity by metals that can contribute to produce ASD symptoms, especially the mercury (Hg).

Objective: To assess hair and urine levels of Hg in children with ASD compared with a control group of healthy children.

Material and methods: 54 children (45 boys and 9 girls) from 2 to 6 years diagnosed with ASD, without another related pathology, were selected. A control group of 54 children without ASD was also included. The ASD diagnosis was made in accordance with diagnostic criteria of DSM-V and, according to data obtained by clinical interview (protocol ADI.R and ADOS test).The severity of autistic symptoms was classified in terms of data obtained in PDD Behaviour Inventory™ and CARS test. Mercury was analyzed by atomic absorption spectrometry with hydride generation system.

Results: The average age in ASD group was 43.7±11.2 months and in the control group was 89,9±21.9 months ($p<0.001$). The distribution by sex in ASD group was 45 boys and 9 girls *vs* 30 boys and 24 girls in the control group (P=0.002). No significant differences between levels of Hg in hair and urine were observed in both groups, after adjusting the analysis by age and sex. No particular correlations were found between Hg levels in hair and urine in either group. Moreover, no correlations between Hg levels in urine and hair with different studied clinical scores, were detected.

Conclusions: In this study with an homogeneus sample of children with ASD, there are no evidences to support the association between the neurotoxicity by mercury and the ethiopathogenesis of ASD.

P15. Characterization of the effect of miR-223 in adipocytes and its possible role as a new biomarker of adipose tissue function

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MicroRNAs are noncoding RNAs that act as negative regulators of gene expression in multiples processes. These mRNA silencers exist both within the cells and in plasma and other biological fluids. Indeed, the circulating levels of specific microRNAs are altered in many diseases and are considered potentially useful biomarkers. In particular, a targeted study in metabolic syndrome patients participating in the CORDIOPREV clinical trial showed that the plasma levels of the microRNA, miR-223, positively correlated with AT dysfunction index (ATI) in this cohort. Herein, we aimed at characterizing the role of miR-223 in adipocytes. To this end, miR-223 levels were measured by RT-PCR during adipogenesis using 3T3-L1 adipocytes and the potential miR-223 targets related to AT function were investigated using *in silico* approaches. Thereafter, overexpression studies using a miR-223 mimic were carried out to test the *in silico* predictions by means of gene and protein expression analyses and functional assays. Our data shows that miR-223 is expressed in 3T3-L1 adipocytes and its levels are increased in late days of differentation. *In silico* research revealed that miR-233 targets participate in insulin signaling (IRS1, FOXO1), vesicular traffic (ATXN2, SNX24, RAB1A, ECT2), cytoskeleton (SEPT2, SEPT6, SEPT10, ACTA1), and, in particular, regulate glucose transporter 4 (GLUT4) traffic. In this line, we analyzed insulin-stimulated GLUT4 translocation (endogenous and exogenously expressed) to the plasma membrane in 3T3-L1 adipocytes overexpressing a miR-223 mimic. These studies showed impaired GLUT4 translocation to the adipocyte membrane in response to insulin in the presence of the miR-223 mimic. However, GLUT4 gene and protein expression levels were not affected, suggesting that others proteins related to this pathway are miR-223 targets. Taken together, our data suggest that miR-223 may play an important role in AT function, specifically by regulating GLUT4 translocation and glucose metabolism.

P16. Healthy diet effect on blood pressure according to the inflammasome genetic variants

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Objective: Inflammasome is a multiprotein complex involved in the activation of inflammatory and apoptotic processes. We aimed to evaluate the role of the inflammasome in metabolic processes by analyzing several single nucleotide polymorphisms (SNPs) located in the inflammasome transcriptional regulator *NRLP3* gene.

Methodology: This study was conducted in 1002 patients included in the CORDIOPREV study (NCT00924937), a randomized dietary intervention with a Mediterranean diet model rich in olive oil (35% fat: 22% monounsaturated fatty acid [MUFA]; 6% polyunsaturated fatty acid [PUFA]; <10% saturated fatty acid [SFA]) compared with a low fat diet (<30% fat: 12-14% MUFA; 6-8% PUFA; <10% SFA). SNPs at the *NLRP3* gene were analyzed by PCR on the OpenArray platform. We analyzed the effect of 5 SNPs in *NRLP3* gene in several metabolic parameters at baseline and at 3 years of dietary intervention.

Results: We observed an interaction between different *NLRP3* gene genotypes and blood pressure after 3 years of dietary intervention. We also observed that the C/G genotype for the SNP rs10754558 (p=0.002), the genotype A/G + A/A for the SNP rs10733113 (p=0.000) and the G/T genotype for the SNP rs4353435 (p=0.003) were associated with a decrease in systolic blood pressure. Likewise, a significant improvement in diastolic blood pressure was observed in those subjects carrying the C/G genotype for the SNP rs10754558 (p=0.002), the G/G and A/G + A/A genotypes for the SNP rs10733113 (p=0.006) and the G/T and T/T genotypes for the SNP rs4353435 (p=0.007, p=0.014).

Conclusions: Our results suggest that the consumption of healthy diets exerts a differential effect of blood pressure according to the genetic variants of the NLRP3 gene. Moreover, our results are consistent with the development of strategies based on personalized nutrition in patients with cardiovascular disease.

P17. Characterization of extracellular components contributing to adipose tissue fibrosis using a 3d-culture system

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The extracellular matrix (ECM) is a highly dynamic structure that regulates cell behaviour and function. It is composed by multiples molecules, including collagens and proteoglycans, which are secreted by the cells. ECM composition and function, which differ between cell types, is dynamically regulated in response to both internal and external changes. In the adipose tissue (AT), ECM is essential to maintain adipocyte differentiation, function, and survival. In obesity, AT ECM undergoes extensive remodelling and ensuing fibrosis, which is considered a major pathogenic mechanism linking obesity to metabolic disease. In this scenario, previous studies from our group revealed increased levels of the proteoglycan, lumican, in subcutaneous fat (SAT) of insulin resistant-obese individuals as compared to normoglycemic obese subjects. Based on these findings, herein we aimed at developing a three-dimensional (3D)-culture system of adipocytes to mimic the *in vivo* environment and to investigate the relevance and role of lumican in ECM physiology and adipocyte function. To this end, we prepared collagen I matrices which we proved suitable for proper 3T3-L1 fibroblast growth and differentiation into lipid-accumulating adipocytes, similar to that observed for 3T3-L1 adipocytes in conventional 2D-cultures. Notably, the presence of lumican at a low dose (10 ng/mL) in the 3D-cultures did not seem to impair adipogenesis, while adipocytes acumulated less lipids and did not acquire a round shape when lumican was added at a higher dose (30 ng/mL). In line with these observations, scanning electron microscopy of the 3D-cultures revealed that both the density and width of collagen fibers surrounding the adipocytes appeared to increase as the concentration of lumican increased in the culture. These observations, together with immunoblotting data suggestive of increased oxidative stress and altered adipokine release by adipocytes in 3D-cultures enriched in lumican, support an important role for this proteoglycan in the regulation of fibrosis and adipocyte (dys) function.

P18. Metabolic and reproductive studies in prenatal, neonatal and post-weaning rat PCOS models: Effects of kisspeptin treatment

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Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder, which affect up to 5-10% women of reproductive age and accounts for approximately 75% of anovulatory infertility. Ovulation failure, hyperandrogenemia and insulin resistance characterizes PCOS, however the pathogenesis and etiology of syndrome is still unclear. Consequently, treatment is palliative rather than curative and focuses on symptomatic approaches.

Kisspeptins, the products of the Kiss1 gene that act via the surface receptor, Gpr54, have emerged in recent years as potent elicitors of gonadotropin secretion, by acting as activators of GnRH neurons. The ability of various forms of kisspeptins to stimulate gonadotropin secretion has been documented in different species, including rodents and primates, even at relatively low doses following central (icv, intra-hypothalamic) or peripheral (intraperitoneal, iv, sc) administration. In fact, single dose or repeated/sustained administration of kisspeptins has been shown to robustly activate the gonadotropin axis.

Rodent models of PCOS based on early androgenisation have been shown to display deregulation of the hypothalamic Kiss1 system, as potential causative factor for altered gonadotropin secretion and ovulation in these models. Recent rodent models display both, reproductive and metabolic disturbances associated with human PCOS.

To reproduce the key features associated with human PCOS, we had been carried out a reproductive and metabolic characterization on three different rat models of PCOS. i) prenatal androgenisation female rats with di-hydrotestosterone (DHT) during 16-19 days of pregnancy (PNA), ii) neonatal androgenisation of one day old female rats with testosterone propionate (NeNA) and iii) pre-pubertal androgenisation female rats with DHT during 90 days after weaning (PWA). Summarizing, altogether the three rat models of PCOS showed similar heterogeneity than human PCOS. After low doses of Kp-54 chronic administration, NeNA model showed significant ovulation data, while PWA model had irreversible signs of anovulation. PNA model represent the PCOS patient without ovulating alterations.

P19. A gut microbiota pattern may predict the development of metabolic syndrome

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Objetive: Recent studies have shown that metabolic syndrome (MetS) is associated with a gut microbial dysbiosis. We hypothesized that gut microbiota composition may be involved in the MetS development. Our aim was to identify a specific microbiota profile that may predict the MetS development.

Materials and Methods: We selected 258 patients from the CORDIOPREV study without MetS at the beginning of the study, 45 from which developed MetS during the first three years of follow-up in the dietary intervention. The patients were randomized to receive an olive oil rich Mediterranean diet (35% of total calories from fat, 22% of monounsaturated fatty acids (MUFAs) or a low-fat diet (<30% of total calories from fat, 12% MUFAs). Feces samples were frozen after collection and DNA extracted by the QIAamp®DNA Stool Mini Kit commercial kit. We analyzed the gut microbiota composition by 16S ribosomal RNA gene (V3 and V4 region) sequencing with the Illumina platform. Sequence analysis was performed by the QIIME software.

Results: We identified a gut microbiota pattern linked to the development of metabolic syndrome through multivariant analysis (Partial Least Square Discriminant Analysis: precision 0.810, R2 0.38 and Q2 0.083), characterized by a high abundance of the *Holdemania* (VIP=1.9) and *Ruminococcus* (VIP=1.7) bacterial genera and a low abundance of the *Erysipelotrichaceae* (VIP=1.8), *Clostridium* (VIP=1.7) and *Prevotella* (VIP=1.7) families. Additionally, a lower richness and diversity of bacterial species was associated with this microbiota profile (P>0.001 y P=0.001, respectively). Moreover, the patients that developed MetS showed lower LBP plasmatic levels (P=0.048) at the beginning of the study than the patients that did not develop it.

Conclusion: Our results suggest that an alteration in the gut microbiota composition precedes and may be associated to the development of metabolic syndrome.

P20. Diagnostic accuracy of waist to height ratio for identifying of obesity in school children

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Childhood obesity is a public health problem of multifactorial etiology, which requires interventions at multiple levels.
In order to facilitate the diagnosis of childhood obesity, it is important to know the relationship between simple anthropometric indices such as the Waist to Height ratio (WHtR) and other estimators of overweight or obesity, such as BMI or body fat percentage (%BF), whose calculation is more complex.
Objective: To evaluate the diagnostic accuracy of waist to height as diagnostic tests for detecting fatness in schoolchildren.
Methods: A cross-sectional analysis of 324 primary and secondary schoolchildren was used. Measurements of weight (kg), height (cm), waist circumference (cm), BMI (Kg/m^2), WHtR, %BF were obtained.
The diagnostic accuracy for detecting excess fatness was evaluated through receiver operating characteristics (ROC) analyses with %BF; is taken as variable test the WHtR and as criterion the status of obesity evaluated by standards of% BF. Sensitivity and specificity, areas under the curve and cut-off points of WHtR that diagnose obesity were calculated.
Results: The areas under the curve reached a value of 0.961 (0.970 in boys and 0.947 in girls), indicating that the WHtR has a high positive predictive power (74.3%) to detect children with obesity. The cutoff points of WHtR that identify obesity are 0.50 in men with a sensitivity of 93.5% and specificity of 90%; and 0.51 for girls with sensitivity and specificity values of 85.4% and 91.3%, respectively.
Conclusion: The Waist to height ratio proved to be a good marker of obesity in children between the ages of 6 and 17 years. Another advantage is that WHtR can be an index with unique cutoff points for boys and girls (0.5-0.51). Since WHtR is a simple anthropometric variable it is recommended to use it in the field of prevention.

P21. Pharmacological characterization of the somastotatin receptor type 3: a novel strategy to treat non-functioning pituitary adenomas

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Non-functioning pituitary adenomas (NFPAs) are the most common type of pituitary tumors. Despite lacking hypersecretion of functionally hormones, NFPAs are mostly macroadenomas (>1cm) at diagnosis and are consequently associated to severe comorbidities related to mass effect. Transsphenoidal surgery is the mainstay of NFPAs treatment, although it is often not definitive because of the invasion of neighboring intracranial structures. Currently, available somatostatin analogs (SSA) with preferential binding affinity for receptor subtypes 2 and 5 (sst2/sst5) serve as a valuable therapy in secreting adenomas. However, this therapy has been shown largely ineffective in NFPAs, which might be explained by the low sst2/sst5 expression levels and predominant expression of sst3. Therefore, the aim of this study was to perform a pharmacological characterization of sst3 in primary cell cultures from human NFPAs, by analyzing the effect of different sst3-specific agonists on key functional parameters, including cell viability, apoptosis/necrosis, hormone secretion and intracellular-signaling pathways. The expression profile of 65 NFPAs and 10 normal pituitaries (NP) was analyzed by quantitative-PCR which revealed a clear alteration in the expression pattern of key hormones and somatostatin-receptors in NFPAs compared to NP samples including a overexpression of sst3 in NFPAs. Immunohistochemistry analysis performed in two cohort of patients revealed that sst3 showed a membranous and cytoplasmic localization. The *in vitro* treatment with sst3-agonists significantly reduced cell-viability and chromogranin-A secretion, regulated expression levels of key genes and increased apoptotic rate by a Ca^{2+}-independent mechanism. Remarkably, silencing of sst3 expression increased cell-viability in a subset of NFPAs. Moreover, we found that the effects of sst3-agonists might be mediated by inhibition of MAPK signaling. Altogether, our study provides new evidences that sst3 plays a relevant functional role in the pathophysiology of NFPAs, and suggest that pharmacological treatments specifically targeting sst3 could be a promising therapeutic alternative for NFPAs.

POSTER SESSION I

Active ageing and fragility. Infectious and Inmunological diseases. Organ transplantation

P22. The combination of ifng+874 t/a and il28b (rs12979860) c/t polymorphisms influence the risk of cmv replication in cmv-seropositive solid organ transplant recipients

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Background: The role of single-nucleotide polymorphisms (SNP) in the risk of cytomegalovirus (CMV) infection is unclear. The aim of this study was to analyze the relationship between the IFNG+874 T/A (rs2430561) and IL28B (rs12979860) C/T polymorphisms, combined and separately, and the risk of CMV replication after solid organ transplantation (SOT).

Methods: CMV-seropositive (R+) transplant patients were included and they were monitored for CMV replication for 6 months following transplantation. Allelic discrimination genotyping with TaqMan real-time PCR was realized. The association between these polymorphisms and the risk of CMV replication was analyzed using logistic regression analysis. Results: A total of 186 CMV-seropositive (R+) patients were enrolled. We observed that the combination of IFNG+874 A/T and IL28B C/T polymorphisms influences the risk CMV replication in these patients. In particular, the AT(IFNG)-CC(IL28B) combination decreased the risk of CMV replication (adjusted OR = 0.05, 95% CI = 0.01–0.39, p = 0.004). Negative and positive predictive values were 96% and 42%, respectively. No association between each polymorphism separately and CMV replication was found.

Conclusions: The AT(IFNG)-CC(IL28B) combination provides useful information for identifying R(+) SOT transplant patients who are protected against CMV replication after transplantation. Hence, the determination of these polymorphisms could be of utility in the clinical setting to guide physicians in the management of CMV infection after SOT.

P23. Prevalence of hepatitis e infection in patients with chronic renal failure on dialysis: preliminary results of a prospective study

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Introduction: Hepatitis E virus (HEV) infection is one of the most common causes of acute hepatitis worldwide. It´s main route of transmission in our environment is the consumption of contaminated foods, mainly products derived from the pig. HEV infection tends to be asymptomatic in most patients, but in immunocompromised individuals the disease has a worse prognosis. In the case of chronic renal failure patients, HEV infection is associated with nephrotic syndrome and acute rejection of the graft after transplantation. Therefore, the aim of this study is to evaluate the prevalence of HEV infection in dialysis patients and the cumulative incidence of VHE infection after renal transplantation.

Methods: CMV-seropositive patients included in the dialysis program and the waiting list of seven spanish hospitals were enrolled. A blood sample was collected in the pre-transplant and +30, +45, +60 and +90 days posttransplant. In all samples the presence of HEV was determined by RT-PCR using standard technique performed in the Laboratory of Infectious Diseases of IMIBIC.

Results: 101 patients were enrolled on dialysis, of which two patients had HEV infection (1.98%; 95% CI: 0.002% -0.069%). 22 of 101 patients were transplanted and followed up during 90 days after transplantation. The cumulative incidence of VHE infection was 4.5% (95% CI: 0.001% -0.228%).

Conclusion: In our study, the prevalence of HEV infection in pretransplant patients was 1.98%. The clinical impact and its influence on transplantation need to be evaluated.

P24. An *in vitro* kidney model of transgenic cyb5r3 over-expression reveals significant changes on autophagic pathway and mitochondrial dynamics markers

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Cytochrome b_5 reductases comprise a family of four flavoproteins (CYB5R1-4) that catalyse the reduction of cytochrome b_5 and alternative acceptors as coenzyme Q using NADH as an electron donor. Also, CYB5R are required for the elongation and desaturation of fatty acids, cholesterol synthesis and monooxygenation of cytochrome P450 enzymes, being these processes associated with protection against metabolic disorders. Previous studies have shown that increased expression of CYB5R3 in a transgenic C57BL/6J mouse line (CYB5R3-Tg) extends longevity, increases insulin sensitivity, improves regulation of glucose homeostasis, and decreases inflammation and oxidative damage. Also, CYB5R3 over-expression decreases mitochondrial membrane potential and protects against induced cancer, suggesting that CYB5R3-based strategies could be a feasible intervention to confer protection against metabolic diseases and to improve long-term health of individuals. In the face of this promising results, additional pathways and specific tissue-effects of this enzyme deserve to be studied in depth.

Autophagy pathway has been pointed out as a crucial phenomenon to explain longevity and healthy aging. By this mechanism, aged subcellular structures which accumulate molecular damage are degraded through a lysosomal pathway and the resulting products are released into the cytosol for recycling or to supply energy during starvation periods. Dysregulation of autophagy has been shown to be involved in the pathogenesis of a number of tissue-specific disorders, including renal issues, many of them closely related to aging. The importance of kidney in understanding the aging process explains why many studies have been targeted to this organ, which has been suggested as one of the major predictors of longevity.

Following these premises, we have developed an *in vitro* kidney model of CYB5R3 overexpression using immortalized mouse proximal tubular TKPTS cells. Our preliminary results in key markers of autophagy and mitochondrial dynamics, show an improvement in the autophagic flux and mitochondrial preservation in CYB5R3-overexpressing TKPTS cells.

P25. Image classification of synaptic dopamine transporters ^{123}I-Ioflupane by machine learning techniques

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Aim Apply machine learning techniques to classify functional brain imaging used in the differential diagnosis of movement disorders.

Material and methods: In this study, SPECT images obtained after iv administration of 5 mCi of ^{123}I-ioflupane in 75 patients were analysed, in order to detect alterations in the distribution of activity (presynaptic dopamine transporter).

The definitive diagnosis identified 51 % of patients with Parkinson's disease (PD) patterns, while the remaining 49 % corresponded to non-ill patients.

Images were preprocessed using the PETRA software to orient and perform spatial normalisation, and a three-dimensional 79x95x69 matrix (i.e. 517,845 voxels) was obtained for each patient.

In order to classify these images, a regularised logistic regression model using all voxels as input data was trained. The regularisation parameter was adjusted by an error estimator based on cross-validation. The model was evaluated by measuring the area under the ROC curve from a 5-fold experimental design.

Results: The number of voxels was reduced to 259 by selecting the 0.05 % of coefficients of the logistic regression model with the highest absolute value.

With these 259 voxels, the model obtained an AUC of 0.9905 with a sensitivity of 94.44 % and a false positive rate of 5.26 %.

The characteristics chosen by the model were consistent with those used by skilled diagnosticians (caudate and putamen areas). However, the model highlighted other voxels outside these regions that were relevant for the task of diagnosis.

Conclusions: The use of machine learning techniques has proved to be plausible for classification SPECT imaging of patients with PD, obtaining results similar to those of medical experts.

The model obtained has shown areas of interest to the diagnostic study of PD beyond the caudate and putamen.

P26. Influence of intra and extra personal variables over motivation, job satisfaction and health in a group of nurses working in a public hospital

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Motivation can determine the quality of nursing performance. As a result, patients´ security could be affected. Low levels of wellbeing at work and job satisfaction could be related to this fact and also affect nurses´ security and health status. This situation could be transformed in a negative effect over the patients.

The main goal of the research project was to identify factors with a relevant influence over motivation and job satisfaction in the nursing staff of the University Clinic Hospital of Valladolid. Motivation was analyzed through the engagement concept through the UWES-17 construct, (Schaufeli & Bakker, 2011). Job satisfaction was studied inside the Herzberg´s Two-Factor Theory (1959) via NTP 394 Overall Job Satisfaction Scale (War, Cook & Wall, 1979). The General health was measured with the General Health Questionnaire (Goldberg, 1979). A quantitative, descriptive, cross-sectional, correlational and comparative design was developed.

For the collection of data, nurses were requested to complete an online survey composed by sociodemographic data, general health, job satisfaction and engagement constructs. The survey was completed by 392 nurses. Data were analyzed using IBM SPSS V.23 software. Data distribution and reliability of the scales were evaluated. Besides, correlational and comparative analyses among the variables were carried out.

The results showed a significant correlation among general health, job satisfaction and engagement. Factors as the work experience, the type of shift, the salary, the destination department, having a nursing specialty or the type of continuous formation, determine engagement and job satisfaction. Besides, two of these variables have a relevant influence over general health: the type of shift and having a nursing specialty. Concerning general health, the results showed scores close to the suggested default threshold to develop health problems. In comparison with previous studies, the average level of engagement was similar, while the levels of job satisfaction were lower.

P27. Involvement of coenzyme Q in the modulation of mitochondrial physiology and autophagy by the flavonol kaempferol in an in vitro kidney cellular model

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Many antioxidant substances suppress the deleterious effects of reactive oxygen species. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a flavonoid found in many edible plants used in traditional medicine, which can behave as antioxidant or pro-oxidant in a concentration-dependent manner. At high concentrations, it can also induce autophagy and apoptosis, whereas at low concentrations it is capable of neutralizing superoxide. Recent results obtained in our research group indicate that this polyphenol produces a significant increase in the levels of coenzyme Q (Q), an endogenous quinone antioxidant, through its participation as a biosynthetic ring precursor of this molecule, particularly in kidney cells. The present study was set to determine how mitochondrial physiology, as well as the autophagy flux, were affected under the experimental conditions previously shown to increase Q, with the aim of elucidating the putative involvement of such increase in the observed effects. For that, we wanted to distinguish those effects mediated by Q by comparing the effects of kaempferol with those of p-hydroxybenzoic acid (pHB), a well-established Q ring precursor, and p-aminobenzoic acid (PABA), a competitive inhibitor of the Coq2 prenyltransferase and hence, a compound known to produce a dramatic decrease of Q levels by shutting-off its endogenous biosynthesis. The effects of combined treatments with these compounds were also assessed. Different mitochondrial oxidative stress-related parameters were measured. The study of autophagy was carried out by western blot measurements of proteins involved in this process. Our results show that Q may mediate some of the kaempferol effects on the mitochondria and autophagy in TKPTS cells at different levels.

P28. Effect of CMV infection and ageing on the expression of CD57, CD300a and CD161 on CD8+ T-cells

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Immunosenescence is a progressive deterioration of the immune system with ageing. It affects both innate and adaptive immunity limiting the response to pathogens and to vaccines. As chronic cytomegalovirus (CMV) infection is probably one of the major driving forces of immunosenescence, the aim of this study was to analyze the effect of CMV-seropositivity and ageing on the expression of CD300a and CD161 inhibitory receptors, along with the expression of CD57 marker on CD8+ T-cells from healthy donors (n = 67) stratified by CMV-serostatus and age. Peripheral blood from each subject was collected and followed by PBMCs isolation from each blood sample and CMV-specific IgG and IgM was determined by ELISA from plasma or sera of each sample. The percentage of cells expressing CD57, CD300a and CD161 was measured on CD8+ T-cells. Samples were acquired by multiparametric flow cytometry and analyzed with FlowJo v X 10.0.7 software. Boolean gating options were performed to analyze the co-expression of CD57, CD161, and CD300a markers and analysis of the phenotype profiles was performed by SPICE5.35 software. Our results showed that CD57+ and CD300+ CD8+T-cells are expanded with age in CMV-seropositive individuals, whereas CD161+ CD8+T-cells decrease. CMV latent infection is associated with the expansion of CD57+ cells, in young CMV-seropositive individuals compare to CMV-seronegative individuals. Moreover, the co-expression analysis of CD57, CD161 and CD300a on CD8+T-cells showed that CD300a is expressed either alone or in combination with CD57 or CD161, while CD161+ T-cells were CD300a+ or CD300a-. On the other hand, the differences of the phenotype profiles of CD8+ T-cells were only due to age, and not to CMV latent infection. Thus, our results show that CMV latent infection and age contribute differentially to the phenotype of CD8+T-cells, highlighting the importance of including CMV serology in any study regarding immunosenescence.

P29. HLA-B, HLA-C and KIR improve the predictive value of IL28B for Hepatitis C spontaneous clearance

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Background: The IL28B genotype has limited value in the prediction of spontaneous viral clearance (SVC) of Hepatitis C Virus (HCV). Accordingly, the identification of additional factors that improve the predictive value of IL28B may be of clinical value. The objective of this study was to analyze the impact of immunological factors, such as HLA-B, HLA-C and KIRs, on SVC as well as their additive effects on the predictive value of the IL28B genotype.

Methods: We conducted a retrospective study of HIV patients that included both SVC and chronic HCV patients. Immunological factors including IL28B, HLA-B, HLA-C and KIRs were determined for all included patients.

Results: In our study, the IL28B CC and non-CC genotypes showed a negative predictive value (NPV) and a positive predictive value (PPV) for SVC of 81.5% and 61.6%, respectively. HLA-B*44, HLA-C*12, and KIR3DS1 were identified as predictive factors for SVC, showing NPVs of 77.4%, 85.7% and 86.2%, respectively. The presence of at least one of these three markers, defined as a genetically unfavorable profile (GUP), in combination with the IL28B non-CC genotype had an NPV for SVC of 100%. The absence of the three markers, defined as a genetically favorable profile (GFP), in addition to the IL28B CC genotype had a PPV for SVC of 74.1%.

Conclusion: The combination of these markers in addition to the IL28B genotype improves the prediction of SVC or viral persistence in the context of acute HCV. Patients with an IL28B non-CC genotype and a GUP could benefit from immediate HCV treatment implementation, while the treatment of patients with an IL28B CC genotype and a GFP could be deferred in the short-term.

P30. Frequent attendance in a primary health care district

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Background. Frequent attendance (FA) in primary healthcare is a phenomenon conditioned not only by factors related to patients, but professionals and health system itself also have an important influence. Likewise it has a major impact economically as well as on quality of health attendance; thus, our aim was to describe the distribution and variability of FA in a primary healthcare district, taking into account facilities and users characteristics.

Methods. An ecological study was performed, including data from 2011 to 2015. Primary care facilities from Cordoba-Guadalquivir Health District have been taken as unit of measurement**.** Defining FA as those subjects who performed 12 or more appointments per year; independently analyzed for nursing, general practice and paediatrics. Prevalence of FA and ***frequent attender/professional ratio*** were used as main outcomes. Demographic characteristics from district population, number of health professionals and general facilities utilization have been studied. Aiming to understand FA distribution, primary health settings have been classified according to facility size and environment location (urban, suburban and rural).

Results. Data from fifty primary care facilities were analyzed. We were faced with a general population of aged users, especially in rural areas. The mean prevalence for frequent utilizers was 10.86% for nursing; general practice 21.70% and for paediatrics 16.96%. Frequent attender/professional ratios comprised from 101.07 for nursing to 239.74 for general practice. Women over used more nursing and general practitioner offices compared to men. It was observed a higher prevalence of FA in smaller settings which were located in rural areas. Although considering frequent attender/professional ratio, medium size practices presented a higher over utilization.

Conclusions. Frequent attenders represent a significant part of primary care users, meaning an important impact. Therefore it is necessary to implement more studies to understand better those factors related with their distribution among different facilities.

P31. Effectiveness of the Prehospital Emergency Service in the care of out-of-hospital cardiac arrest: systematic review and meta-analysis

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Introduction: In Spain, there are Advanced Life Support Units attended by nurses and Advanced Life Support Units attended by physicians and nurses. In other countries, the ambulance crew is composed by paramedics; or by paramedics and physicians. The type of composition of the resuscitation teams leads to the question: the presence of Physicians in out-of-hospital cardiopulmonary resuscitation influences the outcome, measured in terms of survival, return of spontaneous circulation and neurological status. Material and Methods: Systematic review in the databases Medline, Embase and Cochrane; and Meta-analysis; Period from January 1, 2006 to December 31, 2016; Study object: Scientific articles with observational study design; Keywords: "Emergency medical technicians", "Emergencies", "Emergency medical services", "Physicians", "Heart arrest", "Out-of-hospital cardiac arrest", "Death, sudden, cardiac" "Ventricular fibrillation" y "Tachycardia, ventricular". Analysis: descriptive and thematic category; completed with a meta-analysis of random effects. Results: 7 observational studies met Inclusion and quality control criteria. when the physician was present in the ambulance crew, improvement was detected in: the return of spontaneous circulation (OR 1.44, IC 95% 0,93 – 2,23; p=0,10; I2=96%), survival at hospital discharge (OR 1.19, 95% CI 0.52-2.72 p = 0.68, I2 = 68%), survival at 1 month (OR 1.50, 95% CI 1.22-1.85, p <0.001, I2 = 91%) and survival at one month with good neurological status (OR 1.36, 95% CI 0.95 - 1.94, p = 0.09: I2 = 95%). However, a better result was found in the admission of patients to the hospital with return of spontaneous circulation when the physician did not have the presence at the place (OR 0.62, 95% CI 0.37 - 1.05, p = 0.08, I2 = 75%).Conclusions: We can conclude that the presence of the physician in out-of-hospital cardiorespiratory arrest improves the patient's survival and neurological status.

P32. The biological senescence risk score. A practical tool to predict biological senescence status

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Introduction: Cardiovascular disease has been associated with aging, specifically with telomere shortening as the main markers, which is mediated in turn by oxidative stress and inflammation. We aim to develop a predictive model to evaluate the degree of biological senescence.

Matherials and methods: Relative telomere length of 1002 coronary patients from the CORDIOPREV study (NCT00924937) was determined at baseline in addition to different markers of inflammatory response (PCR, MCP-1, IL-6, IL-1B, TNF-alfa, adiponectin, resistin and leptin), and oxidative stress (nitric oxide, lipid peroxidation products (LPO), carbonylated proteins, catalase, total glutathione, reduced glutathione, oxidized glutathione, superoxide dismutase (SOD) and peroxidated glutathione. Biological senescence was considered with relative telomere length in the lower quintile and we developed predictive models using logistic regression analysis.

Results: We selected those patients with all the variables proposed to develop the predictive models (n=353). Statistically significant differences between both groups (Biological senescence vs Non-Biological senescence) were found for total cholesterol, catalase, SOD, IL1b, resistin and leptin. The model with the higher area under the curve ROC to predict biological senescence was calculated using the following variables: age, gender, body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, heart rate, HDL-c, triglycerides, hs-CRP, HOMA-IR, catalase, SOD, LPO, IL1b and resistin (AUC ROC= 0.724).

Conclusions: This predictive model allows us to analyze the degree of biological senescence and establish a pattern of personalized treatment based on clinical and biological profile.

P33. Effect of Human Platelet Lysate Supplemented Medium on Human Mesenchymal Stem Cell Identity and Immunomodulatory Capacity

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Affiliations: GC 14 Terapia Celular Human Bone Marrow derived Mesenchymal stem cells (hMSCs-MO) are promising candidates for cell-based therapies. Large scale in vitro expansion of hMSCs-MO still involves the supplementation of culture media with Fetal Bovine Serum (FBS). However, in order to avoid the risks of contaminants by FBS and xenogenic compounds, respectively, clinical grade stem cell culture should turn to other options. Human platelet lysate (PL) represents an efficient alternative to fetal bovine serum for clinical-scale expansion of MSCs.

Aim: We aimed to compare morphology, proliferation capacity, immunophenotype and immunomodulatory effect between hMSCs-MO cultured under both, FBS and PL supplemented medium.
Methods: hMSCs-MO were cultured under PL or FBS conditions and their biological characteristics were evaluated for cell therapy. A cell flow cytometer was used for determining cells size, cells complexity and immunophenotype. Proliferation rate were calculated and immunomodulatory effect was assessed by lymphocytes proliferation quantification using a co-cultured system.
Results: Under PL supplemented culture conditions, hMSCs-MO exhibited similar fibroblast-like morphology and expression patterns of surface markers than hMSCs-MO cultured under FBS supplemented medium conditions. Besides that, flow cytometer data analysis revealed significant differences in cells size and complexity, cultured with LP showed smaller size and higher complexity than hMSCs-MO cultured with FBS. hMSCs-MO had greater proliferative potential when culturing under PL supplemented medium. However, hMSCs-MO cultured under PL conditions showed a lower immunomodulatory effect compared to those cells cultured under FBS conditions.
Conclusions: hMSCs-MO under PL supplemented culture conditions, presented no change in immunophenotype, despite showed higher complexity, and smaller size than hMSCs-MO cultured under FBS conditions. In addition, hMSCs-MO incubated under PL supplemented medium resulted in higher proliferative capacity. Regarding immunomodulatory capacity both, hMSCs-MO cultured under FBS or PL conditions, were able to inhibit lymphocytes proliferation in vitro. However, this immunomodulatory effect was greater in hMSCs-MO cultured under FBS.

P34. C4D is an important diagnostic tool in membranous nephropathy

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Background and objectives. Idiopathic membranous nephropathy (MN) is the most frequent cause of the nephrotic syndrome in adults. The diagnosis is based on typical findings observed via electron microscopy (EM) and immunofluorescence (IF) studies.
Recent advances have shown that MN is a kidney specific autoimmune disease induced by antibodies specific for podocyte antigens. Complement plays an important role, even if mechanisms of activation have not been clarified yet. C4d is a fragment of C4 that is produced during activation of the classical or lectin complement pathway. We might therefore expect to find C4d deposition as a marker of complement activation in MN.
The aim of our study was to determine whether immunohistochemical detection of C4d in patients with MN could be useful as a diagnostic tool.
Design, setting, participants, & measurements. All adult patients diagnosed with idiopathic minimal change disease (MCD) and MN biopsied in our unit between January 2001-December 2016 were considered for inclusion in the study. Diagnoses of MCD and MN were based on histological assessment of renal biopsy tissue with LM, IF and EM studies. 51 patients with MCD and 91 with MN were finally included.
Results. No C4d deposition was observed in any of the glomeruli of patients with MCD, and 100% of these patients were classified as "negative". C4d was detected in 100% of patients with NM in the form of a uniform granular distribution that outlined all the capillary loops and spared the mesangium.
Detectable C1q deposits by IF were detected in only two patients with MN.
Conclusions. The demonstration of C4d by means of immunohistochemical techniques can thus be used as a tool for the differential diagnosis of MN and MCD.
The deposit of C4d and no C1q deposit suggest that the alternative and/or lectin pathways might be predominantly involved in complement activation and formation of the C5b-9 complex in MN.

P35. Mechanisms of longevity extension in cells overexpressing CYB5R3

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Aging is an irreversible and time-dependent process leading to a deterioration of cell functions, tissues and organs. In accordance with the Free-Radical Theory of Aging, redox imbalance may be an important factor in the aging process and oxidative stress is associated with accelerated aging. On the other hand, consistent with the Theory of Membranes in aging, fatty acids are cellular constituents inversely related to the level of unsaturation in membrane phospholipids. Previous studies carried out in our group have shown that NADH-cytochrome b5 reductase 3 (CYB5R3) plays an important role in aging through mechanisms that appear to be related to a decrease in oxidative damage and alterations in fatty acids composition, consistent with the two previous theories. In transgenic mice overexpressing CYB5R3, those genes related to fatty acid synthesis, as well as long chain polyunsaturated fatty acids (PUFA) levels increase considerably. Accumulation of senescent cells, alterations of proteostasis and defective cell renewal account among the recently recognized hallmarks of aging. However, the effects of CYB5R3 overexpression on cell senescence and autophagy, or the effect of aging on the modulation of cell metabolism by overexpression of CYB5R3 are largely unknown. Based on this, we have developed a cellular model of mouse embryonic fibroblasts (MEFs) that overexpress CYB5R3 to elucidate the mechanisms by which aging can be targeted ***via*** CYB5R3 overexpression. Our main aim is to study the prevention of cell senescence through an ***in vitro*** model in MEFs and to investigate the processes that are altered in CYB5R3-overexpressing cells, paying especial attention to those ways that regulate cell senescence, apoptotic signaling and autophagy.

POSTER SESSION II

Chronic and inflammatory diseases

P36. Role of different factors in methodological quality of systematic reviews and meta-analysis of studies on skin psoriasis

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Background: The exponential growth of publishing and the variable quality of evidence in scientific field such as in psoriasis may put knowledge and decision-making processes at risk. Systematic reviews (SRs) and Meta-analysis (MAs) have become the standard approach in assessing and summarizing applied health research. AMSTAR is a reliable and valid measurement tool to assess the risk of bias of these studies by analyzing their methodological quality.

Objective: To assess the influence of article metadata and bibliometric indexes on methodological quality of SRs and MAs of studies on skin psoriasis.

Methods: A systematic search and quality assessment were performed using AMSTAR tool. Protocol was registred in PROSPERO. MEDLINE, EMBASE, and the Cochrane Database were searched. Only review articles that applied systematic methods of identification, selection and analysis of psoriasis studies were included. Two authors assessed SRs and MAs quality using the same data abstraction forms and the 11-point AMSTAR criteria. The reviews were classified based on AMSTAR score as of low quality, medium quality or high quality. Metadata concerning the study and the journal were extracted. Ordinal logistic regression and Principal Component Analysis (PCA) were performed.

Results: Articles that met eligibility criteria (n=220), were classified as of high (17,2%), medium (55%), or low methodological quality (27,7%) following the AMSTAR criteria. 'Journal Impact Factor' (OR: 1.25; 95%CI: 1.07-5.83), '5-years Impact Factor' (OR: 1.24; 95%CI: 1.05-1.53), 'Article Influence Score' (OR: 14.41; 95%CI: 2.51-87.5), 'Academic or Health Institutions funding' (OR: 5.52; 95%CI: 2.45-13.09), 'Pharmaceutical Industry funding' (OR: 0.40; 95%CI: 0.20-0.77), and 'Number of authors with Conflict of Interest' (OR: 0.89; 95%CI: 0.83-0.96) significantly predicted higher AMSTAR-derived quality levels. Review studied funded by academic of health institutions scored highest in AMSTAR scale, have less authors with conflict of interest, and were mostly published in Q1 journals.

Conclusions: Nature of funding sources and the grade of independence author disclosures to perform SRs may compromise their quality, increasing the risk of methodological bias. By enhancing the efforts to systematically report clearly any funding source or author disclosure in the articles, authors and editors may aid readers to assess the quality of these reviews and to interpret evidence obtained in these articles.

P37. Anti-ds-DNA antibodies regulate atherothrombosis in Systemic Lupus Erythematosus through the induction of NETosis, inflammation and endothelial activation

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Objectives: 1. To analyse in vivo the involvement of anti-dsDNA antibodies in the development of CVD in SLE patients. 2. To evaluate in vitro the mechanisms underlying the effects of anti- dsDNA antibodies in these processes.

Methods: The study was conducted in 50 SLE patients and 38 healthy donors. Endothelial function was assessed by Laser-Doppler. Markers of oxidative stress, cytokines, prothrombotic mediators and NETosis, were quantified in purified leukocytes and plasma. Activation of intracellular pathways was analyzed in monocytes using paths-can array. In vitro, purified leukocytes from healthy donors and endothelial cells (ECs) were treated separately and in co-culture with anti-dsDNA antibodies isolated from SLE patients.

Results: SLE patients showed endothelial dysfunction and altered expression of pro-inflammatory proteins (IL6, IL8, MCP-1 and CRP), prothrombotic molecules (TF), oxidative stress markers and netosis-related molecules (NE, MPO and free-DNA). Monocytes from anti-dsDNA-positive SLE patients showed activation of pathways related to inflammation, thrombosis and apoptosis (ErK, STAT-3, p38, JNK, GSK, Bad and Caspase-3). Association studies demonstrated that those altered molecules were linked to the occurrence of thrombotic events and the presence of anti-dsDNA antibodies. In vitro treatment of purified leukocytes with anti-dsDNA antibodies promoted an increase in the production of NETosis, levels of peroxides and mitochondrial damage, as well as enlarged expression of a number of proinflammatory and prothrombotic molecules. In vitro treatment of HUVEC promoted an increase in endothelial activation molecules (ICAM-1, VCAM-1 and E-selectin). Those effects were even more pronounced when immune and ECs were cocultured.

Conclusions: 1. Positivity for anti-dsDNA antibodies is linked to an increased pro-atherothrombotic status in SLE patients. 2. Anti-dsDNA antibodies, in vitro, promote NETosis on neutrophils, apoptosis on monocytes, modulate the expression of molecules related to inflammation and thrombosis, and induce endothelial activation. Together, that data suggest the involvement of such autoantibodies on atherothrombosis development in SLE.

P38. Effect of eicosapentaenoic acid on mechanosensory and chemosensory behaviors of *Caenorhabditis elegans*

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Eicosapentaenoic acid [EPA; 20:5 (n-3)] is one of several omega-3 fatty acids linked to healthy life. EPA has a protective effect to some diseases including inflammatory diseases, cancer and diabetes. In patients diagnosed with mayor depression a decrease of omega-3 fatty acids has been observed, and this has been related with deficiencies in dopaminergic signaling.

Neurexins and neuroligins are synaptic adhesion molecules present in excitatory and inhibitory synapses. Both proteins have been found to be key factors for optimal neuronal connectivity. Mutations in these genes have been associated with autism spectrum disorder and other neuropsychiatric conditions. In *Caenorhabditis elegans* *nrx-1* and *nlg-1* genes are orthologous to human neurexin and neuroligin, respectively. In fact, in previous studies we have shown that these human genes are functional in the nematode. The *nrx-1* and *nlg-1*-deficient mutants of the nematode have impaired mechanosensory and chemosensory behaviors.

C. elegans is a significant animal model for studying EPA functions because his simple anatomy and easy handling in the laboratory and because it expresses the main enzymes involved in fatty acid biosynthesis. These include fatty acid synthase, acetyl CoA carboxylase and elongase. Previous studies in the nematode have shown that EPA can act like a signaling molecule to modulate the function of sensitive neurons AWC and ASH. EPA also facilitates the function of AWA neurons improving diacetyl chemotaxis. Furthermore, EPA could restore cholinergic signaling in acetylcholinesterase-deficient mutants.

In the present study, we show results that demonstrate that EPA can recover mechanosensory and chemosensory behaviors impaired in *nlg-1* and *nrx-1*-deficient mutants from *C. elegans*. On the other hand, in neurexin mutants an overexpression of *acdh-1*, coding for Acyl CoA DeHydrogenase involved in beta oxidation of fatty acids, was observed. This connection between the effect of EPA and the nervous system improving impairments in these mutants could help to understand physiological mechanisms by which EPA exerts his healthy activities.

P39. Dietary magnesium supplementation prevents and reverses vascular and soft tissue calcifications in uremic rats

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Although magnesium has been shown to prevent vascular calcification *in vitro*, controlled *in vivo* studies in uremic animal models are limited. To determine whether dietary magnesium supplementation protects against the development of vascular calcification, 5/6 nephrectomized Wistar rats were fed diets with different magnesium content increasing from 0.1 to 1.1%. In one study we analyzed bone specimens from rats fed 0.1%, 0.3% and 0.6% magnesium diets, and in another study we evaluated the effect of intraperitoneal magnesium on vascular calcification in 5/6 nephrectomized rats. The effects of magnesium on established vascular calcification were also evaluated in uremic rats fed on diets with either normal (0.1%) or moderately increased magnesium (0.6%) content. The increase in dietary magnesium resulted in a marked reduction in vascular calcification, together with improved mineral metabolism and renal function. Moderately elevated dietary magnesium (0.3%), but not high dietary magnesium (0.6%), improved bone homeostasis as compared to basal dietary magnesium (0.1%). Results of our study also suggested that the protective effect of magnesium on vascular calcification was not limited to its action as an intestinal phosphate binder since magnesium administered intraperitoneally also decreased vascular calcification. Oral magnesium supplementation also reduced blood pressure in uremic rats, and in vitro medium magnesium decreased BMP2 and p65-NFKB in TNF-α treated human umbilical vein endothelial cells. Finally, in uremic rats with established vascular calcification, increasing dietary magnesium from 0.1% magnesium to 0.6% reduced the mortality rate from 52% to 28%, which was associated with reduced vascular calcification. Thus, increasing dietary magnesium reduced both vascular calcification and mortality in uremic rats.

P40. Predicting Mortality in Dialysis Patients using Random Forest: Comparison with Traditional Statistical Methods

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Scientific Program: Chronic and Inflammatory diseases.
Keywords: Mortality, end stage renal disease, statistical methods.

End Stage Renal Disease (ESRD) presents an elevated incidence, with high comorbidity and mortality rates. Recently, based on artificial intelligence, new statistical tools have been used to analyse morbidity and mortality in populational groups. Random Forest (RF) is a new analysis method with the ability to process an unlimited number of variables.

The main objective of this work was to compare the predictive capacity of RF with traditional statistical methods.

An observational study was carried out using a database involving a total of 2038 patients who started dialysis between 1995 and 2015.

The prediction of mortality was calculated using two methods: COX regression and RF. The results from the two systems were compared to each other as well as to the observed mortality.

The mean age of patients was 62.3; and the mean Charlson Comorbidity Index was 4.63.

Mortality prediction models at 1, 1.5, 2 and 3 years were developed based on data at 30, 60, and 90 days after start dialysis. The ability of RF and COX to predict mortality was compared using the area under the curve (AUC) of the corresponding ROC curves. RF showed a greater predictive capacity than COX: the results were statistically significant ($p<0.05$); and the mean advantage was 5.49% (minimum 2.95%, maximum 14.78%) compared to the observed mortality.

In addition, mortality prediction at 1 year calculated using both methods was compared to the observed mortality rate: 3 groups of patients according to the Charlson Index, with RF more closely approximating the real situation.

Our results indicate that for dialysis patients, RF analysis more accurately predicts mortality than the classic model, coming closer to the real data. In conclusion, Random Forest is a useful and reliable statistical method for a high-risk population such as ESRD patients.

P41. Systematic reviews and meta-analyses of psoriasis: influence author-paper affiliation network architecture in the methodological quality

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Background: Systematic reviews (SRs) and meta-analyses (MAs) of randomized clinical trials are currently considered two of the best tools to the summarization of high-quality evidence. However, methodological bias can reduce the validity of conclusions from these types of studies and therefore impair the quality of decision making. The co-authorship is among the most well-documented forms of research collaboration, this work analyze its influence in the methodological quality.

Objetive: To explore whether authors´collaboration methods might influence the methodological quality of SRs and MAs of psoriasis.

Methods: Methodological quality was assessed by two raters who extracted information from full articles. After calculating total and per-item Assessment of Multiple Systematic Reviews (AMSTAR) scores, reviews were classified as low (0-4), medium (5-8), or high (9-11) quality. Article metadata and journal-related bibliometric indices were also obtained.

Results: A total of 741 authors from 520 different institutions and 32 countries published 220 reviews that were classified as high (17.2%), moderate (55%), or low (27.7%) methodological quality. The high methodological quality subnetwork was larger but had a lower connection density than the low and moderate methodological quality subnetworks; specifically, the former contained relatively fewer nodes (authors and reviews), reviews by authors, and collaborators per author. Furthermore, the high methodological quality subnetwork was highly compartmentalized, with several modules representing few poorly interconnected communities.

Conclusions: In conclusion, structural differences in author-paper affiliation network may influence the methodological quality of SRs and MAs on psoriasis. As the author-paper affiliation network structure affects study quality in this research field, authors who maintain an appropriate balance between scientific quality and productivity are more likely to develop higher quality reviews.

P42. How to achieve a global perceived health in patients with cardiovascular disease?

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In developed societies, and specifically in Spain, nowadays cardiovascular disease is the most prevalent chronic disease and the leading cause of death. Scientific evidence indicates that there are many psychosocial determinants that contribute as to its development as equally to its maintenance. The main aim of this research is to analyze the relationship of psychosocial variables (positivity, health specific self-efficacy beliefs and self-regulatory strategies) on perceived global health in patients with cardiovascular disease. Based on theoretical support from previous research, a model of global health was validated with a total of 449 cardiac patients. The participants answered a questionnaire assessing their level of positivity, health specific self-efficacy beliefs (for regulatory negative affect, cardiac self-efficacy and adherence to the Mediterranean diet), anxiety regulation strategies; and their level of global health. The results show adjustment indices proposed for the explanatory model suitable ($\chi 2$ (9, N = 449) = 4.69, p = .860; RMSEA = .001, 95% CI [.001, .03]); GFI = 1.00; AGFI = .99; CFI = 1.00). Thus, the model indicates that positivity, regulation negative affect self-efficacy, cardiac self-efficacy and anxiety regulation strategies are directly related to the global health perceived. These results point to the need to promote psychosocial interventions in order to increase the perception of the general health of cardiac patients and, therefore, to increase their quality of life.

P43. Genetic variants related to inflammation modulate senescence in patients with cardiovascular disease

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Objective: Telomere length has been postulated as a marker of age-related diseases due to a key role in cellular senescence. Leukocyte telomere length attrition has been associated with cardiovascular disease. There is growing evidence about the role that inflammation plays in shortening. We explore whether variability in inflammation genes locus is associated with shorter telomeres.

Material and methods: In the frame of the study of secondary prevention CORDIOPREV (1002 patients, NCT00924937) we determined telomeres length and the genotype of 45 single nucleotide polymorphisms (SNP) of genes associated with inflammation. We divided patients into tertiles based on telomeres length, and we analyzed the distribution of allelic variants in each SNP among patients with telomeres at the longest and the shortest tertile adjusting by confounders (age, sex, body mass index).

Results: Among the carriers of the mutant allele of one SNP of the F2 gene (rs3136441) and other SNP of the Caspase 5 gene (rs61751523) we reported an association with an increased risk of telomeres shortening compared to patients without mutation. F2 gene encodes the coagulation factor 2 that is involved in the coagulation cascade, stimulates inflammation and it is associated with hypercoagulability states that promotes vascular occlusion, whereas Caspase 5 is an apoptosis inducer molecule. We did not observe associations in the other 43 evaluated genetic variants.

Conclusions: Our findings suggest that certain genetic variations in genes associated with the individual inflammatory state are associated with shorter telomeres, and they may lead to an increased susceptibility to premature aging. These interactions could provide a right strategy for personalized preventive measures in CHD patients, in the context of precision medicine.

Keywords: Telomere length, polymorphisms, inflammatory state, genetic variations, premature aging.

P44. Beyond das 28 to prevent structural damage in an early heumatoid arthritis cohort

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects approximately 0.5 percent of the Spanish population. In the last years it has been verified that without specific treatment commonly results in joint destruction, decline in functional status and impairment in the quality of life, culminating in significant morbidity and increased mortality. The recently EULAR guidelines state that treatment should be aimed at reaching a target of remission or low disease activity in every patient, following a tight control protocol and adjusting therapy according to disease activity, often using disease activity score (DAS28) in every visit.
Objectives: To determine whether clinical remission at 5 years as measured by DAS 28 was associated with less structural damage.
Methods: We included 137 patients older than 18 years, diagnosed of RA (according to ACR 1987 criteria) from the early arthritis clinic of the HURS of Córdoba Rheumatology Department, between January 2003 to January 2012. Follow-up 5 years after diagnosis. The demographic, clinical, laboratory and radiological findings were collected. Continuous variables were expressed as mean (standard deviation) and qualitative variables were expressed as percentages (%). Proportions were compared using the Chi square test ($\chi 2$).
Results: The patients were mostly women (69.3%) with a mean age at diagnosis of 52.94 (14.21) years. 67.9% were RF + and 59.1% ACPA +. At the 5-year follow-up, 83 patients (60.6%) were in clinical remission (DAS 28 <2.6). When we compared the presence of erosions in the group of patients who had achieved clinical remission measured by DAS 28 <2.6 at 5 years compared to the group that was not in remission, there were no statistically significant differences in the presence of erosions (48.2% of erosions in the remission group versus 37% in the other group (p = 0.198)).
Conclusions: DAS28 has been established as a fundamental score to evaluate the activity of RA and is the main parameter used in the Treat to Target (T2T) strategy to assess the status of remission and to establish therapeutic decisions in this disease. However, normal values of DAS 28 do not exclude the presence of subclinical activity and the development of erosions which shows the need to use other scores during the follow-up in order to avoid structural damage.

P45. Importance of identifying shared epitope in order to design a therapeutic strategy in patients with rheumatoid arthritis of recent onset r

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Background: Recent research on Rheumatoid Arthritis (RA) has allowed to establish two subtypes of the disease, which can be differentiate by their serological, genetic and severity characteristics. Patients RA seropositive for cyclic citrullinated peptide antibodies (ACPA) develop severe disease. Shared epitope was localized among RA patients in 1987. The alleles coding for this sequence are HLA-DRB1 * 04 and HLA-DRB1 * 01. Subsequent publications have revealed that there is an increased risk of presenting RA in the presence of this amino acid sequence. However, in the last few years we have wondered if the presence of thissequence may also be related to the ability of patients with RA to generate ACPA.

Objectives: to determine if there are differences between ACPA levels and the presence or not of shared epitope in patients with RA of recent onset at the time of diagnosis.

Methods: A total of 101 patients with RA were included were included in the study (ACR 1987 Criteria). They derived from theRheumatology Department of Reina Sofía University Hospital (HURS) in Córdoba, Spain. Patients were classified into two groups according to whether they presented the Shared Epitope or not. Differences between ACPA levels at the time of diagnosis wereanalyzed. Mean and standard deviation of quantitative variables and frequencies and percentages of qualitative variables werecalculated. Bivariate study was performed using the Student's t-test and the Chi-square test ($\chi 2$). Data were analyzed using SPSS v17 program.

Results: Out of the 101 patients in the study, 61.4% were women with a mean age at diagnosis of 51.49 (14.57) years and only19.8% (20 patients) were smokers. Of the 101 patients, 68% were RF positive, 58% were ACPA positive and 59.4% had shared epitope. There was an association between higher levels of ACPA in patients with shared epitope [68.76 (58.69) vs 35.39 (42.46)], ($p<0.003$). Likewise, patients with shared epitope developed more erosive forms of RA at 5 years of follow-up ($p <0.021$).

Conclusions: Our results show that shared epitope is associated with high levels of ACPA, as well as that this is related to moreaggressive forms of disease. Determining the shared epitope at diagnosis may be useful in daily clinical practice in order to identify more aggressive patients and to design a more intense treatment from the beginning.

P46. Connective tissue disease-associated interstitial lung disease treated with intravenous Cyclophosphamide or Rituximab: a unicentre, open-label and comparative study

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Objective: To compare effectiveness of intravenous CYC against RTX as a treatment in patients with CTD-ILD.

Methods: Unicentre and retrospective study in which it was analyzed data from 26 CTD-ILD patients treated with intravenous CYC or RTX.

The prognostic effect of each treatment on stabilization in PFTs or HRTC was evaluated using the Kaplan-Meier method. Baseline characteristics of patients to evaluate predictive factors for the stabilization in lung function were compared by univariate logistic regression. PFTs values were compared at baseline vs. 12 months after the treatment, and direct comparison between the CYC and the RTX groups was performed at 12 months using paired T-test and T-test, respectively.

Results: From the total participants, 14/26 had a diagnosis of Systemic Sclerosis whereas 12/26 had other types of CTD; 15 patients received CYC and 11 RTX. Both groups presented similar baseline characteristics and levels in PFTs.

The Kaplan-Meier method showed an average of months without relapse in CYC and RTX group of 59.79(9.50) and 79.27(7.81) respectively (Long Rank test: p=0.253).

Patients in the CYC group presented a slight deterioration in PFTs levels during the first year of treatment; however, patients in RTX group showed an increase of all PFTs levels, although these differences were non-significatives. Direct comparison between both treatment groups after 12 months showed lower levels of all PFTs in CYC vs. RTX, been DLCO/VA (67.30% and 86.25%, respectively) statistically significative.

Conclusions: This study suggests that CYC treatment stabilizes the lung function, whereas RTX shows a tendency to improve it. However, large scale randomized controlled trials are needed to confirm these results.

P47. Circulating microRNAs as biomarkers for diagnosis and typifying the therothrombotic status in Antiphospholipid Syndrome

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Background: The course of antiphospholipid syndrome (APS) may rapidly progress from asymptomatic to severe manifestations. Thus, timely diagnosis is essential to improve accuracy of therapy.
Objective: To investigate the contribution of circulating miRNAs to the pathogenesis of APS and their potential role as non-invasive biomarkers.
Methods: Ninety APS patients and 42 healthy donors were recruited. Clinical and inflammatory parameters were analysed, along with ankle-brachial-index (ABI) and carotid intima-media thickness (CIMT). miRNA expression profiling was performed in plasma by PCR-Array, and miRNAs and target proteins associated to APS were identified using Ingenuity Pathways analysis software (IPA). Selected miRNAs and proteins were validated by RT-PCR and ELISA/Bioplex. To assess the specificity of the APS-miRNA signature, 23 thrombotic patients without associated autoimmune disease were analyzed. Healthy monocytes and endothelial cells (ECs) were treated in vitro with antiphospholipid antibodies (aPL-IgGs).
Results: The PCR-Array identified 39 miRNAs differentially expressed, including 19 up-regulated and 20 down-regulated in APS. IPA analysis recognized 11 miRNAs as potential modulators of target genes involved in APS physiopathology. Logistic Regression and ROC curve analyses identified a signature of 10 miRNA ratios as biomarkers for diagnosis of APS (AUC:0.81), and 2 miRNA ratios as biomarkers for typifying their atherothrombotic status (pathologic CIMT, AUC:0.76). Patients with thrombosis but without associated autoimmune disease displayed distinct miRNA profiles from APS patients. The miRNA signature was associated to the occurrence of foetal loss and the type of thrombosis suffered, and correlated with parameters of autoimmunity (aPL-IgG titers), inflammation, and thrombosis (ABI, ESR, TF, PAI-1, MCP-1, VEGF-A and VEGF-R1). Treatment of monocytes and ECs with aPL-IgG induced altered levels of the selected miRNAs and target proteins.
Conclusions: Circulating miRNAs in APS patients are potential novel biomarkers for diagnosis and typifying of their atherothrombotic status, thus constituting a useful tool in the prevention and management of the disease.

P48. Hospitalizations due to Ambulatory care sensitive conditions in the area of Reina Sofía Teaching Hospital, Córdoba: time trends 1999-2015

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Introduction: Ambulatory care sensitive conditions (ACSC) are admissions that might have been prevented if services had been delivered effectively in the community (due to the prevention of the onset, treatment of acute illness or control of chronic diseases).
Objectives:
To determine time trends of ACSC hospitalizations in the area of Reina Sofía University Hospital of Córdoba from 1999 to 2015.
Methods: Design: time-series analysis.
Population: All the hospital admissions from 1999 to 2015.
Source of cases: administrative data bases of hospitalizations from 1999 to 2015. ACSC cases were identified by means of ad hoc queries developed using Access and according to the Agency for Healthcare Research and Quality (Preventive Quality Indicators) criteria for ACSC.
Descriptive and Jointpoint regression results are shown.
Results: In 1999 ACSC accounted for 12.1% of all hospitalizations in the hospital. The annual average change was closed to -4% until 2012 (95%CI -4.8% - -3.5%), however the number of ACSC hospitalizations have been increasing since then (not significative). Conditions included under ACSC showing a significative descending trend are: Diabetes Short-Term Complications and Perforated Appendix (both trends in adult population). Conditions with significative rising trends are: Congestive Heart Failure, Bacterial Pneumonia and Asthma (in paediatric and adult populations).
Conclusions: Results show an inversion in trends of ACSC hospitalizations that raises concern with extra-hospital care and with specific disease management. Certain effective interventions to reduce ACSC hospitalisation should be considered.

P49. Burnout in emergency department nursing professionals: a preliminary study

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Background: High levels of burnout exist among emergency department nurses. Workers in this environment can find their engagement is gradually replaced by cynicism, emotional depletion, loss of motivation and reduced commitment, leading to a crisis in professional competence. Numerous studies outline the potential adverse outcomes of burnout for nurses, which include illness and absenteeism, staff conflict, distrust of management, poor coping and substance abuse.
Objectives: To determine the degree of burnout among emergency department nursing staff at a teaching hospital belonging to the Andalusia Public Health System in southern Spain, and to examine potential correlations between burnout levels and social variables.
Design and Method: Observational, descriptive, cross-sectional study of a sample comprising 36 emergency department nurses. An original, specific questionnaire was used to collect social data, and the Maslach Burnout Inventory was administered to all subjects. Data were subjected to descriptive and inferential statistical tests.
Results: In the study sample, 68.57% of the professionals were women. The mean age was 44.26±7.45 years. A 37.14% of nurses were smokers. The level of emotional exhaustion was 8.24 points higher among men professionals when compared to women nurses (p=0.02). As regards personal accomplishment, it cannot be said that smoking women had a higher score than those who did not smoke (p=0.15).
Conclusions: The level of emotional exhaustion and personal accomplishment are average that of depersonalization is high. The variable related to the emotional exhaustion is sex. Other variables such as age, physical exercise, children and smoking consumption are not related to the dimensions of professional burnout.

P50. Serum phosphate modifications are associated with changes in serum fgf23 and c-reactive protein

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Background: Phosphate (P) stimulates FGF23 production, both of them associated with a higher mortality risk in dialysis (HD) patients. However, it remains unclear whether reductions in serum P promotes FGF23 modifications in such population. The aim of the present study was to evaluate in HD patients whether a sustained reduction or elevation in serum P concentration is associated with changes in FGF23.

Methods: We conducted a longitudinal study in 20 stable HD patients. Ten patients had a prolonged elevation in serum P due to poor compliance and 10 additional patients were on strict sustained control of serum P. Patients were evaluated at baseline and after forty weeks. Both FGF23 molecules, intact (i-FGF23) and c-terminal (c-FGF23), parathyroid hormone (PTH), ionized calcium (iCa) and high-sensitive C-reactive Protein (CRP) were measured. FGF23, PTH and hs-CRP were log natural transformed due to skewed distribution.

Results: In patients with a reduction in serum P concentration (mean±sem, from 5.0±0.26 mg/dl to 3.3 ± 0.22, p=0.005), there was a decrease in ln-iFGF23 from 6.5 pg/mL (IQR 5.7-6.8) to 5.4 (4.9-5.8), (p=0.013) and in ln-CRP from 2.2 mg/l (1.6-2.9) to 1.6 (1.1-1.9), (p=0.005). However, c-FGF23 did not significantly decrease (p=0.059). Conversely, in patients with P increase (3.8±0.2 to 5.4±0.3, p=0.008), there was an increase in i-FGF23 from 6.5 pg/ml (5.2-7) to 7.2 (6.8-7.3) p=0.037, in c-FGF23 from 6.9 (6.8-7.3) to 8 (7.5-8.9), (p=0.005), in CRP (p=0.005) and in PTH(p=0.022). Furthermore, including all the patients evaluated, we found significant correlations between percent changes in P and iFGF23 (r=0.877, p<0.001) and between P and cFGF23 (r=0.780, p<0.001). Percent changes in iFGF23 and cFGF23 were also proportional to the change in CRP (r=0.723, p<0.001 and r=0.908, p<0.001, respectively).

Conclusions: Modifications in serum P are associated with changes in serum levels of FGF23 and CRP. Furthermore, changes in CRP and FGF23 are also closely interrelated.

P51. Effects of methotrexate, leflunomide and hydroxychloroquine on the insulin resistance and obesity associated with rheumatoid arthritis: obese mouse models of rheumatoid arthritis

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Objective: To analyze and compare the effects of methotrexate, leflunomide and hydroxychloroquine(disease-modifying antirheumatic drugs (DMARDs)) on the obesity and insulin resistance in an obese collagen-induced arthritis (CIA) mouse model.
Methods: CIA was developed in obese and lean mice.55 C57Bl/6 mice (4-5 weeks) were used. Groups of study: 5 non-diseased lean mice, 9 CIA lean mice, 5 non-diseased OB mice, 9 OB-CIA mice, 9 OB-CIA mice treated with leflunomide, 9 OB-CIA mice treated with methotrexate and 9 OB-CIA mice treated with hydroxychloroquine for 15 days. Mice were weighted and the number of total swollen digits was recorded daily. After treatment,glucose tolerance test (GTT) was performed. Buffy coat, plasma and metabolic tissues (adipose tissue, skeletal muscle and liver) were collected.
Results: CIA obese mice developed arthritis earlier and more severe than CIA lean mice. After 15 days of treatment, the therapies more effective inhibiting the generation of swollen digits were hydroxychloroquine and methotrexate. The development of RA did not have effect on the body weight. Only thetreatment with hydroxychloroquine significantly reduced the body weight. Additionally, glucose tolerance test revealed an improvement of insulin sensitivity after treatment with hydroxychloroquine. Analysis on the adipose tissue revealed that methotrexate and hydroxychloroquine induced changes in the lipid and carbohydrate metabolism. Further studies are currently ongoing in the metabolic tissues in order to completely elucidate the effect of these therapies in the metabolic state.
Conclusions: 1) Obesity accelerates the development and aggravates the outcome of the arthritis in CIA mice. 2) Among the three DMARDs administered, hydroxychloroquine promoted a beneficial effect on the metabolism of CIA obese mice, reducing body weight and improving the insulin sensitivity. These results suggest that hydroxychloroquine could be used as avaluable therapeutic strategy in RA patients to reduce the disease activity and ameliorate the metabolic complications associated.

P52. A New Data Analysis System to Quantify Associations between Biochemical

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Introduction: In hemodialysis patients, deviations from KDIGO recommended values of individual parameters, phosphate, calcium or parathyroid hormone (PTH), are associated with increased mortality. However, it is widely accepted that these parameters are not regulated independently of each other and that therapy aimed to correct one parameter often modifies the others.

Objective: The aim of the present study is to quantify the degree of association between parameters of chronic kidney disease and mineral bone disease (CKD-MBD)

Methods: Data was extracted from a cohort of 1758 adult HD patients between January 2000 and June 2013 obtaining a total of 46.141 records (10 year follow-up). We used an advanced data analysis system called Random Forest (RF) which is based on self-learning procedure with similar axioms to those utilized for the development of artificial intelligence. This new approach is particularly useful when the variables analyzed are closely dependent to each other.

Results: The analysis revealed a strong association between PTH and phosphate that was superior to that of PTH and Calcium. The classical linear regression analysis between PTH and phosphate shows a correlation coefficient is 0.27, $p<0.001$, the possibility to predict PTH changes from phosphate modification is marginal. Alternatively, RF assumes that changes in phosphate will cause modifications in other associated variables (calcium and others) that may also affect PTH values. Using RF the correlation coefficient between changes in serum PTH and phosphate is 0.77, $p<0.001$; thus, the power of prediction is markedly increased. The effect of therapy on biochemical variables was also analyzed using this RF.

Conclussions: Our results suggest that the analysis of the complex interactions between mineral metabolism parameters in CKD-MBD may demand a more advanced data analysis system such as RF

POSTER SESSION II

Cancer (Onocology and Oncohematology)

P53. Molecular characterization of reelin system in low and high-grade astrocytomas

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Reelin is a glycoprotein involved in neuronal migration and plasticity essential for the development and maintenance of brain function. In addition, reelin presence has been assiciated to tumoral pathologies, although, its role in human brain tumors is still unknown. In line with this, astrocytomas comprise a subtype of malignant gliomas graded from low to high agressiveness-features (i.e. from I to IV), being grade IV the most common and aggressive type. The objective of this study was to perform a molecular characterization of reelin and its receptors (ApoER2 and VLDLR) in a cohort of astrocytoma samples obtained from well-characterized patients (n=35: grade II (n=4), III (n=7) and IV (n=24)], and to determine the association of the expression levels of these components with clinical/pathological parameters. Specifically, a tumor piece was collected and quantitative-PCR was used to evaluate the expression levels of the reelin-system components. The results revealed a consistant higher expression of ApoER2 followed by VLDLR and reelin in astrocytoma grade-II, -III and -IV. Moreover, reelin expression was positively correlated to ApoER2 expression (in grade-III and –IV astrocytomas), and ApoER2 was also positively correlated with VLDLR in grade-IV astrocytomas. Interestingly, our data indicated that the expression of the reelin-system components seems to decrease according to a higher tumor-grade and, the expression of reelin seems to be associated to specific brain-tumor areas (i.e. higher expression in the right and left-temporal and right fronto-temporal areas in grade-IV astrocytomas). Additionally, we found a lower expression of ApoER2 in astrocytomas of men vs. women. Moreover, the expression of reelin and the tumor-grade were negatively correlated with the percentage of Ki67 positive cells. Altogether, our results suggest that some components of reelin-system could be used as novel diagnostic biomarker in brain tumors; future studies are currently in progress to fully characterize its functional role in this pathology.

P54. Urinary metabolite panels for prostate cancer detection

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The lack of specificity of the prostate specific antigen (PSA) serum level as a marker for prostate cancer (PCa) drives the search for new biomarkers for its diagnosis, preferably non-invasive biomarkers. In the present research, a comprehensive global urine analysis by LC–QTOF from 62 patients with a clinically significant PCa and 42 healthy individuals, both groups confirmed by biopsy, was applied to search for potential metabolomics biomarkers that may help to understand pathological processes related to PCa. The significant identified metabolites were alternatively combined with PSA measurements in 5-variable panels (5 metabolites or 4 metabolites+PSA) for diagnostic of PCa. The panels were prepared to maximize sensitivity, specificity or accuracy (sensitivity+specificity). The main goal of the panels was to propose a screening tool to complement other diagnostic tests to reduce the false positive cases, which means to reduce the rate of individuals subjected to invasive biopsy. In addition, the panels were characterized by high sensitivity outcomes, which means that they can also detect true positive cases. In fact, the best results achieved in terms of sensitivity and specificity were 97.7% and 100%, respectively. Thus, our diagnostic models suggest that urine metabolomic profiling may have potential for clinical diagnosis of PCa.

P55. Biguanides: an emerging new therapeutic option for aggressive brain tumors

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Gliomas constitute the most frequent type of brain tumors which are characterised by a rapid growth and high diffusion through the brain. In particular, astrocytomas are a subtype of malignant gliomas which are graded from low to high aggressiveness (i.e. grade I, II, III and IV), being grade IV (glioblastoma multiforme, GBM) the most malignant and one of the most common cancers in the brain and CNS. To date, surgery is the first-line therapy combined with chemotherapy or radiotherapy; however, about two-thirds of patients do not have a survival rate greater than two years after diagnosis. Accordingly, identification of new strategies in these tumoral pathologies deem necessary to provide clues for novel therapeutic targets. Biguanides such as metformin (MF; commonly used to treat type-2 diabetes), buformin (BF) and phenformin (PF) have been shown to exert antitumor actions in different tumor types; but, their direct actions in brain tumor cells have not fully investigated. Therefore, the aim of this study was to elucidate the direct effect of different biguanides (i.e. MF, BF and PF at different doses) on key functional parameters such as cell-viability, modulation of the expression of key genes or signaling pathways, etc. Specifically, tumor samples of grades III and IV astrocytomas were collected, dispersed into single cells, cultured and treated with MF, BF and PF. Biguanides treatment significantly reduced cell viability in a dose-dependent manner by a Ca^{2+}-independent mechanism, being the effect of MF lower than BF and PF. Moreover, treatment with MF reduced the expression levels of key genes important in the pathophysiology of brain tumors (i.e. down-regulation of sst2, sst5 and VEGF and up-regulation of DR2T). Altogether, our results suggest that treatment with biguanides may represent a novel clinical tool for the therapeutic treatment of patients with high-grade astrocytomas.

P56. Quantification of Leukemic Stem Cell compartments in AML is a good predictor of progression of disease

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Acute Myeloid Leukemia(AML) is a clonal disorder that modify normal hematopoiesis by neoplastic transformation and clonal expansion of malignant hematopoietic stem cell and progenitors(HSC/P)though changes in genome, epigenome and phenotype markers(Dohner H, 2015). Extensive efforts from many groups are trying to reveal the origin of transformed clone sustaining leukemia but the absence of specific markers on leukemic stem cell(LSC) makes difficult their isolation. Pioneer studios in mouse revealed that LSC from AML is contained within $Lin^{-}CD34^{+}CD38^{-}$ (Dick JE, 1994)compartment and into Side Population(SP)(Goodell MA 1996)compartment. In human, LSC frequency is related with minimal residual disease(MRD)and worse event-free survival in paediatric AML.

In this study, we quantify LSC compartments in 66total patients. 54patients received intensive chemotherapy and only42 reached complete remission(CR). Finally, 17 patients relapsed. As control, we used mononuclear cells from 5 healthy donors. To determine LSC compartments we did flow cytometry using proper markers to identify malignant stem cells. We included chi^2-test, Fisher-test, Student's t-test or Mann-Whitney test and ANOVA. Interestingly, the expression of surface markers on SP cells were heterogeneous and variable among the stage of the disease. Donors-derived SP cells were$CD34^{-}CD45^{-}$. However, AML-derived SP cells were$CD34^{-}$ in most of CR specimens and by contrast SP cells were$CD34^{+}$at diagnosis and relapse specimens. In addition, we found that the percentage of SP cells at diagnosis(0.34%, range 0.01-2.3%) were directly proportional to $CD34^{+}CD38^{-}$ compartment(P=0.015) and higher in patients who did not achieve CR(P=0.007) as well as in patients achieving CR but who presented MRD post induction(P=0.023). Finally, Kaplan-meier curves showed that SP cells were associated with worse Disease Free Survival(DFS) specially in those SP cell expressing CD34.

Herein, we conclude that the presence of LSC-$CD34^{+}$in AML at diagnosis is related to lower CR, clearance of MRD post induction with an impact on the outcome of the AML patients.

P57. Face to face, accuracy and variability comparison between ERPSC and PCPT risk calculator in patients with PSA less than 10ng/ml for clinically significant Prostate cancer

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Objective: To perform a comparison in the prediction of significant PCa (sPCa) between European Randomised Study for Screening of Prostate Cancer (ERSPC) risk calculator (RC) and Prostate Cancer Prevention Trial (PCPT) RC in patients with PSA <10ng/ml by evaluating their accuracy and variability between two consecutive measurements of serum PSA levels.

Methods: An observational study was carried out in patients from the ONCOVER cohort during 2013-2015 (n=1021). All patients had two consecutive serum PSA values before prostate biopsy. PCa and sPCa probabilities were calculated with the two PSA values using ERSPC 3/4+DRE and PCPT v2 + free-PSA RCs. Comparisons for the prediction accuracy of sPCa, by calculating the area under the curve (AUC), were made. Moreover, calibration, discrimination and decision curve analysis (DCA) were studied. Variability between both PSA values and RCs agreement were evaluated using Kappa-test analysis.

Results: A total of 603 patients were analyzed (92 were diagnosed with sPCa). The median PSA varied from 5,2 to 4,9ng/ml between both measures. Both RCs overestimated the risk in the interval of high-risk probabilities, but ERSPC seemed to be better calibrated than PCPT in probabilities under 60%. Discrimination ability for sPCa was similar between models with an AUC of 0.73(0.68-0.79) for ERSPC-RC vs. 0.76(0.71-0.82) for PCPT-RC. DCA also showed that the net benefit was comparable between the two RCs. ERSPC-RC showed less variability than PCPT-RC, with a constant agreement (k=0.7-0.8) for usual range of clinical decision (0-0.3). Finally, our data indicate that a higher number of biopsies could be avoided using the ERSPC-RC but more sPCa could be lost.

Conclusions: Our data indicate that both RCs had similar accuracy for discrimination of sPCa; however, ERSPC-RC had better calibration and stability for intraindividual PSA variations. Finally, a higher rate of biopsies could be avoided with the ERSPC but losing higher rate of sPCa.

P58. Establishment and Molecular Characterization of Patient-derived xenograft (PDX) Models for Colorectal Cancer

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Background and Aims.- Patient-derived xenograft (PDX) models are emerging as useful tools for drug screening, biomarker development, and the preclinical evaluation of personalized medicine strategies. PDX models retain the histopathological and molecular characteristic of their primary tumor and, importantly, they have been shown to be predictive of patient responses to treatment and clinical outcomes. On the other hand, recent studies have identified distinct molecular subtypes of colorectal cancer with evident clinical relevance. Therefore, the aim of this study was, first, the establishment and characterization of PDX models for colorectal cancer (CRC) and, second, their molecular subtyping using immunohistochemistry (IHC) in order to confirm the preservation of the inter-tumoral and intratumoral heterogeneity, as well as the phenotypic and molecular characteristics of the original cancer.

Material and Methods.- Twenty-one fresh surgical tumor samples from CRC patients were subcutaneously transplanted into NOD/SCID mice. Clinicopathological data were recorded for each enrolled patient. Hematoxylin and eosin staining, IHC staining for five markers (CDX2; FRMD6; HTR2B; ZEB1 and KER) and microsatellite instability were performed and scored according to the established criteria.

Results.- Eighteen PDXs models were successfully established, representing an engraftment rate of 85%. The mean latency period (from the day of inoculation to palpable tumor) of xenografts was ± 30 days. With the increase in serial passage number (P1; P2 and P3), the latency period was shorter (7-14 days). The histopathological analysis demonstrated a high concordance between primary tumors and their corresponding xenografts that was maintained between different passages. According to the molecular subtyping of tumors by IHC, PDXs maintained similar expression for the tested markers compared to their parental primary tumors.

Conclusions.- PDXs models of CRC were successfully established and they faithfully recapitulated the molecular diversity, cellular heterogeneity, and histology seen in CRC patient tumors.

P59. Potential effect of biguanides and statins in neuroendocrine tumors

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The incidence of type 2 diabetes (T2DM), hypercholesterolemia and metabolic syndrome is increasing in the general worldwide population and in patients with endocrine-related tumoral pathologies. Recent studies have suggested that biguanides and statins have beneficial effects on various tumor types; however, the particular direct effects and the molecular mechanisms underlying this pathophysiological association are still not well understood in neuroendocrine tumor (NETs).

Objectives: 1) To analyze putative associations between T2DM/hypercholesterolemia treatment with the evolution, prognosis and molecular profile of key endocrine regulatory systems (such as somatostatin/ghrelin-systems) in patients with gastroenteropancreatic (GEP)-NETs (n=100) or lung carcinoids (LCs; n=81); 2) To investigate the potential direct effects of different biguanides (metformin/phenformin/buformin) and statins (simvastatin/atorvastatin/lovastatin/rosuvastatin) on the aggressiveness of NET-cells by evaluating proliferation, migration, secretion, apoptosis and gene-expression in BON1/QGP1 cell-lines.

Results: GEP-NET surgical resection was uncomplete in most T2DM, which may be probably related to a higher tumor size tendency (3,42 cm vs. 2,5 cm; p=0,06); these patients expressed higher SST levels. In lung carcinoids, T2DM patients had lower mortality rate and lower pleura invasion. Any other relation between statins, epidemiological, histological and prognosis variables or molecular expression of ghrelin/SST-systems was observed. Metformin/buphormin/phenformin treatment decreased proliferation-rate in NET cell-lines (at 24/48/72h). However, the effects of statins on proliferation rate were statin-type, cell-type and time dependent. Specifically, only simvastatin/atorvastatin decreased proliferation-rate in BON1-cells (48/72h and 48h, respectively) while, all statins decreased proliferation-rate in QGP1-cells (48/72h). Remarkably, metformin/simvastatin also decreased migration-capacity and increased apoptosis in BON1-cells and, metformin/phenformin reduced serotonin secretion in NET-cells. These antitumor effects were likely mediated by altered expression of key genes involved cancer aggressiveness (i.e. Ki-67, pttg, p53, insulin-R, etc.).

Conclusions: Our results revealed a clear inhibitory effect of biguanides and statins on NET-cell aggressiveness. Given the demonstrated clinical safety of biguanides/statins, our results suggest a potential therapeutic role of these compounds for the treatment of patients with NETs.

Keywords: biguanides, statins, neuroendocrine tumors

P60. Metabolic syndrome and prognostic factors in postmenopausal breast cancer patients

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Objective: To investigate the relationship between metabolic syndrome (MetS), present at the time of diagnosis, and different known prognostic factors of breast cancer (BC) in postmenopausal patients.

Methods: 168 postmenopausal patients with recent diagnosis of postmenopausal BC. We determined whether patients had metabolic syndrome according to classical criteria of ATPIII-NCEP, at diagnosis, and their presence was linked with different well known pathological prognostic factors of breast cancer.

Results: 90 (53.57%) patients met the criteria for MetS at the time of diagnosis and 78 (46.43%) did not. The mean BMI was 28.94 ± 4.74 (30.09 ±4.45 kg/m2 in the MetS group vs. 27.31 ± 4.71 Kg/m2 in the group without MetS, p=0.008). The average ICC was 0.87 ±0.08; 0.88±0.09 in the MetS group vs 0.84±0.06 in the group without MetS; p=0.026). Most of the patients with MetS meet three criteria (68.88%). The most prevalent diagnostic criteria for MetS were the hipertriglicerinemia (86.66%), hyperglycemia (80%), and central obesity (72.22%). We found: A higher proportion of undifferentiated tumors (grade 3) (27.77% vs. 3.84%, p = 0.03), higher percentage of lymphovascular invasion (25.86% vs. 10.0%, p = 0.04), higher rates of Ki-67 positivity (94.11% vs 80.0%, p= 0.03) and their levels, although these were not statistically significant (31.28 vs. 25.47, p =0.30), higher tumor ER (-) (31.11% vs 8.98%, p=0.04) and triple-negative tumors (TN) (34.48% vs 4.76%, p=0.03) among the patients with metabolic syndrome than in group without MetS.

Conclusion: There is a high prevalence of MetS (53.57%) in our postmenopausal patients with BC. The presence, at the time of BC diagnosis, of metabolic syndrome was associated with a more aggressive tumor phenotype in these patients. Diagnosing MetS and BC at the same time may be a key factor for determining prognosis, metastatic potential and adjuvant treatment of choice for these types of tumors.

P61. Integration of tomosynthesis in the population breast screening: detection and recall rates. Is it time to come up with new work strategies?

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Purpose: to evaluate the performance of breast tomosynthesis compared with conventional 2D mammography in terms of breast cancer detection rates and recalls reduction in a population-based screening program.

Materials and Methods: prospective study including women between 50 and 69 years participating in the breast screening program between January 2015 and December 2016. Once the patient has accepted the informed consent, a conventional 2D mammography and a tomosynthesis are performed. After these tests, a synthesized 2D mammography is also obtained. Three interpretation models are carried out by the radiologists: 1) conventional 2D mammography; 2) tomosynthesis + synthesized; 3) tomosynthesis + synthesized + 2D. Six radiologists read independently and blindly each interpretation model.

Results: 16.067 participants with a median age of 57´59 years. We detected 98 breast cancers in 97 participants (one participant had bilateral cancer), because we present data for 16.068 screens. Of the 98 cancers, 70 were detected at both double reading 2D and double reading tomosynthesis (4´3/1.000 screens). 6 cancers were detected with only 2D (76, 4´7/1.000 screens) compared with 22 detected with only tomosynthesis (92, 5´7/1.000 screens). The incremental cancer detection rate attributable to tomosynthesis was 17´4% (95%IC: 10´0-24´8%, p=.004). Tomosynthesis plus synthesized only detected 18 additional cancers (87, 5´4/1.000, increase in 12´6%, p=.043) and tomosynthesis plus 2D only detected 16 additional cancers (81, 5´0/1.000, increase in 6´2%, p=.442). 1.196 women were recalled (7´4%), 323 were recalled at both 2D and tomosynthesis. 487 recalls with only 2D (810 recalls, 5´04%) compared with 386 recalls with only tomosynthesis (709 recalls, 4´4%). The decrease recall rates attributable to tomosynthesis were 12´5% (95%IC: 6´0-19´0%, p = .001). 482 recalls occurred at tomosynthesis plus synthesized (decrease in 40´5%, p<.001) and 450 recalls occurred at tomosynthesis plus 2D (decrease in 44´5%, p<.001).

Conclusion: the use of tomosynthesis in a screening program results in a significantly higher cancer detection rate and decreased recall rate. Synthesized may replace 2D to avoid a higher dose of radiation. Single reading of tomosynthesis has shown a higher detection rate and reduction recalls rate to double reading 2D.

P62. Relationship of neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and lymphocyte-monocyte ratio with the progression and recurrence of non-muscle-invasive bladder cancer

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Introduction: Nowadays, the role of inflammation in tumor progression has been under discussion, playing an important role in the prognosis of cancer. Thus, the objective of this study was to analyze the association between inflammation, measured as neutrophil / lymphocyte ratio (NLR), platelet / lymphocyte ratio (PLR) and lymphocyte / monocyte ratio (LMR) and the behavior of high grade non-muscle-invasive bladder cancer (NMIBC), measured as free-recurrence survival (FRS), free-progression survival (FPS), overall survival(OS) and cancer specific survival (CSS).

Material and methods: We retrospectively analyzed 65 patients, diagnosed between 2009 and 2013 of primary CVNMI cT1G3, treated with BCG protocol. The ratios were calculated with the absolute values of the presurgical hematimetry. They were categorized using cut-off points calculated with ROC curves for RNL, RPL and RLM. Kaplan Meier survival tests were performed, and multivariate Cox regression was adjusted for the following clinical-pathological variables: Re-RTU, multifocality, tumor size and in situ carcinoma.

Results: High values of NLR (> 2.6) were significantly associated with worse OS (p = 0.004). It was independent of the clinical-pathological variables studied and the progression in the multivariate analysis (p = 0.002).

High values of PLR (> 102) were associated with lower OS (p = 0.026), worse FPS (p = 0.024) and worse FRS (p = 0.030). In the multivariate analysis, this tendency persisted, although without reaching statistical significance (p = 0.100, p = 0.090 and p = 0.050, respectively).

A LMR> 5 was associated with a lower FPS (p = 0.031) and CSS (p = 0.050), behaving as an independent risk factor in the multivariate analysis (p = 0.041 and p = 0.048 respectively).

Conclusions: Presurgical LNR, LPR and MLR are a low-cost, safe and accessible tool that could provide prognostic information in patients with non-muscle-invasive bladder cancer.

P63. Advanced feature extraction and machine learning models to melanoma and Breslow index detection

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Melanoma is a type of cancer that develops from the pigment-containing cells known as melanocytes. Usually occurring on the skin, early detection and diagnosis is strongly related to survival rates. In the present work, we propose a system combining image analysis and machine learning to detect melanoma presence and severity. The severity is assessed in terms of melanoma thickness, which is measured by the Breslow index. Previous works have mainly focused on the binary problem of detecting the presence of melanoma, while the proposed system goes a step further by including also the stage of the melanoma into the classification task.

The images are preprocessed to extract 100 features considering the shape, colour, pigment network and texture of the benign and malignant lesions. The features designed for characterizing melanomas are based on clinical findings in the literature.

From the machine learning perspective, the problem is tackled as a five-class classification problem, where the first class represents benign lesions and the remaining four classes represent the different stages of the melanoma (measured by the Breslow index). Based on its definition, we identify the problem as a partial order problem in which the lesions of these four categories (melanoma stages) show an order relationship, but where no order arrangement is found with respect to the benign lesion category. Under this assumption about the data topology, we design several proposals to exploit this structure and build better models, experimentally confirming the partial order hypothesis.

The experimental study is conducted with clinician-curated images from the Interactive Atlas of Dermoscopy. In the melanoma detection problem we obtain 86.61/90.5% of sensitivity/specificity, whereas in the five-class problem we achieve 66.5% of accuracy and 0.777 of averaged mean average error, this last metric showing that the mean error is below one category in the ordinal scale.

P64. Determination of RAS mutational status in metastatic colorectal cancer patients using liquid biopsy

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Anti-EGFR monoclonal antibodies should only be considered for treatment of metastatic colorectal cancer (mCRC) patients whose tumors are wild type for RAS genes. FFPE (formalin-fixed paraffin-embedded) tumor tissue is currently used as Standard of Care for RAS testing, however, it is an invasive process and could involve some risks for patients. Liquid biopsy, which is based on the analysis of cell free DNA (cfDNA), could solve these problems. In patients with cancer, a fraction of cfDNA is tumor derived and is termed circulating tumor DNA (ctDNA). Because tumor-specific alterations in ctDNA are not present in normal cells, they offer an exquisitely sensitive and specific approach for cancer detection. The assay of ctDNA allows the analysis of tumors that are in areas of difficult access and lesions with insufficient material available for genotyping. Also, heterogeneity, both intratumoral and between primary and metastases, could be solved through liquid biopsy. The objective of this study was to determine the level of concordance between plasma and tissue RAS mutation status in patients with mCRC. RAS testing was performed on 62 plasma samples using the highly sensitive technology BEAMing (beads, emulsion, amplification and magnetics), which is based on digital PCR, and compared with data of corresponding FFPE tumor samples. The prevalence of RAS mutations detected in plasma and in tumor tissue was equal (66,67%). The positive agreement between plasma and tumor RAS status was 88,8% (32/36), the negative agreement was 91,67% (22/24), and the overall agreement (concordance) was 87,09% (54/62). The high concordance between plasma and tissue results demonstrates that BEAMing is a blood-based RAS mutation test with comparable capacity to detect RAS mutation as tissue-based RAS determination. Therefore, testing of RAS mutational status in plasma is a powerful tool for the diagnosis and clinical management of mCRC patients, that could replace tumor tissue analysis.

P65. Development of a diagnosis tool for colorectal cancer based on gut microbiota

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Introduction: Colorectal cancer (CRC) is one of the most common cancers in developed countries. In the last few years, several studies have suggested that the development of CRC may be related with changes in gut microbiota. Based on this, we hypothesized that gut microbiota composition may be useful as a tool for diagnosis of CRC. Our study is focused in the development of a screening test for the people in risk, valid and safe, improving the screening prognosis. This microbiota-based diagnosis tool would be more specific and sensitive than the current method, fecal occult blood. In fact, our method would be able to distinguish whether fecal occult blood is due to fissures, not related with CRC (which are positive for fecal occult blood, false positive) and is more sensitive (sometimes polyps do not leak blood).

Objective: We aimed to assess whether a bioinformatics model is able to discriminate between cases with polyps and CRC, based in the gut microbiota composition data.

Materials and Methods: A total of 65 patients with CRC and 65 with adenomas (polyps) were included into the study. Feces samples were frozen and DNA extracted by the QIAamp®DNA Stool Mini Kit commercial kit. We analyzed the gut microbiota composition by 16S ribosomal RNA gene (V3 and V4 region) sequencing with the Illumina platform. Sequence analysis was performed by the Metagenonic 16S software.

Results: The receiver-operating characteristic (ROC) analysis showed a high yield distinguishing between polyps and CCR patients (area under curves 0.928, and 95% CI 0.880 and 0.975).

Conclusion: Our results suggest that microbiota composition may be used as a tool for diagnosis of CRC. In addition, our diagnosis tool could be directly performed in the Illumina platform, which is already in use for many hospitals through all the national geography.

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