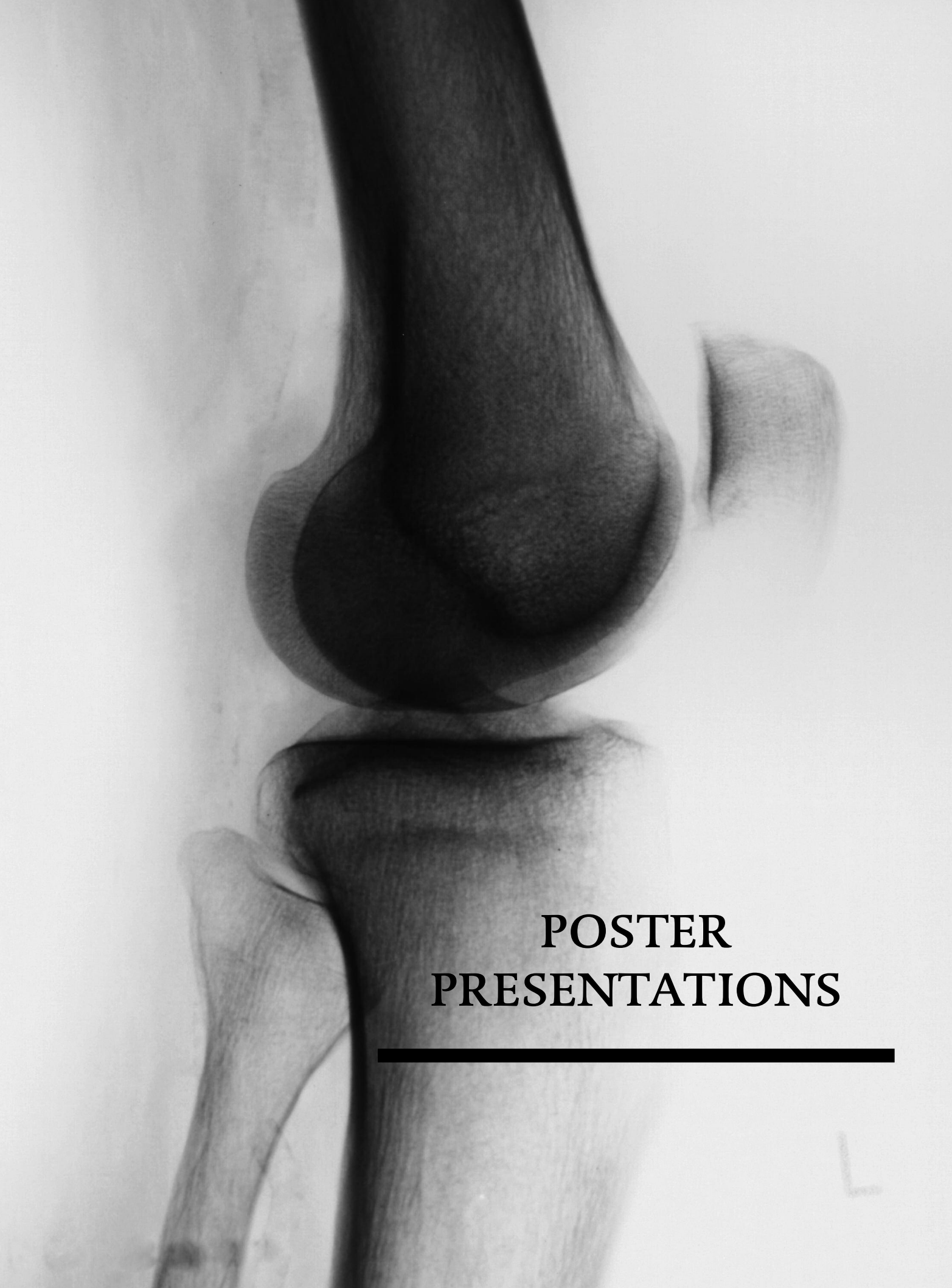

# POSTER PRESENTATIONS

# Poster Presentations

ACTA REUMATOL PORT. 2014:39:27-49 (SUP)

## P1

### EPISTATIC INTERACTION OF *ERAP1* AND HLA-B IN BEHÇET DISEASE IN THE SPANISH POPULATION

Montes-Cano M.A., Conde-Jaldón M., García-Lozano J.R., Ortiz-Fernández L., Ortego-Centeno N., González-León R., Espinosa G., Graña-Gil G., Sánchez-Bursón J., González-Gay M.A., Barnosi-Marín A.C., Solans R., Fanlo P., Rodríguez Carballeira M., Camps T., Castañeda S., Martín J., González-Escribano M.F.
Servicio de Inmunología, IBiS, HU Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, Spain

**Introduction:** Behçet's disease (BD) is a multifactorial disorder associated with the HLA region. The ERAP1 gene has been proposed as a susceptibility locus with a recessive model and with epistatic interaction with HLA-B51. ERAP1 trims peptides in the endoplasmic reticulum to optimize their length for MHC-I binding. Polymorphisms in this gene have been related with other immune-mediated diseases.
**Aim:** To replicate the association described in the Turkish population between ERAP1 (rs17482078) and BD and to analyze four additional SNPs (rs27044, rs10050860, rs30187 and rs2287987) associated with other diseases related to HLA class I and the haplotype blocks in this gene region.
**Materials and Methods:** A total of 362 BD patients and 460 healthy controls were genotyped using real-time PCR.
**Results and Discussion:** Frequencies of the homozygous genotypes for the minor alleles of all the SNPs were increased among patients and the OR values were higher in the subgroup of patients with the HLA-B risk factors, although the differences were not statistically significant. Linkage disequilibrium among these 5 SNPs was found in the Spanish population and four haplotypes (named H1 to H4) with frequencies >0.05 were detected. The rs27044G, a risk factor for AS, tags the haplotype H2, whereas the rs17482078T, described as a risk factor in BD, and also the rs100504860T and rs2287987C tag the haplotype H3. The three haplotypes containing mutations: H2, H3 and H4 were slightly more common among patients. The presence of the same set of mutations in both chromosomes increased the OR values from 4.51 to 10.72 in individuals carrying the HLA-B risk factors ($p<10^{-5}$).
**Conclusions:** Our data were consistent with an association between *ERAP1* and BD as well as with an epistatic interaction between *ERAP1* and HLA-B in the Spanish population.

## P2

### INDUCTION OF LONG-TERM TOLERANCE TO FACTOR VIII IN HEMOPHILIC A MICE

Costa-Santos I., Oliveira V., Graça L.
Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal

The development of inhibitory immunoglobulins to human recombinant factor VIII (hFVIII) is a major complication of hemophilia A therapy, affecting approximately 30% of patients with severe and moderately severe forms. Protocols for immune tolerance induction (ITI) have been assessed, in patients and animal models, to control the production of inhibitors, but all lacked consistently effective results.

Mucosal tolerance has been described as a strategy to induce tolerance to orally or nasally introduced antigens. Although most studies exploring mucosal tolerance have focused on oral tolerance induction, we consider the nasal route as more attractive as it requires less antigen and may be more effective with children. We used full FVIII and combination of rapamycin that is known to boost regulatory mechanisms that support tolerance induction while reducing the risk of immune activation following antigen administration.

Our preliminary data has shown intranasal exposure to hFVIII can prevent the generation of FVIII-inhibitors in FVIII-deficient mice, leading to long-term tolerance to multiple infusions of FVIII over three months. We are now investigating the impact of nasal tolerance induction in pre-sensitized mice, as well as using mice with

key genetic defects to establish the cellular and molecular mechanisms underlying induction of mucosal tolerance to FVIII.

## P3

### IL-9 EXPRESSION BY INVARIANT NATURAL KILLER T (INKT) CELLS IS IMPRINTED OUTSIDE THE THYMUS

Monteiro M.[1,2], Almeida C.F.[1,2], Agua-Doce A.[1,2], Graca L.[1,2]

1. Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon
2. Instituto Gulbenkian de Ciências, Oeiras, Portugal

Invariant Natural Killer T (iNKT)-cell development in the thymus has been shown to lead to distinct committed effector lineages, namely NKT-1, NKT-2 and NKT-17. However, nothing is known about the functional commitment of iNKT cells towards IL-9 expression. Here we report that although iNKT cells isolated from the thymus of naïve mice do not express IL-9, stimulation in presence of TGF-b and IL-4 induces polarization of iNKT cells towards IL-9 secretion. The same effect was observed in human iNKT cells stimulated under a similar cytokine conditions. Acquisition of IL-9 production was observed in different iNKT subsets defined by CD4, NK1.1 and Nrp-1, indicating that distinct functional subpopulations are receptive to IL-9 polarization. Factors regulating IL-9 expression in iNKT and CD4 T cells are similar, suggesting they share common mechanisms of IL-9 induction. Importantly, adoptive transfer of an enriched IL-9$^+$ iNKT cell population lead to exacerbated allergic inflammation in the lungs upon intra-nasal immunization with house dust mite. This suggests that IL-9-producing iNKT cells are able to mediate pro-inflammatory effects in vivo, namely granulocyte and mast-cell recruitment to the lungs. Taken together, our data show that peripheral iNKT cells retain the capacity of shaping their function in response to environmental cues, namely TGF-β and IL-4, adopting distinct functional characteristics.

## P4

### WHOLE-EXOME SEQUENCING AS A DIAGNOSTIC TOOL FOR SEVERE IMMUNODEFICIENCIES

Wikström A.C.[1], Marits P.[1], Sundin M.[2], Wirta V.[3], Stranneheim H.[4], Omazic B.[1], Uzunel M.[1], Thunberg S.[1], Hauzenberger D.[1], Winqvist O.[1], Wedell A.[3,4]

1. Clinical Immunology and Transfusion Medicine
2. Division of Pediatrics
3. Clinical Genomics, Science for Life Laboratory, Stockholm, Sweden
4. Centre for Inherited Metabolic Diseases (CMMS), Karolinska University Hospital

**Introduction:** Severe primary immunodeficiencies (PID) are life-threatening disorders that often require hematopoietic stem cell transplantation. Early transplantation is important for patient outcome. Whole exome sequencing (WES), has enabled an improved diagnostic procedure for PIDs, with rapid identification of mutations in candidate genes. We here report the first eight patients analyzed by WES.

**Materials and Methods:** Genomic DNA from eight patients was prepared from EDTA blood, fragmented to an average length of 300 bp and exome enriched using the SureSelect XT Human All Exon v5 technology (Agilent). Sequencing was carried out to average 150-fold coverage using PE 2x100 bp sequencing (Illumina HiSeq 2500). The bioinformatic analysis was carried out using the Mutation Identification Pipeline MIP (Stranneheim, submitted). Results were presented in an interactive browser-based visualization tool (Scout), developed in-house. The clinical interpretation of the results was restricted to a predefined set of 233 genes known to be involved in primary immunodeficiencies. The candidate variants were then evaluated in relation to clinical presentation and confirmed by Sanger sequencing in relevant cases.

**Results:** In a 2½ year old girl with progressive SCID, compound heterozygote mutations in DCLRE1C (Artemis) were identified. In a boy, presenting with T-B-SCID, a non-sense mutation in IL2RG (common γ-chain), generating a premature STOP codon in exon 7, was found. In the remaining six patients, the identified exonic sequence variants were not considered diagnostic.

**Discussion:** The MIP, combined with Scout, made WES feasible for clinical diagnostics. However, downstream evaluation of identified sequence variants remained demanding. In ambiguous cases, confirmatory functional assays are mandatory.

**Conclusions:** Using a selective WES approach, causative genes were identified in 25% of cases. With a continuously increasing number of genes implicated in PID, exome sequencing has the potential to great-

ly improve diagnosis in these patients.

## P5

### DENSE GENOME-WIDE DNA METHYLATION ANALYSIS IN MONOZYGOTIC TWINS WITH INFLAMMATORY BOWEL DISEASE AND PRIMARY SCLEROSING CHOLANGITIS

Selmi C.[1,2], Barlassina C.[2,3], Cavaciocchi C.[1,2], Ceribelli A.[1,2], Barcella M.[3], Miozzo M.[2], Annese V.[4], Pacini G.[4], Cusi D.[2,3], Danese S.[1], Gershwin M.E.[5]
1. Humanitas Research Hospital
2. University of Milan
3. Filarete Foundation
4. University of Florence, Italy
5. University of California, Davis, USA

**Introduction/Aim:** Ulcerative colitis (UC), Crohn's disease (CD), and primary sclerosing cholangitis (PSC) represent the three main manifestations in the spectrum of chronic inflammatory diseases of the digestive tract, and intestinal and biliary involvements frequently coexist. Genome-wide association studies demonstrated some degree of shared genomic susceptibility for these diseases, but UC and CD disease concordance rates in monozygotic twins are largely incomplete and this supports a possible role of epigenetic changes.
**Materials and Methods:** We herein took advantage of a unique series of monozygotic twins with UC (3 discordant pairs), CD (2 concordant, 2 discordant pairs), and PSC (1 concordant, 1 discordant pair) and performed a dense 450k DNA methylation array. We utilized the newest Illumina Infinium Human Methylation 450K BeadChip (Illumina Inc.). Pathway analysis was performed to arbitrarily select candidate genes to be investigated for methylation differences by pyrosequencing. The analysis included UCSC genome browser, Ingenuity pathway analysis, and enrichment and disease pathway analysis.
**Results:** Based on the detected differentially methylated loci we identified 32 differentially mehtylated genes (DMG) and determined their functional pathway and disease enrichment for all conditions. Seven DMG in association with CD (C20orf195), UC (FAM153C, LHFPL2, GOLGA2), and PSC (GSTT1, RAP2B, BAZ2B) were arbitrarily selected following pathway analysis to be further investigated by pyrosequencing. We confirmed a significant difference in CpG island methylation for the BAZ2B gene in PSC discordant twins but could not confirm the methylation differences of other candidate genes.
**Conclusions:** Our data suggest that different DNA methylation of candidate genes such as BAZ2B may play a role in the susceptibility to chronic inflammatory diseases of the digestive tract.

## P6

### GENE EXPRESSION PROFILING IN PERIPHERAL BLOOD MONONUCLEAR CELLS AND MODULATION OF T AND B CELLS FOLLOWING INTRAVENOUS IMMUNOGLOBULIN THERAPY IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

Dolcino M.[1*], Patuzzo G.[2*], Barbieri A.[2], Rizzi M.[2], Beri R.[2], Argentino G.[2], Ottria A.[3], Lunardi C.[2], Puccetti A.[1,3]
1. Institute G. Gaslini, Genova
2. University of Verona
3. University of Genova, Italy
*Equal contributors

**Introduction:** Regular intravenous immunoglobulin treatment is used to replace antibody deficiency in primary immunodeficiency diseases; however the therapeutic effect seems to be related not only to antibody replacement but also to an active role in the modulation of the immune response. Common variable immunodeficiency is the most frequent primary immunodeficiency seen in clinical practice.
**Methods:** We have studied the effect of intravenous immunoglobulin replacement in patients with common variable immunodeficiency by evaluating the gene-expression profiles from Affimetrix HG-U133A. The gene array results were validated by real time RT--PCR and by the measurement of circulating cytokines and chemokines by ELISA. Moreover we performed FACS analysis of blood mononuclear cells from the patients enrolled in the study.
**Results:** A series of genes involved in innate and acquired immune responses were markedly up- or down-modulated before therapy. Such genes included CD14, CD36, LEPR, IRF-5, RGS-1, CD38, TNFRSF25, IL-4, CXCR4, CCR3, IL-8. Most of these modulated genes showed an expression similar to that of normal controls after immunoglobulin replacement. Real time RT-PCR of selected genes and serum levels of IL-4, CXCR4 before and after therapy changed accordingly to gene array results.

FACS analysis showed a marked decrease of CD8+ T cells and an increase of CD4+ T cells following treatment. Moreover we observed a marked increase of $CD23^{-}CD27^{-}IgM^{-}IgG^{-}$ B cells (centrocytes).

**Conclusions:** Our results provide further support to the hypothesis that the benefits of intravenous immunoglobulin therapy are not only related to antibody replacement but also to its ability to modulate the immune response in common variable immunodeficiency.

## P7

### PEPTIDOGLYCAN RECOGNITION PROTEIN 4 AS A NEGATIVE REGULATOR OF IL1BETA

Shrivastav A.[1], Conrad C.[1], Baal N.[2], Wachtendorf A.[2], Dziarski R.[3], Reppe K.[1], Witzenrath M.[1], Hackstein H.[2], N'Guessan P.[1], Suttorp N.[1], Aly S.[1]
1. Charité University, Dept. of Internal Medicine, Infectious Diseases and Pulmonary Medicine, Berlin
2. Justus-Liebig-University, Institute for Clinical Immunology and Transfusion Medicine, Gießen, Germany
3. Indiana University School of Medicine Northwest, Gary, Indiana, USA

**Introduction:** Peptidoglycan recognition proteins (PGLYRPs) are a novel group of antimicrobial peptides and in mammals these proteins have been reported to have bactericidal properties.

**Aim:** To assess the role of these proteins in a murine infection model with *Streptococcus pneumoniae*, a major causative agent of pneumonia.

**Materials and Methods:** Wild type (WT), PGLYRP 3 knock out (PGLYRP 3 KO) and PGLYRP 4 knock out (PGLYRP 4 KO) mice (SPF) were infected with *Streptococcus pneumoniae*. Post-infection the bacterial load and the immune response in the lungs were analyzed.

**Results:** Determination of the bacterial load in the lungs revealed that there was a striking decrease in the bacterial load obtained from the lungs of PGLYRP 4 KO mice when compared to the WT and PGLYRP 3 KO mice. FACS analysis of the lungs showed minor differences in the recruitment of cells between WT, PGLYRP 3KO and PGLYRP 4KO mice. Interestingly in the absence of PGLYRP 4, neutrophils infected with *Streptococcus pneumoniae* showed an increased expression of IL1beta.

**Discussion:** Although PGLYRPs have been reported in other infection models to exhibit bactericidal activity, in our infection model the absence of PGLYRP 4 resulted in a significantly stronger clearance of *Streptococcus pneumoniae* when compared to the WT mice. Our initial results indicate an anti-inflammatory function of this protein. This anti-inflammatory activity seems to involve the down regulation of IL1beta.

**Conclusion:** This activity of negative regulation of IL1beta by PGLYRP 4 has not been reported thus far. Hence, this needs to be further investigated, as it might help in identifying new pathways involved in the regulation of innate immunity of the lung.

## P8

### PRO-RESOLVING MEDIATORS MODULATING APC METABOLISM TOWARD A TOLEROGENIC PROFILE ALLOWED TREATMENT OF INFLAMMATION IN COLLAGEN-INDUCED ARTHRITIC MICE

Bonnefoy F., Vallion R., Couturier M., Daoui A., Perruche S.
INSERM UMR1098 - EFS BFC - University of FC, Besançon, France

Pro-resolving mediators produced by macrophages eliminating apoptotic effector cells during the resolution of inflammation have been shown to stop inflammation, favor tissue repair and return to homeostasis. In immune-mediated inflammatory diseases, in which resolution is defective, one may believe that reintroducing pro-resolving mediators would allow the control of the disease. Therefore, we evaluated the pro-resolving capacities of the soluble factors issued from the culture of macrophages eliminating apoptotic cells. The effect of this supernatant, called SuperMApo, was evaluated i) on conventional and plasmacytoid dendritic cell (cDC and pDC) and macrophage maturation before or after TLR-ligand stimulation, ii) T cell polarization and iii) *in vivo* in the treatment of collagen-induced arthritis (CIA).

Macrophages, cDC and pDC submitted to SuperMApo demonstrated robust insensitivity to TLR ligand-induced activation, particularly in terms of co-stimulatory molecules expression.

In addition, once stimulated by TLR ligands, activated cDC, pDC and macrophages demonstrated a strong decrease of costimulatory molecule expression suggesting that SuperMApo treatment both prevented from TLR-induced activation and counteract TLR-in-

duced activation. SuperMApo-incubated cDC, pDC and macrophages were then cultivated with naïve $CD4^+CD25^+$ RAG/OTII T cells in an $OVA_{323-339}$-specific assay, and pDC and macrophages facilitated a solid Treg polarization of naïve T cells, whereas cDC limited Th1 polarization. Similarly, spleen cells cultured with SuperMApo demonstrated a strong Treg induction. Finally, CIA mice received 1 injection of SuperMApo and demonstrated a significant long term decrease of CIA. This was associated with a higher suppressive function of Treg, fewer sensitivity of cDC, pDC and macrophages to TLR ligands and acquisition of a pro-tolerogenic profile (induction of Treg) by the latest. Such pro-tolerogenic properties of APC followed metabolism modification by SuperMApo. Our study demonstrated that macrophages eliminating apoptotic cells produced pro-resolving mediators affecting APC metabolism therefore allowing the control of inflammatory disease.

## P9

### EFFECTS OF THE CHINESE HERBAL MIXTURE *BAIZHU HUANGQI* TANG ON THE EXPRESSION OF CYTOKINES IN LPS-STIMULATED U937 CELLS

Fan D.P.[1], Bian Y.Q.[1], Nikles S.[2], Bauer R.[2], He X.J.[1], Lu A.P.[1,3]

1. Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medicine Sciences, Beijing, China
2. Institute of Pharmaceutical Sciences, Dept. of Pharmacognosy, University of Graz, Graz, Austria
3. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China

**Introduction:** *Baizhu Huangqi Tang* (BHT), an ancient prescription, consists of *Atractylodes macrocephala*, *Astragalus membranaceus* and *Glycyrrhiza uralensis* with the proportion of 10:7:3. It has been used for some chronic inflammatory diseases such as ulcerative colitis. However, the active constituents and mechanism of action are still unknown.

**Objective:** To explore the effects of different BHT extracts on expression of TNF-α, IL-1β, IL-4 and IL-6 in LPS-stimulated U937 cells.

**Methods:** The mixture of BHT has been extracted successively with dichloromethane (DCM), ethylacetate (EtOAc), n-butanol and water. U937 cells were pretreated with PMA (10 ng/mL) for 48 hours to induce

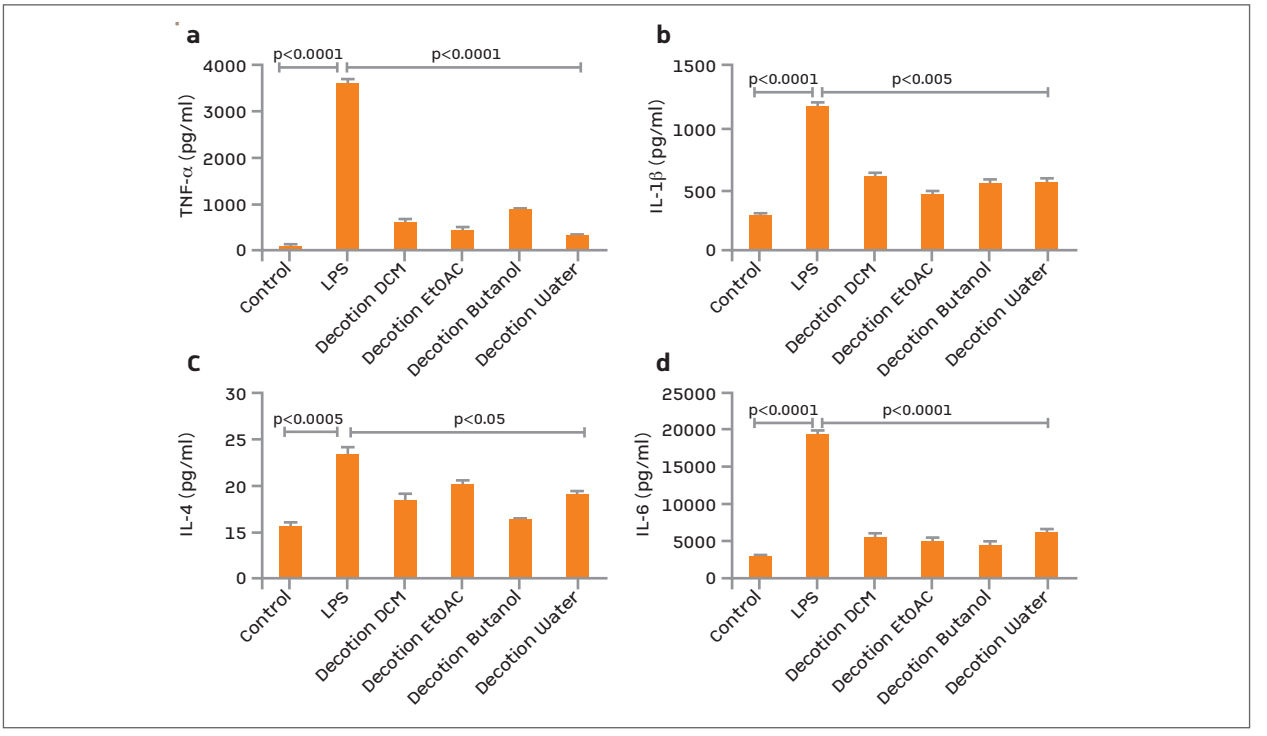


**FIGURE 1.** Fig.1 (a-d) Effects of different BHT extracts on LPS-induced cytokine production in U937 cells

macrophages. Then, different concentrations of the BHT extracts were added. CCK-8 assay was used to evaluate the viability of U937 cells. Furthermore, U937 cells induced by PMA were stimulated with LPS (1 g/mL) with or without appropriate concentration of different BHT extracts for 48 hours. The supernatant was collected, and TNF-α, IL-1β, IL-4 and IL-6 was assessed by ELISA.

**Results:** The CCK-8 assay showed that different BHT extracts didn't exhibit cytotoxicity at the concentrations of 25-100 g/ml. Subsequently, the concentration of 25 g/ml was used for the further experiments. We found that expression of TNF-α, IL-1β, IL-4 and IL-6 was significantly increased in the supernatant of LPS--stimulated U937 cells compared to the control group. Various BHT extracts could significantly lower the levels of TNF-α, IL-1β, IL-4 and IL-6 compared with the LPS group (Fig. 1).

**Conclusion:** The mixture of BHT fractionated with four solvents of different polarity could inhibit the production of TNF-α, IL-1β, IL-4 and IL-6 in U937 cells stimulated by LPS and therefore showed anti-inflammatory effects. This indicates that a range of constituents are responsible for the effect.

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## P10

### ANTI-INFLAMMATORY AND ANTI-ANGIOGENESIS EFFECTS OF TRIPTOLIDE IN RATS WITH COLLAGEN-INDUCED ARTHRITIS

*Zheng K.[1,2], Li J.[1,2], Zhang C.[1], Lu A.P.[1,3], He X.J.[1]*

1. China Academy of Chinese Medicine Sciences, Institute of Basic Research in Clinical Medicine, Beijing
2. School of Life Science and Engineering, Southwest Jiaotong University, Chengdu
3. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China

**Objective:** To explore the anti-inflammatory and anti-angiogenesis effects of Triptolide in collagen-induced arthritis (CIA) rats.

**Methods:** Forty males SD rats were randomly divided into four groups (n=10): normal group, model group, Triptolide high dose group (TH) and Triptolide low dose group (TL). Arthritis was induced by a standard protocol with type II bovine collagen in incomplete Freund's adjuvant. On day 15 (when arthritis had become established), rats were given different treatments for 28 days as follows: TH group (18.62 g/kg), TL group (9.31 g/kg), normal group (physiological saline) and model group (physiological saline). Paw swelling was assessed every three days until the rats were killed; serum were collected for cytokines assay, and joints were separated for histology and immunohistochemistry analysis.

**Results:** Treatment with high dose and low dose of Triptolide after the onset of arthritis significantly reduced the severity of CIA rats. Histological analysis demonstrated that Triptolide also significantly reduced the inflammatory responses and cartilage damage in the joint tissues. Systemic levels of IL-17A, IL-1β and TNF-α were significantly decreased in rats treated with Triptolide when compared with the model rats. Moreover, fewer expressions of HIF-1α and VEGF were detected in the synovium after treatment with Triptolide.

**Conclusion:** These results demonstrated that Triptolide had anti-inflammatory and anti-angiogenesis effects in CIA rats.

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## P11

### FOXP3+ INKTREG CELLS PROMOTE LOCAL IMMUNESSUPRESSION LEADING TO LONG-TERM SURVIVAL OF INTRA-PORTAL ISLET ALLOGRAFTS IN DIABETIC MICE

Almeida S.C.P., Graca L.

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

**Introduction/Aim:** Currently, prevention of transplant rejection relies on immunosuppressive drugs with systemic effects. We found Foxp3 expression can be induced in invariant NKT (iNKT) cells leading to immunosuppressive Foxp3$^+$iNKT regulatory (iNKTreg) cells. When adoptively transferred i.v. iNKTreg cells preferentially accumulate in the liver. We tested the hypothesis that iNKTreg cells can be used as a cellular

therapy supporting organ-restricted immunosuppression.

**Materials and Methods:** Splenic iNKT cells were isolated with CD1d-loaded-tetramer as $Tet^{+}TCRb^{int}CD25^{-}CD2^{-}$ from Foxp3-huCD2 reporter mice. Upon *in vitro* activation, using aCD3, aCD28 and TGF-b, $Foxp3^{+}$iNKTreg cells were sort purified and transferred into streptozotocin (STZ)-induced-diabetic mice transplanted with OVA-expressing islet allografts through the intra-portal route.

**Results:** We confirmed $Foxp3^{+}$iNKTreg cells administered i.v. migrate into the liver. Furthermore, adoptive transfer of $2.5x10^{5}$ $Foxp3^{+}$iNKTreg cells led to prolonged survival of islet allografts, with restoration of normo-glycemic levels (around 200 mg/dl) in some animals. In contrast, mice treated with control $Foxp3^{-}$iNKT cell populations or in the absence of cellular therapy rapidly rejected islet allografts and developed hyperglycemia.

**Discussion:** $Foxp3^{+}$iNKTreg cells supported prolonged survival of islet-allografts in STZ-induced-diabetic mice. The intra-portal route of OVA-islet administration is clinically relevant, as it is one of the routes of choice in many clinical trials, where the transplanted islets are indeed located within the liver.

**Conclusions:** Our results confirmed iNKTreg cells, due to their preferential hepatic accumulation, act as a cellular therapy inducing an immunosuppressive environment in the liver.

## P12

### ARTHRITIS INDUCES EARLY BONE HIGH TURNOVER, STRUCTURAL DEGRADATION AND MECHANICAL WEAKNESS

Vidal B.[1], Cascão R.[1], Vale A.C.[3], Cavaleiro I.[4], Vaz M.F.[3,5], Brito J.A.[4], Canhão H.[1,2], Fonseca J.E.[1,2]

1. Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon
2. Rheumatology Dept., Lisbon Academic Medical Centre
3. ICEMS, Instituto Superior Técnico, University of Lisbon, Lisbon
4. Instituto Superior de Ciências da Saúde Egas Moniz – Campus Universitário, Quinta da Granja, Caparica
5. Dept. de Engenharia Mecânica, Instituto Superior Técnico, UL, Lisbon, Portugal

**Background:** We have previously found in the chronic SKG mouse model of arthritis that long standing (5 and 8 months) inflammation directly leads to high collagen bone turnover, disorganization of the collagen network, disturbed bone microstructure and degradation of bone biomechanical properties. The main goal of the present work was to study the effects of the first days of the inflammatory process on the microarchitecture and mechanical properties of bone.

**Methods:** Twenty eight Wistar adjuvant induced arthritis (AIA) rats were monitored during 22 days after disease induction for the inflammatory score, ankle perimeter and body weight.

Healthy nonarthritic rats were used as controls for comparison. After 22 days of disease progression rats were sacrificed and bone samples were collected for histomorphometrical, 3 point bending and energy dispersive X ray spectroscopical analysis. Blood samples were also collected for bone turnover markers and cytokine quantification.

**Results:** AIA rats had an increased bone turnover (as inferred from increased P1NP and CTX1, $p = 0.0010$ and $p = 0.0002$, respectively) and this was paralleled by a decreased mineral content (calcium $p = 0.0046$ and phosphorus $p = 0.0046$). Histomorphometry showed a lower trabecular thickness ($p = 0.0002$) and bone volume ($p = 0.0003$) and higher trabecular separation ($p = 0.0009$) in the arthritic group as compared with controls. In addition, bone mechanical tests showed evidence of fragility as depicted by diminished values of yield stress and ultimate fracture point ($p = 0.0061$ and $p = 0.0279$, respectively) in the arthritic group.

**Conclusions:** We have shown in an AIA rat model that arthritis induces early bone high turnover, structural degradation and mechanical weakness.

## P13

### EFFECTS OF LIGUSTRAZINE ON RHEUMATOID ARTHRITIS BASED ON NETWORK PHARMACOLOGY RESEARCH

Bian Y.Q.[1,2], Fan D.P.[1], Niu X.Y.[1], Jiang M.[1], He X.J.[1*], Lu A.P.[1,3]

1. China Academy of Chinese Medicine Sciences, Institute of Basic Research in Clinical Medicine, Beijing
2. Shanghai University of Traditional Chinese Medicine, Shanghai
3. School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

**Introduction:** Ligustrazine, an extract from a herb

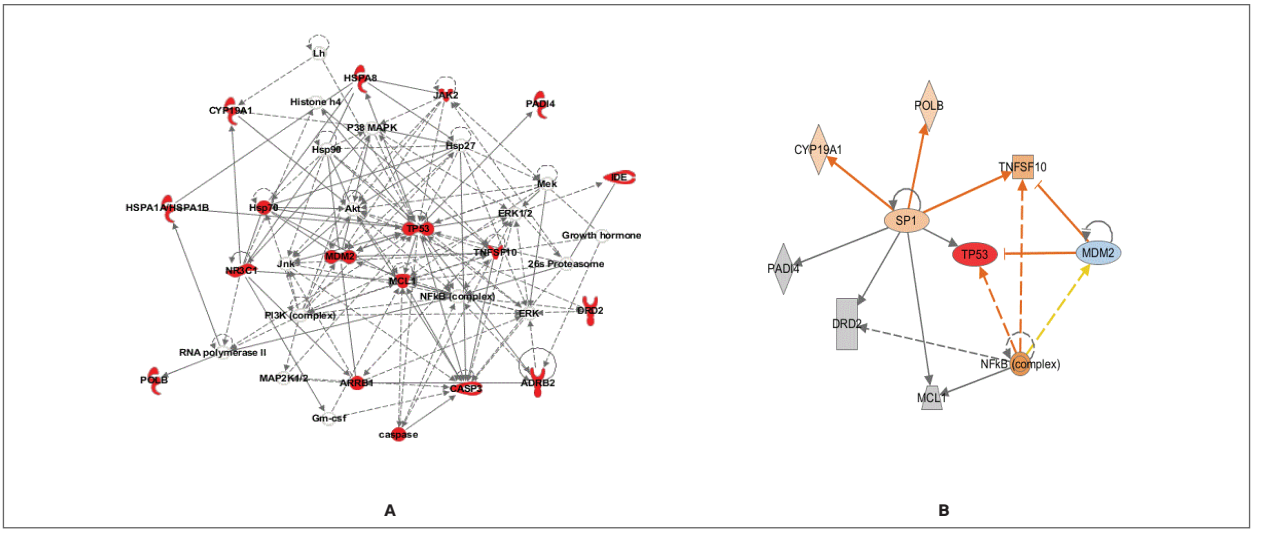


**FIGURE 1.** The shared molecular network between RA human genes and Ligustrazine human protein targets
**A)** Shared molecular network based on the shared 20 molecules between RA human genes and Ligustrazine human protein targets by IPA software; Red nodes represented the shared 20 molecules between RA human genes and Ligustrazine human protein targets
**B)** The shared upstream regulator network between RA and Ligustrazine. Orange or blue nodes represented activated or inhibited molecules regulated by TP53 respectively

*Szechuan Lovage Rhizome* (*Rhizoma Chuanxiong*), is a clinical common used drug for cerebrovascular disease and proved to have an anticoagulation effect. Whether Ligustrazine can be used to treat RA is still largely unknown. Therefore, this study aims to predict the potential effects and mechanism of Ligustrazine on RA by network pharmacology methods.

**Materials and Methods:** The human protein targets of Ligustrazine were searched in PubChem database. The obtained data were imported into bioinformatic platform to build and analyze the pharmacological networks of Ligustrazine on RA.

**Results:** Twenty protein targets of Ligustrazine were found and shared with RA. The 4 top biological function of Ligustrazine protein targets network were found, including blood coagulation, signal transduction, inflammation and angiogenesis. TP53, MDM2, MCL1 and TNFSP10 were the key nodes in the protein targets network of Ligustrazine on RA (Fig 1A).

Further assay revealed that the shared upstream regulator TP53 protein was important in molecular network regulation of Ligustrazine on RA treatment (Fig 1B).

**Conclusion:** This study indicated that Ligustrazine might play a role in RA therapy by inhibiting blood coagulation, inflammation and angiogenesis. TP53 might a potential target of Ligustrazine in RA treatment.

## P14

### SLEEP DISTURBANCES IN RHEUMATOID ARTHRITIS: RELATIONSHIP WITH THE DISEASE SEVERITY AND PSYCHO-EMOTIONAL STATUS

Sikalo J.K., Stanislavchuk M.A.
Vinnytsia National Medical University, Internal Medicine Chair #1, Vinnytsia, Ukraine

The **aim** of our study was to evaluate sleep disturbances and the related variables in patients with rheumatoid arthritis (RA).

**Methods:** One hundred forty six RA patients and seventy-one healthy controls were enrolled in the study. The average age of patients was 49.9±12.9 years, there were 80.1 % of women, duration of disease was 8.7±7.2 years. Disease activity was assessed through the Disease Activity Score (DAS28) and the Clinical Disease Activity Index (CDAI) scales. Functional disability was assessed using the Health Assessment Questionnaire (HAQ) scale. Evaluation of the sleep disturbance was measured by semi-structured clinical interview using the Visual Analog Scale (VAS), the Insomnia Severity Index (ISI) and Subjective sleep characteristics scale. Psychometric testing was including abbreviated form MMPI (Mini-Mult).

**Results:** The patients with RA had significantly higher scores in all sleep disturbances scales. The incidence of severe and very severe sleep disorders in patients with RA according to the ISI were in 6.5 times higher compared to the healthy control group. According to the results of Spearman's analysis, there was a significantly correlation between all sleep disturbances scales and DAS28 ($r_S$=0.38-0.42, p<0.01), CDAI ($r_S$=0.37--0.40, p<0.01), number of tender joints ($r_S$=0.29-0.32, p<0,01), number of swollen joints ($r_S$=0.33-0.39, p<0.01), VAS pain ($r_S$=0.24-0.27, p<0.01), HAQ ($r_S$=0.18-0.24, p<0.05). Also we defined that RA patients had significantly correlation between severity of sleep disorders and scales of the MMPI score such as hypochondriasis (Hs) ($r_S$=0.18-0.25, p<0.05), depression (D), ($r_S$=0.16-0.19, p<0.05) hypomania (Hy) ($r_S$=-0.19-0.22, p<0.05) and schizophrenia (Sh) ($r_S$=0.19-0.20, p<0.05).

**Conclusion:** For patients with RA the main factor of sleep disturbances is high disease activity and pain. Related changes in psycho-emotional status are not decisive in the genesis of sleep disorders.

## P15

### SEX DIFFERENCES IN PSORIATIC DISEASES CLINICAL FEATURES AND DRUG SURVIVAL

Selmi C.[1,2], Generali E.[1,2], Carrara G.[3], Scirè C.A.[3]
1. Rheumatology, Humanitas Research Hospital
2. University of Milan
3. Italian Society for Rheumatology, Italy

**Introduction/Aim:** Psoriasis (PsO) and Psoriatic Arthritis (PsA) affect both sexes equally, different from other autoimmune diseases. Aim of this study was to find sex differences in disease characteristics.

**Materials and Methods:** The data were extracted from the Regional Health Databases from 2004 to 2013 and patients with PsO and PsA identified with International Classification of Disease, Ninth Revision, Clinical Modification (ICD9-CM) codes 045.696.0 and 696.1 assigned by specialists. PsA (n=2799, 53.2% women) and PsO (n=761, 50.3% women) cases were included in the analysis. The collected data have been linked to methotrexate (MTX) prescriptions (ATC code L01BA01). Drug survival was defined as the time between first and last prescriptions. The influence of demographic, clinical, and therapeutic factors on drug survival was evaluated with the Cox-proportional hazard model and the results are presented as hazard ratio (HR) and 95% confidence interval (95%CI).

**Results:** Statistically significant differences were observed between women and men in age at diagnosis of PsA (women 55.6 ±12.5 years, men 53.1 ± 12.7, p<0.001), but not PsO (women 57.9 ± 14.1 years, men 56.2 ± 14.5, p=0.05). No statistically significant difference was observed in disease duration between women and men in both PsA (women 3.9±3.8 years, men 3.7±3.9, p=0.11) and PsO (women 3.5±4.5 years, men 3.8±4.4, p=0.10). In this population, MTX has been prescribed to 48%(363/761) of PsO and 77% (2168/2799) of PsA cases and, in both populations, women received significantly less MTX prescription than men (in PsO 40% vs. 56%, p<0.001, in PsA 74% vs. 82%, p<0.001). Male sex was also associated with MTX drug survival, adjusted for age at diagnosis and disease duration (HR 95%CI 1.14 [1.05, 1.24]).

**Conclusion:** Women are significantly older at PsA diagnosis, receive less frequent MTX prescription and manifest shorter drug survival. This may reflect different views by physicians.

## P16

### CELL-SURFACE EXPRESSION OF CHEMOKINE RECEPTORS CCR1 AND CCR2 IN PB B-LYMPHOCYTES OF PATIENTS WITH RHEUMATOID ARTHRITIS, OSTEOARTHRITIS, AND B-CELL LYMPHOPROLIFERATIVE DISORDER

Kholodnyuk Holodnuka I.[1], Spaka I.[1], Kadisa A.[1,2], Studers P.[3], Vilkaite A.[1], Spaks A.[1], Gravelsina S.[1], Rivkina A.[1,2,4], Lejniece S.[2,4], Lajnieks A.[2,4], Murovska M.[1]
1. A. Kirchenstein Inst. of Microbiology and Virology
2. Dept. of Internal Diseases
3. Research Lab. of Traumatology and Orthopedics
4. Riga East University Hospital, Riga Stradins University, Riga, Latvia

**Background:** In the synovial tissue of patients with rheumatoid arthritis (RA), there is abundant expression of chemokines-ligands of CCR1 and CCR2. Experiments in rodent models show that collagen-induced arthritis is accelerated when CCR2 is genetically inactivated. B-cells are involved in severity of RA by production of auto-antibodies and aberrant antigen presentation however the role of CCR1/CCR2 is largely unknown.

**Objective:** To examine the CCR1/CCR2 expression on PB B-lymphocytes of patients with RA and osteoarthritis (OA) in comparison with healthy individuals (HI) and patients with B-cell lymphoproliferative disorder (LPD) to verify whether their expression play a role in the severity of RA.
**Patients and Methods:** PB samples were obtained from 10 patients with RA, 8 with OA, 12 with B-cell LPD, 2 patients with acute infections (AI), and 12 HI. CCR1/CCR2 expression was studied by 6-color FC. Presence of viral DNA (HHV6 and parvovirus-B19) was examined by nested-PCR. B19 infection status was analyzed by RecomLine (IgG and IgM) tests. Cytokines were assayed by ELISA.
**Results:** In HI and CLL patients, PB B-cells were CCR1/CCR2-negative. In RA patients, CCR1/CCR2 were expressed on PB B-cells of patients with high level of RF (>200 U/mL) only, both on mature and naïve B-cells. Expression of CCR1/CCR2 correlated with the increased level of IL-10 (>300 pg/mL) and IL-6 (>200 pg/mL) in plasma. Active HHV6 infection was identified in two OA patients. In contrast to other OA patients, in these two, the increased number of CCR1+/CCR2+ PB B-cells was detected (25/17 % and 30/12 %). PB B-cells in AI also expressed CCR1/CCR2.
**Conclusion:** CCR1/CCR2 expression on PB B-cells is found in the cases of infection and/or inflammation.

**ACKNOWLEDGEMENT**
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## P17

### SEVERE XEROSTOMIA IN MEN: A DIAGNOSTIC CHALLENGE

Martins A.S., Nunes M., Palmela P., Salvado F.
Oral Surgery and Stomatology Dept., Hospital de Santa Maria, Lisbon, Portugal

A 59-year-old man with hypertension and dyslipidemia was sent to our Oral Medicine Consult, with a three month history of moderate xerostomia, history of bilateral enlargement of submaxillary glands six months before with self improvement, malaise, moderate eosinophilia, weight loss of 24kg in the last 8 months and an admission to Pneumology department due to acute respiratory 3 months ago. Oral examination revealed moderate xerostomia, ulcerative eritroplasic lesion on the hard palate. At 4-week after biopsy, xerostomia was severe with mucosal ulcerations and incipient dental caries. Primary results of pathology revealed epithelial atrophy of minor salivary glands, chronic inflammatory lymphoplasmacytic infiltrate with eosinophils; follicular lymphoid hyperplasia, periductal fibrosis; and one imunohystochemistry Cytomegalovirus (CMV) positive macrophage. Plasma serum results revealed 1060 eosinophils/microL, IgG4 on serum of 461mg/dL, IgM+ and IgG+ for CMV, with negative HIV tests. A definitive diagnosis of IgG4-related disease was finally made with a more than 50 per cent IgG4+/IgG plasmocytes ratio and more than 10 IgG4 cells per high-power field on pathology sample. Xerostomia is a frequent complaint with different etiologies and a higher prevalence in women. IgG4-related disease has a low prevalence and its association with xerostomia and constitutional symptoms like malaise is not common. In contrast with other autoimmune diseases that it mimicks, it has a male predominance, with the exception of IgG4-related disease with isolated affection of salivary or lacrimal glands. Diagnostic criteria include serum IgG4>135mg/dL and organ-specific histopathological criteria. Differential diagnosis with concomitant local causes of local enlargement and lymphocytic infiltrate should be made, especially concerning its association with higher prevalence of solid cancer and lymphoma.

## P18

### TRIPTOLIDE MIGHT TREAT RHEUMATOID ARTHRITIS BY REGULATING PROINFLAMMATORY RESPONSE THROUGH TREM1 SIGNAL PATHWAY

Bian Y.Q.[1,2], Niu X.Y.[1], Jiang M.[1], Zhang C.[1], He X.J.[1], Lu A.P.[1,3]
1. Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medicine Sciences, Beijing
2. Shanghai University of Traditional Chinese Medicine, Shanghai
3. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China

**Background:** Triptolide (TP), a diterpenoid triepoxide from *Tripterygium wilfordii Hook F*, is widely used to treat autoimmune and inflammatory diseases, especially rheumatoid arthritis (RA). However, the underlying molecular mechanisms of TP on RA is still largely unknown.

Therefore, this study is to overview the molecular

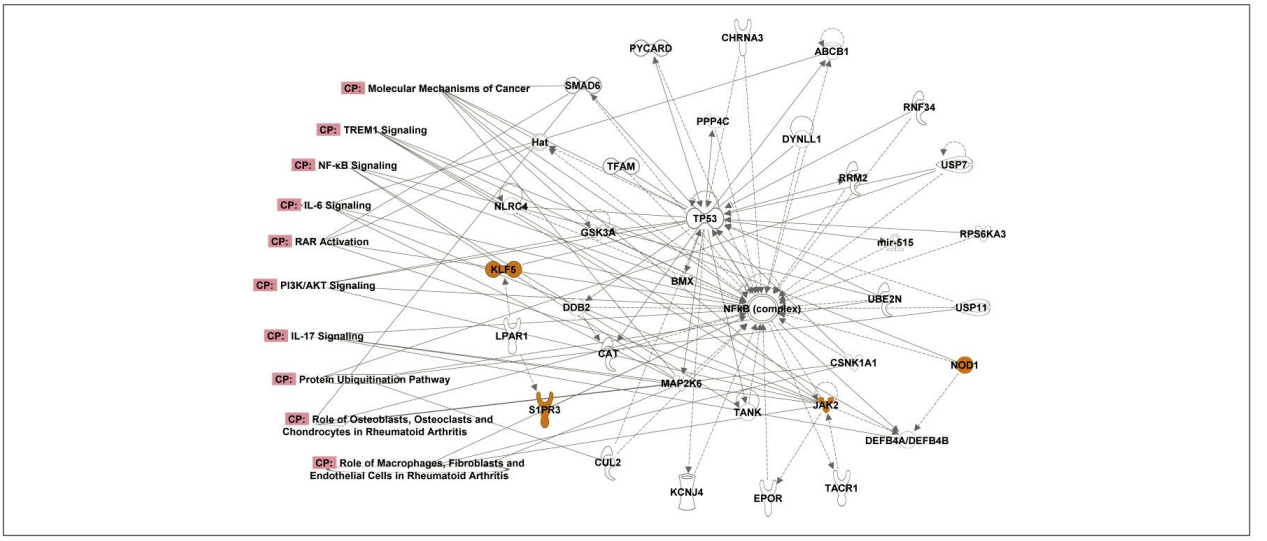


**FIGURE 1.** The molecular networks of TP protein targets.
Orange nodes represented the protein targets of TP.

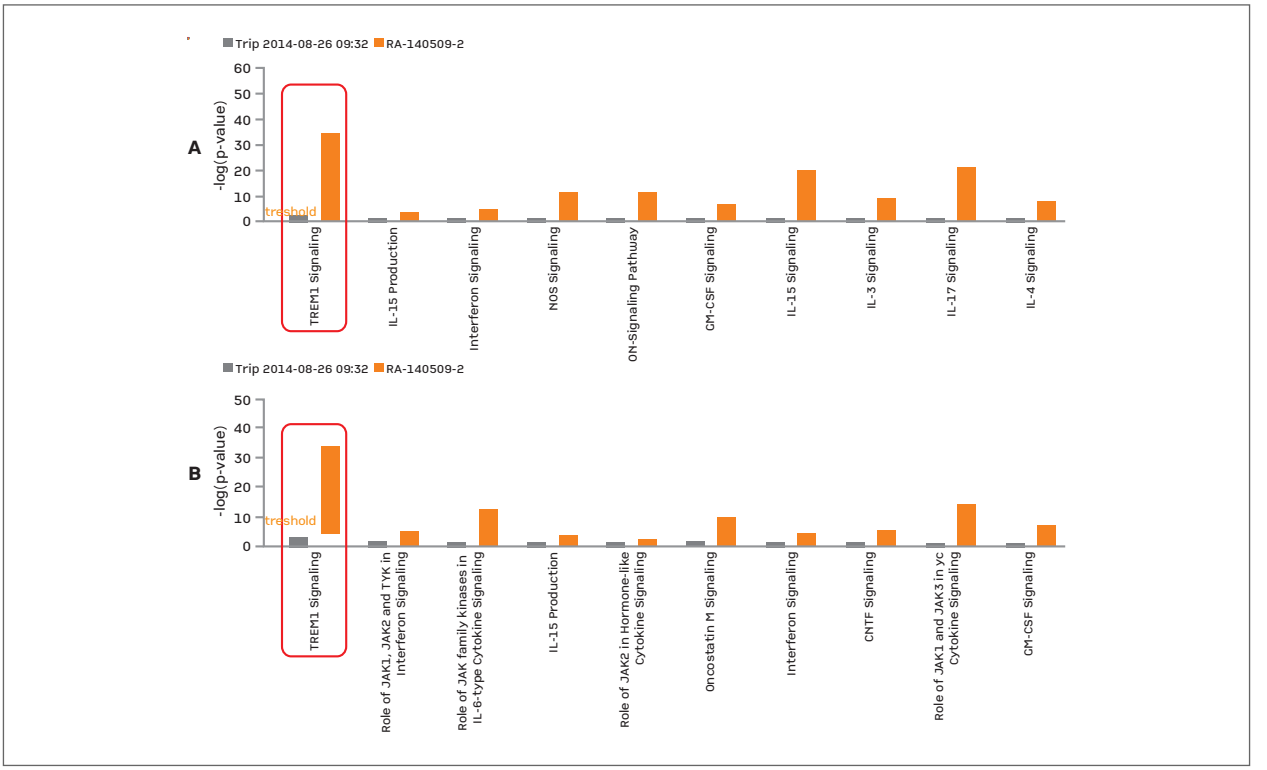


**FIGURE 1.** Fig 2. Shared signaling pathways between gene molecular network related with RA and protein targets molecular network of TP in cytokine and cellular immune signaling. **A)** Cellular immune signaling. **B)** Cytokine signaling.

mechanism of TP on RA in global based on bioinformatics approach.

**Methods:** The human protein targets of TP were searched in PubChem database, and the human genes of RA were searched in Gene database from NCBI. These two dataset were imported into Ingenuity Path-

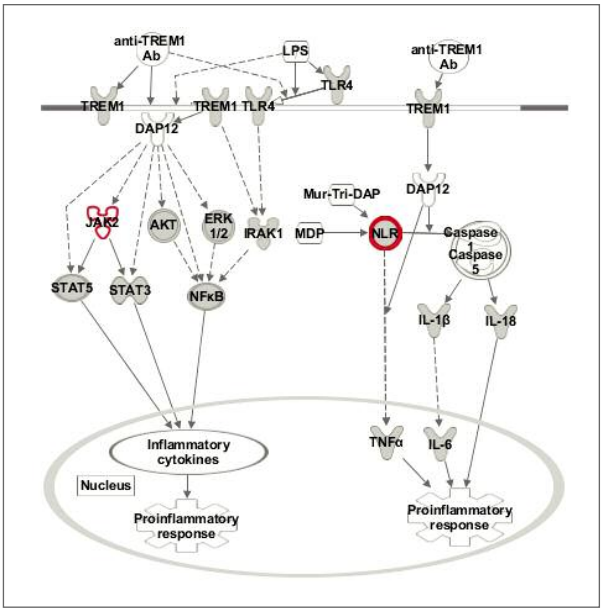


**FIGURE 3.** The key molecule targets of TP on RA in TREM1 signaling pathway
The gray nodes represented the RA genes; the red circles represented the protein targets of TP

way Analysis (IPA) software on line, by which we can build and analysis the molecular network of TP on RA.
**Results:** Eight hundred and thirty two genes related with RA and nine protein targets of TP were found. The top 15 signaling pathways of genes related with RA were focused on cellular immune response, cytokine signaling, humoral immune response, and intercellular and second messenger signaling. The main canonical signal pathways of TP protein targets network were mainly focused on cytokine and cellular immune signaling (Fig 1). Further comparative analysis showed that TREM1 signaling was detected to be the top 1 shared signaling pathway and involved in the cytokine and cellular immune signaling (Fig 2). From the TREM1 signal pathway, the results predict that TP is likely to regulate the proinflammatory response by JAK2 and NLR molecules (Fig 3).
**Conclusion:** TP might treat RA by regulating proinflammatory response through TREM1 signal pathway. The results still need to be confirmed in future.

#### ACKNOWLEDGMENTS
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## P19

### RHEUMATOID ARTHRITIS: A DISEASE ASSOCIATED WITH CARDIOVASCULAR RISK

Hernández R., Rodriguez S., Uceda J., Marenco J.L.
Valme University Hospital, Seville, Spain

**Introduction:** Patients with rheumatoid arthritis (RA) have an increased cardiovascular morbidity and mortality (CV) remains leading cause of death. The SCORE (Systematic Coronary Risk Evaluation) are tables that allow us estimate the risk of CV death.
**Objective:** To describe the characteristics in terms of risk factors in patients with rheumatoid arthritis (RA) for cardiovascular disease. We calculate SCORE CV mortality risk at 10 years.
**Material and Methods:** Observational study of a case series of patients diagnosed with RA tracking Valme hospital area. We collected the following variables: Sex, age, smoking habit, systolic blood pressure (mm Hg) and total cholesterol (mg /dl). The SCORE risk adapted for southern European people.
**Results:** we reviewed 107 patients. Among cardiovascular risk factors we found that 69.5% were obese (BMI > 25), 54.7 % had dyslipidemia, 41% hypertension, 22.1 % were smokers and finally, 13,2 % are diagnosed with diabetes mellitus.

| Variable | Sample |
|---|---|
| Current age (median DS) | 56,78 (12,3) |
| Female (%) | 70,1% |
| Smoker (%) | 22,1 |
| Sistolic Pressure (mmhg mean DS) | 127,8 (16,4) |
| Dyslipemia (mg/dl mean DS) | 195,54 (38,0) |
| SCORE (mean DS) | 2,93 (3,5) |

**Conclusions:** We find a low risk of mortality (median SCORE <5). This could be justified by the application of primary and secondary measures of prevention. However, about 25% of patients have a high cardiovascular risk. In some ways this was expected since most of them were obese and had hyperlipidemia and hypertension. It seems clear we need to develop specific action strategies that allow us to remove the modifiable risk factors.

## P20

### CHRONIC INFLAMMATION AND CARDIOVASCULAR DISEASE IN RHEUMATOID ARTHRITIS: ROLE OF ANTI CITRULINATED PEPTIDE ANTIBODIES

Hernández R., Uceda J., Rodriguez S., Marenco J.L.
Rheumatology, Valme University Hospital, Seville, Spain

**Objective:** To assess whether the presence of anti-cyclic citrullinated peptide antibodies (ACPA) is related to increased frequency of cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA).
**Methods:** Observational analytical case-control study nested in a cohort of patients with a diagnosis of AR tracking Valme hospital during the period from March 2009 to January 2014. Cases are considered as patients who developed a myocardial infarction or stroke, ischemic heart disease or heart failure, and controls RA patients without CVD. They all determine ACPA levels and risk estimation of CV death by SCORE (Systematic Coronary Risk Evaluation).
**Results:** 107 patients diagnosed with RA were reviewed. No statistically significant differences in socio-demographic, disease characteristics and cardiovascular risk factors among ACPA (+)Vs. ACPA(-) patients were found. We also found no differences in the occurrence of CV events in ACPA + (7%) Vs. ACPA - (14%), neither in SCORE between the two groups.
**Conclusions:** In our sample probably due to the small size and number of CVD, we found no statistically significant differences in SCORE of the appearance of CVD in ACPA (+) Vs. ACPA (-) patients.

| N: 107 patients | ACPA + (67%) | ACPA - |
|---|---|---|
| Sex (female %) | 66.2 | 77.1 |
| Median age (years) | 56.63 (11.8) | 57.14 (13.6) |
| FR+ (%) | 88.6 | 14.3 |
| DAS 28 (mean/ DS) | 3 (1.2) | 2.5 (1.1) |
| FAME (%) | 81.7 | 82.9 |
| Biologics (%) | 22.4 | 6.7 |
| Obesity (%) | 73.2 | 61.8 |
| DM (%) | 12.7 | 14.3 |
| HTA (%) | 40.8 | 41.2 |
| Dyslipemia (%) | 49.3 | 65.7 |
| Smoker %) | 22.9 | 20.6 |
| Cardiovascular disease (%) | 7 | 14.3 |
| Score >/= 5 (%) | 23.5 | 27.3 |

## P21

### CHANGES IN VIDEOCAPILLAROSCOPY IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS (JIA): AN OBSERVATIONAL STUDY

Uceda J., Velloso M.L., Martínez R., Marenco J.L.
Rheumatology Unit, Valme University Hospital, Seville, Spain

**Introduction:** Videocapillaroscopy allows for the detection of changes in microcirculation characteristic of connective tissue diseases. Differences in the capillaroscopic findings have been found between healthy adults and healthy children. Currently there is no defined capilaroscopic pattern in JIA.
**Objective:** identify the capillaroscopic pattern in JIA and assess its relationship with JIA forms.
**Material and Methods:** Medical records of patients less than 18 years old were reviewed. We identified 14 patients, current mean age of 11.35, and predominantly female. All videocapillaroscopies were performed by the same rheumatologist.
**Results:** Microvascular alterations were found in 12 patients. All had tortuous capillaries, 4 had bushy morphology and 1 had twister morphology. Venus plexus was very visible in 9 patients. Dilated capillaries were found in 6 and 6 other patients showed a lower capillary density. Data analysed by JIA forms showed more frequent capillary dilation in the oligoarticular and enthesitis related forms and less capillary density in the oligoarticular form.
**Conclusions:** In JIA, only nonspecific changes have been described, such as capillary tortuosity and elongation, microhemorrhages and increased venular plexus visibility. We found morphological alterations, high visibility of subpapilar venous plexus and a disorganized periungueal area in the majority of the patients regardless of JIA form. Our study confirms the existence of nonspecific capillary changes in JIA and highlights the possibility that capillary dilation is a specific finding in oligoarticular and enthesitis related forms. Further studies are needed to clarify these findings.

## P22

### REFRACTORY CHRONIC ERYTHEMA NODOSUM AND ANTI TNF

Uceda J., Hernández R., González P., Marenco J.L.
Rheumatology Unit, Valme University Hospital, Seville, Spain

**Introduction:** Erythema nodosum (EN) a septal panniculitis without vasculitis. Remission of lesions occurs within 1-6 weeks without scarring or residual atrophy. There are, however, some cases which become chronic or reoccur and are treated with oral potassium iodide, corticosteroids, colchicine, hydroxychloroquine or immunosuppressive agents.
**Objectives:** Description of the cases of refractory chronic erythema nodosum and review of the literature.
**Material and Methods:** Selection of patients with refractory chronic erythema nodosum undergoing treatment with anti TNF in the Rheumatology unit. Literature search using PubMed with keywords: erythema nodosum and Adalimumab, Etanercept, Infliximab.
**Results:**

| Patient | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Sex | Female | Female | Male | Female |
| Age | 38 | 32 | 40 | 43 |
| Diagnosis | Behçet's disease | Behçet's disease | Sarcoidosis | Psoriatic arthritis |
| Pre-treatment | Prednisone<br>Azathioprine<br>NSAIDS | Prednisone<br>Azathioprine | Prednisone<br>Azathioprine<br>NSAIDS | Prednisone<br>Azathioprine<br>NSAIDS |
| Anti TNF | Infliximab 300 mg every 8 weeks<br>Etanercept 50 mg weekly | Infliximab 300 mg every 8 weeks | Infliximab 375 mg every 8 weeks | Infliximab 300 mg every 8 weeks |
| Evolution | Improvement of erythema nodosum with both anti TNF treatments a month after start of treatment | Asymptomatic after the second infusion | Asymptomatic after the first infusion | Asymptomatic after the 4th infusion |

**Conclusions:** In our sample, all cases have responded favorably to treatment with anti TNF. No adverse events were observed, except the occurrence of cutaneous psoriasis in one patients after infliximab treatment. In reviewing the literature we find that anti TNF paradoxically brings about an immediate response in EN patients, however provokes EN and others skin manifestations.

## P23

### PERIUNGUEAL CAPILLAROSCOPY FINDINGS IN A SERIES OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS: AN OBSERVATIONAL STUDY

Uceda J., Martínez R., Velloso M.L., Marenco J.L.
Rheumatology Unit, Valme University Hospital, Seville, Spain

**Introduction:** Microvascular alteration is a fundamental factor in the development in systemic lupus erythematous (SLE). Currently we do not have a typical capillaroscopic pattern in this disease. At capillaroscopic examination, the most frequent changes in SLE patients were morphological alterations.
**Objectives:** The aim was to assess the capillaroscopic findings in patients with SLE and to determine its relationship with the presence of Raynaud's phenomenon (RP).
**Materials and Methods:** A descriptive cross-sectional observational study was carried out.
A total of 10 patients diagnosed with SLE were included. Demographic variables, clinical manifestations were collected. A periungueal capillaroscopy in the 3rd, 4th and 5th fingers of both hands was conducted.

**Results:** From the sample, 8 were women. The mean age was 36. 7 patients had major organ involvement and 5 patients had RP. The most frequent capillaroscopic changes were the presence of increased tortuosity. Formation of new vessels was observed in half of the sample. In 5 patients the subpapilar venous plexus was visible. Dilated capillary loops were found in 50 % of patients. No significant differences were observed between patients with RP and those without, however.
**Conclusions:** According to the results, most of the patients had tortuous capillaries and 50% had capillary dilations and visible subpapillary venous plexus. Findings were similar to those reported in the literature. In our sample, we found no differences in findings between capillaroscopic SLE patients presenting RP and those without RP. These findings were discordant with previously published data.

## P24

### CROSSREACTIVE AUTOANTIBODIES DIRECTED AGAINST CUTANEOUS AND JOINT ANTIGENS ARE A HALLMARK OF PSORIATIC ARTHRITIS

Ottria A.[1*], Dolcino M.[2*], Patuzzo G.[3*], Lunardi C.[3§], Puccetti A.[1,2§]
1. University of Genova
2. Institute G. Gaslini, Genova
3. University of Verona, Italy
*§Equal contributors

**Introduction:** Psoriatic arthritis (PsA) is a chronic inflammatory disease of unknown origin, characterized by erosions and new bone formation. Diagnosis of PsA is mainly clinical and there are no biomarkers available. Moreover in PsA autoantibodies have not been described so far. Indeed an autoimmune origin has been suggested but never proven. Aim of the study was to attempt to identify the autoimmune aspects of the disease.
**Methods:** We used pooled IgG immunoglobulins derived from 30 patients with PsA to screen a random peptide library in order to identify disease relevant autoantigen peptides.
**Results:** Among the selected peptides, one was recognised by nearly all the patients' sera. The identified peptide (PsA peptide) shows sequence similarities with skin autoantigens, such as fibrillin 3, a constituent of actin microfibrils, desmocollin 3, a constituent of the desmosomes and keratin 78, a component of epithelial cytoskeleton. Interestingly the PsA peptide shares homology with the nebulin-related anchoring protein (N-RAP), a protein localized in the enthesis (point of insertion of a tendon or ligament to the bone), which represents the first affected site during early PsA. Antibodies affinity purified against the PsA peptide recognize fibrillin, desmocollin, keratin and N-RAP. Moreover antibodies directed against the PsA peptide are detectable in 85/100 (85%) PsA patients. Such antibodies are not present in healthy donors and are present in 13/100 patients with seroposive rheumatoid arthritis (RA). In seronegative RA these antibodies are detectable only in 3/100 patients.
**Conclusions:** Our results indicate that PsA may have an autoimmune origin, and that it is characterized by the presence of serum autoantibodies crossreacting with an epitope shared by skin and joint antigens.

## P25

### GENE EXPRESSION PROFILING IN PERIPHERAL BLOOD CELLS AND SYNOVIAL MEMBRANES OF PATIENTS WITH PSORIATIC ARTHRITIS

Ottria A.[1*], Dolcino M.[2*], Barbieri A.[3], Argentino G.[3], Beri R.[3], Lunardi C.[3§], Puccetti A.[1,2§]
1. University of Genova
2. Institute G. Gaslini, Genova; 3University of Verona, Italy
*§Equal contributors

**Introduction:** Psoriatic arthritis (PsA) is an inflammatory arthritis of unknown origin characterized by erosions and new bone formation. Diagnosis of PsA is clinical and there are no diagnostic biomarkers available. Disease pathogenesis is poorly understood. We aimed at clarifying some aspects of PsA pathogenesis and at identifying gene signatures in paired peripheral blood cells (PBC) and synovial biopsies of PsA patients. Moreover we searched for disease biomarkers to be used in clinical practice.
**Methods:** We performed gene expression analysis in PBC and synovial biopsies of 10 patients with PsA using Affymetrix arrays. Expression data were validated by Q-PCR, FACS analysis and detection of soluble mediators.
**Results:** Approximately 200 genes were modulated in synovial biopsies of patients when compared to synovial biopsies of healthy donors. Differentially expressed genes (DEGs) indicate upregulation of Th17 related genes and overexpression of type I interferon (IFN) inducible genes. Th17 polarization was confirmed by FACS analysis. The synovial trascriptome identifies gene clusters (bone remodeling, angiogenesis and inflammation) involved in the pathogenesis of typical features of PsA. Interestingly 90 genes are modulated in both PBC and synovium indicating that signature pathways in PBC reproduce gene signatures of the inflamed synovium. The osteoactivin gene was upregulated in both PBC and synovial biopsies, where it shows a very high level of induction. Interestingly high levels of osteoactivin are detectable in PsA sera but not in other inflammatory arthritides.
**Conclusions:** We report here the first analysis of the trascriptome of paired synovium and PBC of PsA patients. The study provides a better understanding of PsA pathogenesis and suggests an autoimmune origin of PsA since it is well-known that coactivity of IFN and Th17 pathways is typical of autoimmunity. Finally this

approach has allowed the identification of a potential disease biomarker, osteoactivin, easily detectable in PsA serum.

## P26

### RECEPTOR ACTIVATOR OF NF-KB EXPRESSION IS REDUCED IN CIRCULATING MONOCYTES FROM ANKYLOSING SPONDYLITIS PATIENTS

Perpétuo I.P.[1], Caetano-Lopes J.[1,2], Vieira-Sousa E.[1,3], Campanilho-Marques R.[1,3], Ponte C.[1,3], Canhão H.[1,3], Ainola M.[4], Fonseca J.E.[1,3]

1. Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon Academic Medical Centre, Lisboa, Portugal
2. Dept. of Orthopedic Research, Children's Hospital Boston and Genetics Dept., Harvard Medical School, Boston, MA, USA
3. Rheumatology and Bone Metabolic Diseases Dept., Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, EPE, Lisbon Academic Medical Centre, Lisboa, Portugal
4. Musculoskeletal Diseases and Inflammation Research Group, Biomedicum Helsinki, Faculty of Medicine, Institute of Clinical Medicine, University of Helsinki, Finland

**Introduction/Aim:** Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are systemic, immune-mediated diseases. RA's main targets are the peripheral joints while AS has the axial skeleton and enthesis as the principal affected areas. RA is characterized by bone erosions and impaired repair whilst AS is typified by bone overgrowth. The causes for these differences are not yet understood; however we hypothesize that AS patients' monocytes receive reduced osteoclastogenic stimuli and/or have abnormal capacity to respond to them.

The aim of this study was to characterize bone remodeling and pro-osteoclastogenesis inflammatory environment and monocyte phenotypes in AS patients as compared to RA patients and healthy controls.

**Materials and Methods:** Untreated AS (n=14) or RA (n=21) patients with active disease and 14 age and gender-matched healthy donors were recruited for this study. Blood was collected for flow cytometry measurements of RANKL expression and monocyte subpopulation characterization. Serum was collected for cytokine and bone remodeling factors quantification.

**Results:** We found that RANKL lymphocyte expression was higher in AS and RA patients when compared to healthy donors. However, both in classical and intermediate monocyte subpopulations RANK expression was lower in AS patients as compared to RA patients and healthy controls. In accordance, CTX-I serum level was also lower in AS patients than in healthy controls.

**Conclusions:** Despite comparable osteoclastogenic stimuli in AS and RA patients, RANK expression is reduced in AS circulating monocytes which may contribute to the bone forming phenotype observed in AS patients.

## P27

### PREDICTORS OF REMISSION IN AXIAL SPONDYLOARTHRITIS PATIENTS TREATED WITH NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Furtado C.[1], Fernandes S.[1], Rodrigues A.M.[3], Martins F.[2], Miranda Rosa C.[1], Pereira da Silva J.A.[1], Vieira-Sousa E.[1]

1. Rheumatology Dept., Hospital de Santa Maria
2. Portuguese Society of Rheumatology, Lisbon
3. Rheumatology Research Unit, Instituto de Medicina Molecular, Lisbon, Portugal

**Introduction:** First-line therapy for axial spondyloarthritis (axSpA) has not yet changed in the biologic era, and non-steroidal anti-inflammatory drugs (NSAIDs) are still considered the cornerstone treatment. The aim of this work was to identify predictors of remission in axSpA patients, treated exclusively with NSAIDs.

**Patients and Methods:** AxSpA patients treated exclusively with NSAIDs, registered at the Rheumatic Diseases Portuguese Register - Reuma.pt, were included. Demographic and clinical parameters of disease activity as well as NSAIDs therapy [category (nonselective vs selective), dose, duration, regime (continuous vs on-demand)] were analyzed. Ankylosing spondylitis disease activity score (ASDAS) inactive disease, at the last visit, was defined as remission (primary outcome).

**Results:** 50 patients fulfilling the Assessment in Spondyloarthritis (ASAS) classification criteria for axial SpA were included. This cohort had a mean age of 48.5±14.2 years, a mean disease duration of 20.7±13.4 years and a mean follow up of 19.5±17.3 months, with female predominance (58%). Peripheral involvement was documented in 22%, uveitis in 22%

and enthesitis in 28% of patients. 60% were HLAB27 positive. The mean BASDAI (2.8±2.1; 2.2±1.7) BASFI (2.7±2.4; 2.6±2.4), CRP (0.7±1.0; 6±0.7mg/dl) and ASDAS (2.0±1.0; 1.7±0.9) on baseline and last available visits, respectively. All patients were treated with NSAIDs, on average for 6.2±8.5 years, approximately half on demand (52%). 20% were receiving maximum recommended doses of NSAIDs. On the last visit 36% of patients showed inactive disease. On univariate analysis male gender, disease duration and baseline ASDAS were negatively associated with ASDAS inactive disease. On a multivariate analysis ASDAS at baseline was the only independent predictor of ASDAS inactive disease. The covariate considered were gender, age, disease duration, presence of sacroiliitis and HLAB27 and NSAIDs dose.

**Conclusions:** ASDAS at baseline was the only independent predictor of remission in axSpA patients treated exclusively with NSAIDs.

## P28

### ARE ARTHROSCOPIC GUIDED SYNOVIAL BIOPSIES STILL RELEVANT IN RHEUMATOLOGY PRACTICE?

Vieira-Sousa E.[1,2], Narciso L.[1], Saraiva F.[1], Pereira da Silva J.A.[1], Fonseca J.E.[1,2]

1. Rheumatology and Bone Metabolic Diseases Dept., HSM, CAML

2. Rheumatology Research Unit, IMM, FMUL, CAML, Portugal

**Aim:** We aim to analyze the outcomes of the first 24 knee arthroscopic guided synovial biopsies (AGSB) recently performed at our centre for diagnosis and (non-orthopedic) therapeutic purposes.

**Methods:** AGSB were performed according to standardized procedures. Twenty-two for diagnostic and two for therapeutic purposes. A macroscopic scoring for vascularization and proliferation was applied and synovial tissue processed for routine histology and microbiology assessment.

**Results:** 24 patients were submitted to AGSB. Ten had a previous diagnosis of inflammatory arthritis: RA (3), JIA (3), PsA (3) and SLE (1). In all cases, patients were in joint remission with the exception of knee arthritis. AGSB allowed the exclusion of joint infection in 9 of 10 patients and septic arthritis was the established diagnosis in one case. This favored rapid therapeutic adjustment. The remaining patients had no previous rheumatic disease and presented with monoarthritis (9) or oligoarthritis (5) of unknown etiology. Considering the monoarthritis group, *Mycobacterium tuberculosis* was isolated in two patients. For the remaining seven, infection was excluded and a definite diagnosis of osteoarthritis (1), crystal induced arthritis (3), psoriatic arthritis (1) and lipoma arborescens (2) was established. In the oligoarthritis group, neither synovial membrane macroscopic, histology nor microbiology analysis helped to clarify the diagnosis.

**Conclusions:** In inflammatory arthritis, persistent synovitis of a single joint can be a red flag for joint infection. In this small series however, the majority of patients exhibited uncontrolled disease activity. The rate of joint tuberculosis was high (8%) highlighting its endemic occurrence. Synovial membrane crystal deposits could be visualized in patients in whom they were not detected in the synovial fluid. For oligoarthritis patients, undifferentiated arthritis was the final diagnosis, indicating that further synovial tissue biomarkers for inflammatory arthritis diagnosis are required. AGSF allowed the establishment of a definite in the large majority (79%) of patients favoring decisions in clinical practice.

## P29

### A MATHEMATICAL APPROACH TO UNDERSTAND THE DISEASE BIOLOGY: RA BLOOD BASED DISEASE ACTIVITY SCORE (RABBDAS)

Aggarwal A.[1,2], Sharma A.[3], Bhatnagar A.[1]

1. Dept. of Biochemistry, Panjab University, Chandigarh

2. Centre of Excellence in HIV Care, Postgraduate Institute of Medical Education and Research, Chandigarh

3. Dept. of Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

**Introduction:** Methods of early diagnosis and improved disease severity prediction markers are key to take therapeutic decisions and better outcomes. Existing Disease Activity Score 28, Simplified Disease Activity Index and Clinical Disease Activity Index are influenced by intra and inter-assessor variability.

**Aim:** 1. To elucidate the role of oxidative stress and immunobiology of NK and NKT cells.

2. To design a mathematical model for measuring disease severity in RA patients.

**Methods:** 50 RA patients (39 females, 11 males) were recruited from the rheumatology Clinic of PGIMER

Chandigarh with informed consent. 50 age and sex matched healthy volunteers (41 females, 9 males) were also enrolled. The biochemical parameters investigated in serum samples were Lipid Peroxidation, Reduced Glutathione, Catalase, Superoxide dismutase, Glutathione peroxidise, IL-18 and TNF-α. NK and NKT cells related intracellular parameters (DNA damage, caspase-3, perforin, granzyme A and B, IL-4, IFN-γ and IL-8 expression) were measured using multicolour flow cytometer. Mathematical model equation was developed for predicting RA blood based disease activity score (RABBDAS) using multiple linear regression analysis. The ethical approval was obtained from the IEC of PGIMER Chandigarh.

**Results:** Antioxidants were severely compromised. NK and NKT cells were reduced with elevated apoptotic markers in RA patients. Granzyme A, perforin and IL-8 were independently associated with RABBDAS as:

**RABBDAS = $-1.115 + 0.054X_1 + 0.032X_2 + 0.14X_3$ ($r^2 = 0.95$)**

Where $X_1$: NK_GranA; $X_2$: NKT_Perf; $X_3$: NKT_IL-8

**Discussion**: There is a state of profound oxidative stress in RA patients. Immunobiology of NK and NKT cells were severely compromised.

**Significance and Innovation:** This novel severity index may serve as an un-separable tool in quantifying the severity index of RA in patients. A patent application "**Diagnostic Kit For In Vitro Diagnosis Of Rheumatoid Arthritis (Ra) And Method Of Use Thereof**" has been filed.

## P30

### IL-1RA SECRETED BY MESENCHYMAL STEM CELLS EXERTS A KEY IMMUNOMODULATORY ROLE ON THE MATURATION OF B CELLS AND MACROPHAGES

Luz-Crawford P.[1,2], Djouad F.[1,2], Toupet K.[1,2], Franquesa M.[3], Hoogduijn M.J.[3], Jorgensen C.[1,2], Noël D.[1,2]

1. Inserm U 844, Montpellier
2. Université Montpellier 1, Montpellier, France
3. Erasmus Medical Center, Rotterdam, The Netherlands

**Introduction:** The efficacy of interleukin-1-receptor antagonist (IL-1RA) treatment has been documented in several autoimmune diseases and in particular, in rheumatoid arthritis (RA). IL-1RA is an endogenous specific receptor antagonist that competes with the pro-inflammatory signals triggered by IL-1α or IL-1β and participates to the immune system homeostasis through the maintenance of IL-1/IL-1RA balance. IL-1RA is also secreted by mesenchymal stem cells (MSCs) that are potent immunosuppressive cells. The aim of this study was to evaluate how IL-1RA contributes to the therapeutic effect of MSCs.

**Materials and Methods:** MSCs were isolated from the bone marrow of mice knockout for IL-1RA (IL-1RA$^{-/-}$ MSCs) and wild type mice (wt MSCs). One million of cells were injected (day 18 and 24) via the tail vein in collagen induced arthritic mice (CIA).

**Results:** In contrast to wt MSCs that inhibited CIA progression, the injection of IL-1RA$^{-/-}$ MSCs did not decrease the percentage of Th1 and Th17 cells in spleens and lymph nodes of treated mice. The loss of therapeutic effect could not be associated to the incapacity to inhibit the proliferation of T cells. IL-1RA$^{-/-}$ MSCs were less efficient than wt MSCs to reduce the maturation of macrophages as evidenced by a significantly higher percentage of F4/80$^+$CD86$^+$MHCII$^+$ cells, which expressed higher levels of TNF-α and lower amounts of IL-10. Moreover in contrast to wt MSCs, IL-1RA$^{-/-}$ MSCs were not able to reduce the proliferation and maturation of IgG-secreting B lymphocytes which secreted lower amounts of IL-10.

Indeed, IL-1RA is involved both in the switch from a pro-inflammatory *M1* macrophage polarization state to an anti-inflammatory *M2* macrophage subtype and the generation of IL-10-secreting B cells.

**Conclusion:** In summary, the present data showed a new insight into the mechanism of immunosuppression exerted by MSCs to inhibit arthritis progression and highlighted the role of IL-1RA in this effect.

## P31

### MESENCHYMAL STEM CELLS INDUCE NON-CLASSICAL IL10-PRODUCING REGULATORY TH17 CELLS IN ARTHRITIS: ROLE OF GILZ

Djouad F.[1,2], Luz-Crawford P.[1,2], Tejedor G.[1,2], Bonnefont A.L.[1,2], Morand E.[4], Beaulieu E.[4], Jorgensen C.[1,2,3], Noël D.[1,2]

1. Inserm, U 844, Montpellier
2. Université Montpellier 1, UFR de Médecine, Montpellier
3. Service d'Immuno-Rhumatologie, Hôpital Lapeyronie, Montpellier, France

4. Monash University, Melbourne, Australia

**Background and Objectives:** Regulatory T (Treg) cells have a critical inhibitory effect in rheumatoid arthritis (RA) and are a potential target for therapies that suppress inflammation. Induction of Treg cells by mesenchymal stem cells (MSC) is proposed as one mechanism through which they exert immunosuppressive effects. The aim of this study was to evaluate the molecular mechanisms involved in the therapeutic potential of MSC in a murine model of RA and to better understand how the immune *balance* between Th1/*Th17* and *Treg* cells is regulated following MSC injection. We focused our study on the role of Gilz which was recently described as an inhibitor of immune-inflammatory responses in RA.
**Materials and Methods:** MSC deficient for Gilz ($Gilz^{-/-}$ MSC) were isolated from knockout mice. Their therapeutic effect after intravenous administration was compared to that of WT MSC in collagen-*induced arthritis* (CIA). The *in vivo* suppressive mechanism on effector T cell proliferation and the generation of regulatory T cells were also investigated.
**Results:** Analysis of clinical and immunological parameters revealed that Gilz expression is crucial for the ability of MSC to control the progression of experimental arthritis. Moreover, we showed that while MSC reduce the frequency of Th1 and Th17 cells in the draining lymph nodes during arthritis development, $Gilz^{-/-}$ MSC did not. This significant decrease of Th1 and Th17 frequency following MSC injection was associated with the generation of IL-10-producing regulatory Th17 cells, an effect absent in $Gilz^{-/-}$ MSC treated mice.
**Conclusion:** Our results demonstrate the requirement for Gilz in the therapeutic effect of MSC in a model of RA. We propose that the suppressive effect of MSC is mediated by the Gilz-dependent generation of new population of functional regulatory T cells bearing the $CD4^{+}ROR\gamma T^{+}IL17^{Low}IL10^{+}$ signature.

## P32

### THE LONG-TERM EFFECTIVENESS OF RITUXIMAB IN RHEUMATOID ARTHRITIS DEPENDS ON THE TREATMENT SEQUENCING STRATEGY, PATIENT AGE AND DURATION OF DISEASE

Espinoza F., LeBlay P., Ferreira R., Pers Y.M., Jorgensen C.
Clinical Immunology and Osteoarticular Diseases Therapeutic Unit, CHU Lapeyronie, Montpellier, France

**Background:** To analyse whether the sequencing strategy of Rituximab (RTX) administration and other clinical factors affects RTX long-term effectiveness in patients with rheumatoid arthritis (RA).
**Methods:** The retrospective analysis involved 70 patients who were treated with RTX and were followed for at least 36 months. All patients satisfy the 2010 ACR/EULAR clinical criteria for RA. Change in the 28--joint Disease Activity Score (DAS28), European League Against Rheumatism (EULAR) response and the proportion of patients achieving remission were used to evaluate the efficacy of RTX at baseline and until the end of follow-up. Paired t test, two-way ANOVA and logistic regression tests were performed.
**Results:** 77.7% of patients were females. 81.4% of patients have a positive anti-citrullinated protein antibody. All patients were received an anti-TNF agent as first bDMARD. After 6 months, DAS28 decreased significantly from 5.1± 0.16to 3.3 ± 0.19 ($p<0.0001$, 95% CI-2.29, -1.25), 30% of the patients achieved disease remission and 70% of patients achieved good EULAR response. After 36 months of follow-up, 61.4% of patients continued RTX treatment with a DAS 28 of 2.82 ± 0.34. In this group of patients, the effectiveness of RTX was significantly better when it was used as a second-line therapy in comparison to third-line therapy agent (47% vs 11% achieving remission, $p<0,001$). Other clinical characteristics associated with RTX long--term efficacy were: younger patients (OR=1.78, p=0.03), duration of disease up to 5 years (OR=2.45, p=0.002) and anti-CCP positivity (OR=1.32, p=0.05).
**Conclusion:** In this observational cohort study, we show that long-term effectiveness of RTX is better when it used as a second-line biological treatment after one anti-TNF alpha failure. Others factors involved in a better clinical outcome after 36 months of treatment were age and RA duration.

## P33

### CLINICAL AND PATHOLOGICAL DIFFERENCES OF ELDERLY- AND YOUNGER-ONSET RHEUMATOID ARTHRITIS IN AN EARLY ARTHRITIS COHORT

Romão V.C.[1,2], DiCicco M.[1], Mahto A.[1], Lazarou I.[1], Fonseca J.E.[2], Kelly S.[1], Humby F.[1], Pitzalis C.[1]
1. Experimental Medicine & Rheumatology, Queen Mary

University of London, London, UK
2. Rheumatology Research Unit, Instituto de Medicina Molecular – Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal

**Introduction:** Elderly-onset rheumatoid arthritis (EORA), starting after the age of 60, is a subset of disease with different characteristics from the classic younger-onset RA (YORA).

We aimed to compare clinical and pathological features between these two disease subsets in the Barts Early Arthritis Cohort (BEAC).

**Material and Methods:** We included BEAC patients fulfilling ACR 2010 criteria and assessed clinical and pathological variables at baseline and at 6 months of DMARD therapy.

The primary outcome was the achievement of low disease activity (LDA) according to DAS28 at 6 months. We used multivariate logistic regression to determine predictors of LDA at 6 months.

**Results:** We included 140 patients, 99 YORA and 41 EORA. EORA patients were more frequently male, Caucasian, had more commonly proximal joint involvement at presentation and abrupt/polymyalgia rheumatica (PMR)-like onset, weight loss, anemia, extra-articular manifestations and cardiovascular comorbidity. At baseline, disease activity parameters were similar between both groups, including ESR, CRP, joint counts, DAS28, CDAI and SDAI.

Synovial pathotype classification (ectopic lymphoid-like structures positive (ELS+); diffuse myeloid infiltrate or non-inflammatory/pauci-immune) did not reveal differences between groups as a whole (p=0.12). However, EORA patients presented pauci-immune pathotype less frequently (25.0 vs. 44.3, p=0.045). Macrophage, B-cell, T-cell and plasmocyte semi-quantitative scores were also similar. At 6 months (n=105), EORA (OR=0.28, p=0.047), female gender (OR=0.23, p=0.016), current smoking (OR=0.11, p=0.002) and high baseline DAS28 (OR=0.41, p<0.001) were negatively associated with reaching DAS28 LDA.

Contrastingly, PMR-like onset was strongly predictive of DAS28 LDA (OR=63.9, p=0.004).

**Conclusions:** In an early arthritis cohort, EORA patients presented different clinical features at presentation, but similar synovial pathological characteristics. Adjusting for other significant factors such as gender, smoking, baseline DAS28 and PMR-like onset, EORA was negatively associated with the achievement of LDA at 6 months according to DAS28.

## P34

### HEPATITIS B SEROLOGIC PROFILE AND REACTIVATION IN RHEUMATIC PATIENTS TREATED WITH BIOLOGICAL THERAPIES – SINGLE CENTRE EXPERIENCE

Romão V.C.[1,2], Saavedra M.J.[1], Costa M.[1], Macieira C.[1], Vieira-Sousa E.[1,2], Ramos F.[1], Resende C.[1], Capela S.[1], Barros R.[1], Polido-Pereira J.[1,2], Rodrigues A.M.[1,2], Madruga-Dias J.[1,2], Marques R.[1,2], Ponte C.[1,2], Castro A.[1,2], Furtado C.[1,2], Fernandes S.[1,2], Gonçalves M.J.[1,2], Pereira da Silva J.A.[1], Canhão H.[1,2], Fonseca J.E.[1,2]
1. Rheumatology Dept., Santa Maria Hospital, Lisbon
2. Rheumatology Research Unit, Instituto de Medicina Molecular – FMUL, Lisbon, Portugal

**Introduction:** Biologic therapy for rheumatic diseases has been associated with increased risk of hepatitis B (HB) reactivation. We aimed to evaluate the serologic profile and incidence of HB reactivation in biologic-treated rheumatic patients from a single centre.

**Methods:** We collected electronically available HB serology of rheumatic patients that ever started biological therapy at our department. We reviewed the clinical course to identify reactivation cases, defined as raising viral load and transaminases.

**Results:** We included 288 patients with available electronic data on HB serologies. Mean age was 43.9±15.7 years (3.1 to 82.3 years), disease duration was 10.9±9.5 years, 63.9% of patients were female and the most common rheumatic disease was rheumatoid arthritis (38.9%). As first biologic, 254 patients (88.2%) started TNF inhibitors, 14 (4.9%) rituximab, 11 (3.8%) tocilizumab and 9 patients (3.1%) another biologic. 30 patients (10.4%) eventually stopped biologic treatment. 185 patients (64.2%) were treated with concomitant methotrexate, 81 (28.1%) with other DMARDs and 169 (58.7%) were treated with corticosteroids (58.7%). All of the 288 patients were HBsAg- and 23 were anti-HBc+ (9%). The anti-HBc+ patients did not differ significantly from the overall population in terms of disease or treatment characteristics. No patient received prophylactic antiviral therapy and there were no cases of reactivation or isolated rise in viral load during a cumulative biologic exposure of 1005.6 patient-years (Figure 1).

**Conclusions:** In our cohort, there were no cases of HB reactivation on the 288 patients treated with biologic therapy. Anti-HBc positivity was infrequent, viral load

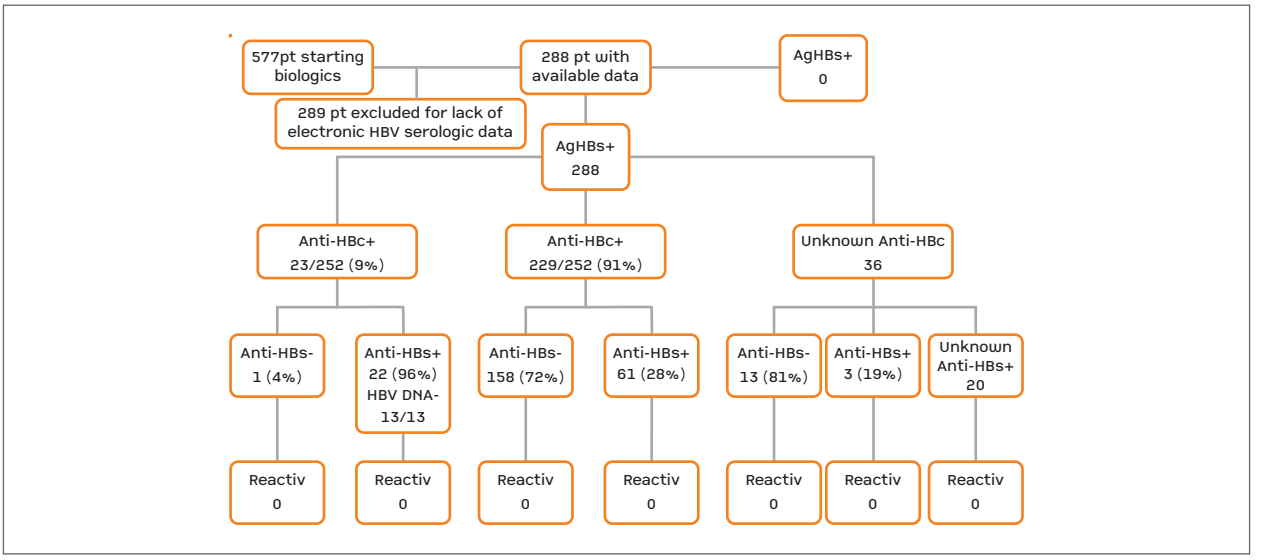


**FIGURE 1.** Hepatitis B serologic pattern of rheumatic patients starting biological therapy

was undetectable in all cases and no chronic HB cases were detected.

## P35

### ANTI-RIBO-P AND ANTI-KU ANTIBODIES AS SYSTEMIC LUPUS ERYTHEMATOSUS DIAGNOSTIC BIOMARKERS

Carmona-Fernandes D.[1], Schulte-Pelkum J.[2], Albesa R.[2], Mahler M.[2], Santos M.J.[1]

1. Rheumatology Research Unit, Instituto de Medicina Molecular, Lisbon, Portugal
2. Inova Diagnostics Inc., San Diego, CA, USA

**Introduction:** Systemic Lupus Erythematosus (SLE) is characterized by the production of several autoantibodies. Beside anti-dsDNA antibodies, which are included in the classification criteria, anti-ribosomal P (Ribo-P) were found to be highly specific for SLE and recently anti-Ku autoantibodies were also described in lupus patients.

We assessed the performance of anti-dsDNA, anti--Ribo-P and anti-Ku antibodies for the diagnosis of SLE as well as their clinical associations.

**Patients and Methods:** We included 125 SLE patients, 92 healthy controls and 247 patients with other rheumatic diseases (rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis and psoriatic arthritis). Demographic and clinical characteristics were recorded at the time of blood collection. For SLE patients, information regarding disease activity and duration, clinical features and medication was also obtained.

We used a chemiluminescent immunoassay (CIA, QUANTA Flash®) for the determination of autoantibody levels.

**Results:** Anti-dsDNA, anti-Ribo-P and anti-Ku titers were significantly higher in SLE patients (119.87± 16.10, 25.31±4.93 and 23.87±7.09, respectively) than in healthy (14.77±1.16, 5.98±0.29 and 3.23±0.45, respectively) or disease control population (17.95±2.14, 12.13±1.20 and 3.55±0.48, respectively).

In total, 46.4% of SLE patients were positive for anti--dsDNA (sensitivity 46.4%, specificity 95.6%, PPV 79.5%, NPV 82.9%), 29.6% for anti-Ribo-P (sensitivity 29.6%, specificity 95.6%, PPV 71.2%, NPV 78.6%) and 16.0% for anti-Ku antibodies (sensitivity 16.0%, specificity 97.5%, PPV 74.1%, NPV72.2%). Among anti-dsDNA negative patients, 6.8% tested positive for anti-Ribo-P only and 8.7% for anti-Ku.

Anti-dsDNA titers were independently associated with age (r=-0.195), disease activity (r=0.476) and erythrocyte sedimentation rate (ESR) (r=0.268). Anti--Ribo-P titers correlated to ESR (r=0.223) and anti-Ku titers with disease activity (r=0.278) and pericarditis (r=0.273).

**Discussion:** Anti-Ribo-P and anti-Ku antibodies are

specific for SLE diagnosis and can be positive in anti--dsDNA negative patients. Antibody titers are higher in active lupus.

**Conclusions:** Our data confirm anti-Rib-P and anti--Ku autoantibodies as potentially useful biomarkers for SLE diagnosis. Further studies are needed to understand their role in disease activity monitoring.

## P36

### CARDIOVASCULAR RISK IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS EVALUATED WITH CAROTID ULTRASONOGRAPHY

Martinez Perez R.[1], Velloso Feijoo M.L.[2], Uceda Montañes J.[2], Marenco de la Fuente J.L.[2]

1. Rheumatology Unit, Virgen del Rocio University Hospital, Seville

2. Rheumatology Unit, Valme University Hospital, Seville. Spain

**Background:** The frequency of stroke in SLE patients between 18-44 years is 2 times the frequency in general population of the same age and sex. Clinical atherosclerosis is detected in 6-12% of patients with SLE. Some researchers have suggested that the development of atherosclerosis may be related to the systemic inflammation. The objective of our study is to assess cardiovascular risk in patients with SLE by measuring carotid intima-media thickness and identifying the presence of plaques.

**Material and Methods:** Cross-sectional study on a series of 10 SLE patients without dyslipidemia. The presence of plaques and/or an intima-media thickness higher than 0.9 mm, evaluated with a high-resolution B mode ultrasound, reflect high cardiovascular risk.

**Results:** Of the 10 patients, 8 were women. The mean age was 36 ± 1.2 years and the mean age at diagnosis of the disease 23.2 ± 7.37 years.

Mean intima-media thickness in the right carotid was 580.72 ± 103.83 mm and in the left 544.76 ± 202.17 mm. Of the 10 patients, 3 had plaques, 2 of them were smokers and had been treated with Rituximab after unsuccessfully treatment with several immunosuppressants.

**Conclusions:** The 30% of our patients had plaques, even without having classical cardiovascular risk factors. The use of carotid ultrasound may predict cardiovascular events. Carotid study may be considered for assessing the existence of subclinical aterosclerosis in SLE patients and treating with statins when necessary.

| **Patient's characteristics** | Frequency | **Analytical data** | Values |
|---|---|---|---|
| **Major organ involvement** | 7/10 | **Total cholesterol** | 186.71±32.54 |
| **DM** | 0/10 | **LDL** | 98.71±15.43 |
| **Arterial Hypertension** | 1/10 | **HDL** | 85.46±17.60 |
| **Previous stroke** | 1/10 | **Triglycerides** | 129.71±25.2 |
| **Smoking** | 2/10 | **RCP** | 21.2±4.3 |
| **Corticosteroids (mean dosis)** | 5/10 (7.5 mgr/day) | **ESR** | 17.43±7.59 |
| **Inmunosuppressants** | 5/10 | **C3** | 77.14±27.9 |
| **Rituximab** | 7/10 | **C4** | 18.48±4.52 |