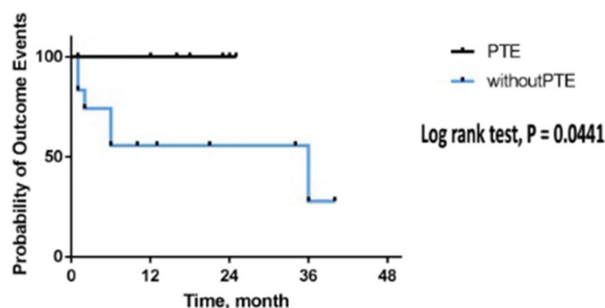
baseline WHO FC have no effects on the prognosis of CTEPH in patients with APS.



Abstract OP0355 – Figure 1 Kaplan-Meier survival curves of APS patients associated CTEPH after treatment (anticoagulation and/or PTE).

Conclusions: After a full specialised and multidisciplinary risk-benefit evaluation to limit the risk of thrombosis or bleeding and to manage possible thrombocytopenia, for those CTEPH developed in APS patients, PTE is a curative resolution.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.4044

OP0356 PLASMABLAST PROLIFERATION IS ASSOCIATED WITH TOLL LIKE RECEPTOR 7 POLYMORPHISMS AND UPREGULATION OF TYPE I INTERFERON, CONTRIBUTING TO THE ANTIBODY PRODUCTION IN ANTIPHOSPHOLIPID SYNDROME

R. Hisada, M. Kato, E. Sugawara, Y. Fujieda, K. Oku, T. Bohgaki, O. Amengual, S. Yasuda, T. Atsumi. *Department of Rheumatology, Endocrinology and Nephrology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo City, Japan*

Background: Antiphospholipid antibodies (aPL) as pathogenic autoantibodies in systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) are supported by a number of clinical, *ex vivo* and animal studies. Nevertheless, aPL are not eliminated by corticosteroid administration or immunosuppression. Novel therapy targeting aPL production is currently unmet needs, in contrast, little is known on its pathological mechanism.
Objectives: This study aimed to clarify the mechanism of aPL production by lymphocyte subset analysis, genomic analysis and *ex vivo* experiments.
Methods: B cell and T cell subsets, a total of 21 subsets, were evaluated in peripheral blood mononuclear cells (PBMC) of 26 primary APS (PAPS), 18 SLE-associated APS (SLE/APS) patients and 10 healthy controls by flow cytometry. Twenty-one single nucleotide polymorphisms (SNP), which were shown to be associated with autoimmune or thrombotic diseases, were analysed in genomic DNA of those patients by TaqMan genotyping assay. Interferon (IFN) score was calculated based on the mRNA expression of Ly6e, Mx1, IFIT1 and IFIT3 in PBMC. To evaluate the aPL-producing capability of plasmablasts, PBMC obtained from APS patients were cultured following depletion of CD19 +CD20+or CD19+CD20 cells and the culture supernatants were applied to aPL measurements by enzyme-linked immunosorbent assay and cell assay using β2GPI/HLA-DR7 overexpressing HEK293T cells.[1]
Results: In PAPS and SLE/APS patients, plasmablasts, Th2 cells and Th17 cells were increased while pre- and post- switched memory B cells, regulatory B cells and regulatory T cells were decreased compared to healthy controls. Genomic analysis revealed that the increase of plasmablasts (p=0.032) and the decrease of memory B cells (p=0.013) were more pronounced in patients with a risk allele of SNP in toll like receptor 7 (TLR7) gene (rs3853839). IFN score was significantly higher in the TLR7 SNP risk allele carriers, confirming the downstream signalling of TLR7 (p=0.029). *Ex vivo* experiments showed that aPL, including anti-cardiolipin/β2-glycoprotein I-HLA class II complexes -IgG and -IgM, were present in the culture supernatant of CD19 +CD20+depleted PBMC from APS patients, but not in that of CD19 +CD20 depleted cells.
Conclusions: Our data indicate an important role of plasmablasts in the production of aPL. Furthermore, plasmablast proliferation was associated with TLR 7 and type I IFN, suggesting a common pathophysiology in SLE and APS. Targeting plasmablasts might be a novel, immunological therapeutic approach in the treatment of APS.

REFERENCE:

[1] Tanimura K, et al. β2-Glycoprotein I/HLA class II complexes are novel autoantigens in antiphospholipid syndrome. Blood 2015.

Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.2816

SATURDAY, 16 JUNE 2018

The links between gout and kidney function

OP0357 HYPERECHOIC DEPOSITS IN THE RENAL MEDULLA ARE ASSOCIATED WITH SEVERE GOUT AND DECREASED EGFR: A TRANSVERSAL STUDY IN 503 VIETNAMESE PATIENTS

T. Bardin[1,2], K.M. Tran[1], Q.D. Nguyen[1], N.H. Le [1], P. Richette[2], P. Le Van[3,4], J.-M. Correas[5], M. Resche-Rigon[6]. [1]*French-Vietnamese Gout Research Center, Vien Gut general clinic, Ho Chi Minh City, Viet Nam*; [2]*Rheumatology, hôpital Lariboisière, Paris, France*; [3]*Radiology, Cho Ray Hospital*; [4]*university of Medicine and Pharmacy, Ho Chi Minh City, Viet Nam*; [5]*radiology, Hôpital Necker*; [6]*Biostatistics, hôpital Saint Louis, Paris, France*

Background: Renal medulla crystal deposits have been demonstrated by pathology in severe gout but little studied by ultrasound (US) scan.
Objectives: To assess the frequency of hyperechoic renal medulla (HERM) in gouty patients and factors associated with their development.
Methods: Renal US scan using a Ecube 9 echograph (Alpinion S. Korea), was performed in gout patients (ACR/EULAR criteria) consecutively seen at the Vien Gut general clinic, Ho Chi Minh City, Vietnam, and receiving no ULT at presentation. Age and sex of patients, gout features, associated diseases, serum (S) uric acid (UA), eGFR (MDRD), urinary lab stick parameters, urine UA/creatinine ratio, and fractional clearance of urate (FCU) were recorded. Patients with HERM were counted and compared with those who had no medullary deposits by the Wilcoxon rank sum test for continuous varables and the Fischer exact test for categorical variables. Multivariable logistic model was used to assess relation between variables at inclusion in the study and presence of medulla deposits.
Results: 503 consecutive patients (500 males) were included. They had a median age of 46 years, median BMI of 25 kg/m2, median gout duration of 4 years. 280 (56%) had clinical tophi, 154 (31%) urate arthropathy, 28;[5]6%) urolithiasis, 112 (22%) hypertension, 58 (11.5%) type 2 diabetes, 5 (1%) coronary heart disease. Their median eGFR was 78 ml/min, SUA 423 micromol/L, FCU 0.063, urine UA/creatinine ratio 0.253, urinary pH 6.
Diffuse and bilateral HERM on the B mode with frequent twinkling artefacts on the Doppler mode was identified in 181 (36%) of the 503 patients. Univariate analysis showed that HERM associated with higher age, longer duration of gout, clinical tophi, urate arthropathy (p<0.0001 for each of the variables), higher uricemia (p=0.001), hypertension (p=0.0008), CHD (p=0.0006), lower eGFR (p<0.0001), leucocyturia (p=0.02), proteinuria (p=0.02). No association with US-diagnosed urolithiasis, hematuria, urine UA/creatinine ratio, FCU and urinary pH was found. In multivariate analysis, log of the duration of gout (0R: 2.22 (CI: 1.63–3.08), p<0.001), clinical tophi (OR: 8.21 (4.23–16.91) p<0.001), urate arthropathy (OR: 3.74 (2.18–6.52, p<0.001), and lower eGFR (OR: 0.86 (0.75–0.99) for each 10 ml/min decrease, p=0.04) were significantly associated with HERM.
Conclusions: In our gout population, HERM was observed in 36% of patients, correlated with decreased renal function, and clearly associated with severe gout, but not with features of uric acic lithiasis.
Disclosure of Interest: T. Bardin Consultant for: Grunnenthal, Ipsen Menarini, Astrazeneca, NovartisSobi, K. M. Tran: None declared, Q. D. Nguyen: None declared, N. H. Le : None declared, P. Richette Consultant for: Grunnenthal, Ipsen Menarini, Astrazeneca, NovartisSobi, P. Le Van: None declared, J.-M. Correas: None declared, M. Resche-Rigon: None declared
DOI: 10.1136/annrheumdis-2018-eular.5027

OP0358 WHY DO NOT ALL CHRONIC KIDNEY DISEASE PATIENTS GET GOUT? IMPAIRED NEUTROPHIL CHEMOTAXIS IN HYPERURICEMIA-RELATED CKD

Q. Ma[1], M. Sellmayr[1], F. Preitner[2], H.-J. Anders[1], S. Steiger[1]. [1]*Klinikum der Universität München, Medizinische Klinik und Poliklinik IV, Nephrologisches Zentrum, München, Germany*; [2]*University of Lausanne, Center for Integrative Genomics, Lausanne, Switzerland*

Background: One characteristic feature of acute gout is the infiltration of neutrophils into the inflamed joints, where they recognise monosodium urate (MSU) crystals leading to an acute inflammatory response. The development of chronic kidney disease (CKD) is associated with increased serum uric acid (UA) levels also known as hyperuricemia, a major risk factor for gout. Despite hyperuricemia, acute gout is less frequent in CKD patients. However, the effects of hyperuricemia on leukocyte chemotaxis in CKD are not fully understood.

Ann Rheum Dis: first published as 10.1136/annrheumdis-2018-eular.4500 on 12 June 2018. Downloaded from http://ard.bmj.com/ on June 24, 2024 by guest. Protected by copyright.

Objectives: We hypothesised that hyperuricemia affects neutrophil chemotaxis in CKD patients. Furthermore, we made use of a novel mouse model of chronic uric acid nephropathy.

Methods: ***Human study***: Serum was collected and neutrophils isolated from CKD patients or healthy subjects. Serum BUN (blood urea nitrogen), creatinine and uric acid levels were measured. Neutrophil transwell assays were carried out and the number of migrated neutrophils towards fMLP, human IL-8 determined by flow cytomentry. ***Animal study*:** Six week old Alb-creERT2;Glut9$^{lox/lox}$ mice (ki/ki) and mice without active Cre (+/+) were injected with tamoxifen. The ki/ki mice received either a high fat diet with Inosine (HFD+Ino) to induce hyperuricemia-associated CKD or a chow diet with Inosine (Chow+Ino) to induce only hyperuricemia without CKD. Control +/+mice either received HFD+Ino or Chow+Ino diet. After two weeks, all groups were injected either with MSU crystals or vehicle into a preexisting air pouch, a mouse model for acute gouty arthritis. After 12 hours, neutrophil infiltration and the extent of inflammation were assessed via flow cytometry, ELISA, and colorimetric assays.

Results: ***Human study*:** Compared to healthy subjects, CKD stage 5 patients presented with significant higher levels of serum BUN (14.1 vs 52.1 mg/dl, p=0.001), creatinine (1.5 vs 9.3 mg/dl, p=0.001) and UA (2.3 vs 10.3 mg/dl, p=0.001). Neutrophils from CKD patients showed an impaired migratory ability due to the down-regulation of the adhesion molecules P-Selectin and αβIntegrin. ***Animal study*:** Two weeks post-HFD +Ino, ki/ki mice developed hyperuricemia-associated CKD (serum UA: 10–14 mg/dl; BUN: 80 mg/dl), whereas the ki/ki mice on Chow+Ino diet became hyperuricemic without CKD. Control +/+mice on both diets did neither develop hyperuricemia nor CKD. Interestingly, the number of infiltrating neutrophils into the air pouch was reduced in hyperuricemic ki/ki mice with CKD as well as in hyperuricemic ki/ki mice without CKD compared to +/+control mice. We observed less inflammation indicated by decreased IL-1β, TNFα, CXCL1 and myeloperoxidase levels, and a down-regulation of adhesion molecules on infiltrated neutrophils in hyperuricemic ki/ki mice with CKD compared to +/+control mice.

Conclusions: Our data show that neutrophils from CKD patients are less able to migrate, which was consistent with data from our novel mouse model demonstrating that hyperuricemia impairs neutrophil chemotaxis in MSU crystal-induced inflammation. This indicated that the mechanism for defective neutrophil migration might be responsible for the lower incidence of acute gouty arthritis in hyperuricemic CKD patients.

Acknowledgements: This work was supported by grants from the Deutsche Forschungsgemeinschaft and the LMUexcellent junior researcher fund.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.4500

SATURDAY, 16 JUNE 2018

Big data in pre-clinical research

OP0359 EXPLORATION OF T-CELL SIGNATURES FOLLOWING TCR STIMULATION USING SINGLE CELL RNA-SEQ TO INFORM TREATMENT RESPONSE STUDIES IN RHEUMATOID ARTHRITIS

P. Martin[1], J. Ding[1], B. Mulhearn[1], S. Viatte[1], S. Eyre[1,2]. [1]*Arthritis Research UK Centre for Genetics and Genomics, Centre for Musculoskeletal Research, Manchester Academic Health Science Centre, The University of Manchester*; [2]*NIHR Manchester Musculoskelatal Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester, UK*

Background: For rheumatoid arthritis (RA), as with many other rheumatic diseases, the importance of determining which therapy will work best, early in disease, to prevent further progression, is an important area of research. Progress in treatment response has been limited, possibly due to the complex interplay between various cell types. As such, specific T-cell signatures, determined by single cell RNA-Seq (scRNA-Seq), could be predictive of future response to treatments such as anti-TNF biologic therapies.

Objectives: Our aim was therefore to determine the optimal study design and to assess the potential of scRNA-Seq to identify T-cell signatures under resting and stimulated conditions to inform future studies.

Methods: Primary CD4 +T cells were either stimulated using anti-CD3/CD28 beads or subjected to the same conditions without stimulation for 4 hours. Single cells were isolated using the 10X Genomics Chromium Controller with a target recovery of 6000 cells. Each scRNA-Seq library was sequenced on 4 Illumina HiSeq 4000 lanes (~200K reads/cell) and processed using the cellranger pipeline. Further quality control and cluster analysis was performed using Seurat.

Results: For the unstimulated sample 5,586 cells were recovered and after quality control and filtering, 5,387 cells remained. Similarly, for the stimulated sample, 4,621 cells were recovered and 4473 remained. This resulted in an average of 1094 and 1456 genes per cell. Similar clusters were seen after downsampling the stimulated dataset to 1 lane (~379M reads,~82K reads/cell), suggesting that CD4 +T cells are defined by large gene expression changes rather than subtle variations, consistent with protein expression data. Cluster exploration allowed the identification of several typical CD4 +T cell populations, including naïve, helper and regulatory. Furthermore, alignment of the two conditions in Seurat, identified classical and non-classical markers of activation, such as CD69, CCR7, MYC and PIM3. Finally, the relative cluster location and the expression of indicative markers suggested evidence of a progression from a naïve cell state to an 'active' effector state.

Conclusions: This data has provided important insights into future study design and confirmed the potential of scRNA-Seq to identify T-cell signatures. Importantly, despite obvious expression changes, cluster identity was maintained between stimulatory conditions. This implies it is possible to directly compare scRNA-Seq expression profiles between patient samples showing different disease activity without confounding the conclusions and enable the use of scRNA-Seq to investigate its predictive potential in RA treatment response. We are therefore in the process of expanding this work to study patient samples and different cell types. For example we have already generated similar data for monocytes on 3 RA samples and 3 healthy samples.

Acknowledgements: We would like to acknowledge the Faculty of Biology, Medicine and Health Genomics Facility, the assistance given by IT Services and the use of the Computational Shared Facility at The University of Manchester. This work was supported by the Wellcome Trust [105610/Z/14/Z].

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.3236

OP0360 RNA-SEQUENCING OF 800 HUMAN BLOOD SAMPLES REVEALS SHARED AND UNIQUE EXPRESSION PROFILES ACROSS SEVEN SYSTEMIC AUTOIMMUNE DISEASES

S. Babaei[1], Z. Makowska[1], S. Hayat[1], A. Buttgereit[1], G. Barturen[2], J. Kageyama[1], B. Weiss[1], J. Wojcik[3], R. Lesche[1], M. Alarcón Riquelme[2], F. McDonald[1], on behalf of Precisesads Clinical Consortium. [1]*BAYER AG, Berlin, Germany*; [2]*GENYO (Centre for Genomics and Oncological Research), Granada, Spain*; [3]*Quartz Bio, Geneva, Switzerland*

Background: Systemic autoimmune diseases (SADs) are chronic inflammatory conditions with limited treatment options. Although SADs encompass different clinical diagnoses, many of them share common pathophysiological mechanisms and have similar clinical manifestations. Therefore, defining a precise diagnosis and consequently an appropriate treatment is complex.

Objectives: We aimed to identify characteristic expression profiles for patients diagnosed with different SADs and find specific biomarkers for each disease based on whole blood RNA-seq data.

Methods: As part of the ongoing IMI PreciseSADS project we generated and analysed globin-depleted, polyA-selected RNA-seq data from an initial subset of 800 peripheral blood samples of healthy controls and patients with seven different SADs: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), Sjögren`s syndrome (SSj), mixed connective tissue disease (MCTD), undifferentiated connective tissue disease (UCTD) and primary antiphospholipid syndrome (PAPS). Differential gene expression analysis was performed with DESeq2 and biomarker discovery was achieved using linear support vector classifier-based feature elimination and logistic regression-based estimator with cross-validation.

Results: We identified unique and common genes that were differentially expressed between controls and each of the seven SADs. The greatest extent of transcriptional dysregulation was found in SLE and MCTD patients, while UCTD, SSc and RA showed fewest differentially expressed genes. We found large and statistically significant overlaps between the lists of differentially expressed genes for each disease, with SLE, MCTD, SjS and UCTD showing most pronounced similarity at the gene expression level. The overlapping genes were enriched in interferon signalling pathway and the classical complement pathway. Low overlap was found between SjS and RA, and SjS and SSc.

We also looked for unique gene expression patterns in each disease with the aim of identifying potential biomarkers. We were able to define gene signatures differentiating between SADs and controls and also between SAD pairs.

Conclusions: Even though we were able to identify a limited number of disease specific signature genes, there are extensive and statistically significant overlaps in gene expression profiles of the seven investigated SADs. Similar to the clinical manifestations, the data presented here suggest that also on the molecular level, these diseases share a large portion of their pathophysiology. Work is ongoing to expand the dataset for confirmation of these preliminary data.

Acknowledgements: The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115565, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in kind contribution.

Disclosure of Interest: None declared

Ann Rheum Dis: first published as 10.1136/annrheumdis-2018-eular.4500 on 12 June 2018. Downloaded from http://ard.bmj.com/ on June 24, 2024 by guest. Protected by copyright.