flow cytometry, in relation to clinical parameters and previously established LN classes assessed according to the ISN/RPS 2003 classification.

Results Lymphocytes percentages in class IV were different from classes III, V or a combination of III and V. In the latter classes, the percentages of the Tregs and Th17 cells were significantly lower, whereas in class IV the increase in FOXP3 in the Tregs and Th17 cells over six months period was significantly higher (Table 1). Changes in glomerular filtration rate and SLEDAI within 5 years did not correlate with single or repeated Tregs measurements.

Conclusions Differences in lymphocyte proportions between class IV and other classes may suggest its distinct pathogenesis and warrants further investigations on their role as LN biomarker.

272 CLINICAL SIGNIFICANCE OF ANTI-DNA/NR2 ANTIBODIES IN DE NOVO NPSLE AND POST-STEROID NPSLE

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10.1136/lupus-2017-000215.272

Background and aims Anti-DNA/NR2 antibodies are a subset of anti DNA autoantibodies that cross-react with the extracellular domain of the GluN2A/GluN2B subunits of the N-methyl-d-aspartate receptor 2 (NR2), which induce apoptosis of hippocampus neurons and psychiatric disorder in mice and humans. Neuropsychiatric SLE (NPSLE) can develop after initiation of steroid (post-steroid neuropsychiatric manifestation: PSNP) or before treatment (*de novo* NPSLE). The objective of this study was to clarify the prevalence of anti-DNA/NR2 antibodies in PSNP-SLE and *de novo* NPSLE

Methods This study involved a cohort of patients with NPSLE who were admitted to Hokkaido University Hospital. NPSLE patients were classified into two groups, *de novo* NPSLE and PSNP-SLE. Serum anti-DNA antibodies and anti-DNA/NR2 antibodies were measured using in-house ELISAs.

Results Serum samples were obtained from 29 patients with *de novo* NPSLE, 26 with PSNP-SLE and 83 healthy controls (HC). The levels of anti-DNA antibodies in patients with *de novo* NPSLE and PSNP-SLE were significantly higher than those in healthy controls (*de novo* NPSLE, PSNP-SLE, HC: 1.34±0.09, 1.40±0.14, 0.33±0.03, $p<0.0001$). The levels of anti-DNA/NR2 antibodies were highest in *de novo* NPSLE and in PSNP-SLE and HC (*de novo* NPSLE, PSNP-SLE, HC: 0.75 ±0.10, 0.60±0.07, 0.49±0.03). In PSNP-SLE, the frequency of mood disorders was higher than that in *de novo* NPSLE (58% vs 31% $p<0.05$).

Conclusions The levels of anti-DNA/NR2 in PSNP-SLE are lower than in *de novo* NPSLE, indicating the differences in the pathogenesis of these two conditions.

273 URINARY TWEAK LEVELS AS BIOMARKER OF LUPUS NEPHRITIS IN COLOMBIAN SLE PATIENTS

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10.1136/lupus-2017-000215.273

Background and aims TNF-like WEAK inducer of apoptosis (TWEAK), a TNF ligand superfamily is mainly produced by monocytes/macrophages, and is widely expressed at the RNA level in tissues including kidneys. The usefulness of urinary TWEAK (uTWEAK) to identify renal involvement in Mestizo and African-Latin American (ALA) SLE patients has not been examined yet.

Methods Patients meeting the revised ACR criteria for SLE were recruited from 2 different centres at Medellín and Baranquilla, Colombia. uTWEAK were measured using an ELISA kit (R and D system, USA)

Results 158 SLE patients were recruited (89% female) with median age of 32.8±12.1 years and median disease duration of 7.27±6.6 years. Mestizo (77%) and ALA (20%) were majority. 64% of patients had lupus nephritis (LN). 50 out of 71 biopsy proven LN had proliferative forms. Mean SLEDAI score was 8.5±8.7. LN patients (2803±6086 vs 672±1042, $p=0.013$) (Fig 1A) and ALA patients (3995±9656 vs 1618 ±2653, $p=0.002$) had significant higher levels of uTWEAK. uTWEAK levels were higher in patients with active LN and in Class V LN (Fig. 1B). uTWEAK levels were significantly correlated with 24 hours proteinuria, SLEDAI (Fig. 1C) and serum anti-C1q titers. An ROC curve constructed showed a good level of sensitivity and specificity (Fig. 1D)

Conclusions In our cohort of Colombian SLE patients, uTWEAK levels were 4 and 2 times higher in LN patients and ALA respectively. uTWEAK were significantly higher in active LN and were correlated with disease activity, proteinuria and anti-C1q antibodies.

274 PREVALENCE OF ANTI-DFS70 ANTIBODIES IN A COLOMBIAN COHORT: A CASE-CONTROL STUDY

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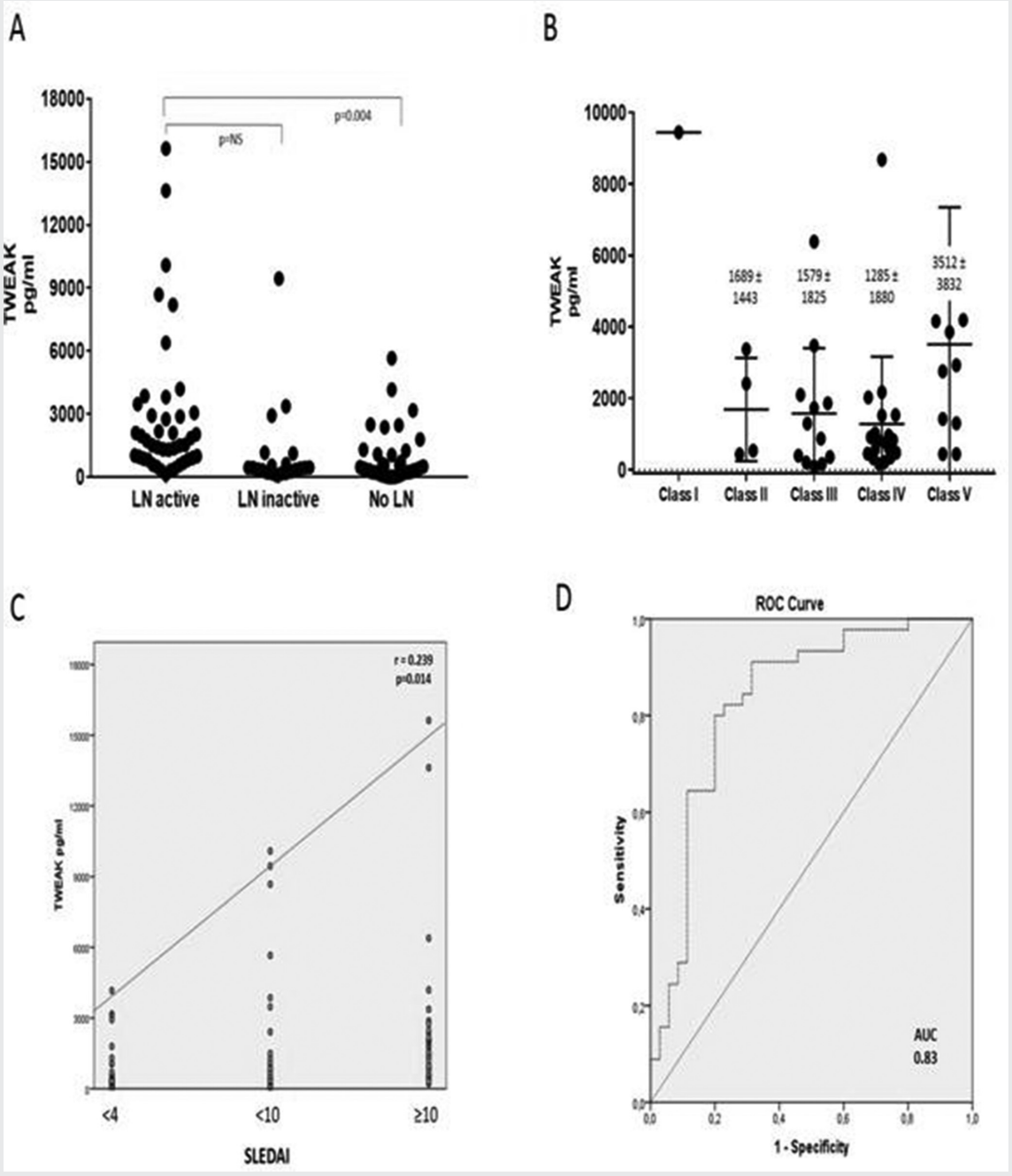
10.1136/lupus-2017-000215.274

Background and aims Anti-dense fine speckled 70 (anti-DFS70) antibodies were initially identified as an ANA IIF pattern from patients with interstitial cystitis; however, some recent studies showed that anti-DFS70 antibodies are common among ANA

Abstract 273 Figure 1



positive individuals with no evidence of systemic autoimmune disease (SAD)(Mahler M. 2012). Information of anti-DFS70 in Latin-American countries is very limited. We determined the prevalence of Anti-DFS70 antibodies in a Colombian cohort.

Methods We evaluated individuals≥18 years old, including 100 SLE patients, 102 SADs, 200 healthy controls, and 56 subjects suspected of having autoimmune disease with ANA positive and negative anti ds-DNA antibodies. The presence of anti-DFS70 antibodies was determined by QUANTA Flash by chemiluminescent techniques (Inova/Werfen, San Diego)

Results Our final cohort included 458 samples. The mean age of SLE patients was 33±12 years, for SADs was 41±19 and for healthy controls was 36±10 years. The main diagnoses of SAD were: Vasculitis (n=28), RA (n=21), Systemic sclerosis (n=12), primary antiphospholipid syndrome (n=11), dermatomyositis (n=10) among others. Racial/ethnic breakdown was: 76% Mestizo and 20% Afro-latin Americans. Anti-DFS70 antibodies were positive in 1.8% of subjects with ANAs positive/anti DNA negative, in 1% of SLE patients, 0.9% of patients with other SADs and in 0.5% of healthy controls. Given the low prevalence of anti-DFS70 antibodies, no clinical correlations were possible.

Conclusions Despite anti-DFS70 antibodies are a good diagnostic tool for discrimination among healthy individuals and SADs (including SLE), we found a very low prevalence of anti-DFS70 antibodies in our Colombian cohort.

275 IDENTIFICATION OF MICRORNA PREDICTIVE OF TREATMENT RESPONSE IN LUPUS NEPHRITIS

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10.1136/lupus-2017-000215.275

Background and aims High dose corticosteroids and cyclophosphamide are commonly used to treat LN. Although effective in preventing end stage renal disease (ESRD) in most cases, significant long-term side effects such as infections, increased risk of malignancy, and infertility are common and are related to the duration of therapy or the cumulative dose of medications. There are currently no markers that can reliably determine response or refractoriness to treatment at an individual level. MicroRNAs are small, non-coding RNAs responsible for post-transcriptional regulation, have been shown to have altered expression levels in a variety of diseases suggesting their potential use as biomarkers. We propose miRNAs can be predictive markers for response to cyclophosphamide.
Methods RNA was isolated and analysed via TaqMan Array MicroRNA 384 well Cards, from formalin-fixed paraffin embedded (FFPE) renal biopsies of two cohorts of patients with LN who were subsequently treated with cyclophosphamide with at least 2 years of follow up history. Patients who responded to cyclophosphamide based on urinalysis criteria of no active urinary sediments, no RBCs or WBCs in urine, and proteinuria less than 1 gram were classified as responders while those that did not fit the criteria were classified as non-responders. Significantly deferentially expressed miRNAs, determined via $2^{\Delta\Delta Ct}$ method, from the first cohort were validated by the second cohort.
Results Six significantly up-regulated miRNAs, hsa-miR-30c-2-3p, hsa-miR-29b-1-5p, hsa-miR-195-3p, hsa-miR-424-3p, hsa-miR-1260a, and hsa-miR-1248 were found in responders.
Conclusions These miRNAs may act as prognostic markers of renal outcomes and treatment response, which can establish a more personalised treatment of lupus nephritis in the future.

276 LOW-DOSE IL-2 CIRCUMVENTED MTOR SIGNALLINGSIGNALING IN T CELLS IN THE TREATMENT OF SLE

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10.1136/lupus-2017-000215.276

Background and aims mTOR signalling is proved to be one of the most important pathway in the pathogenesis in SLE. However, in patients with SLE, whether mTOR pathway can be activated by low-dose IL-2 remained unclear.This study is to clarify the effects of low-dose IL-2 therapy on mTOR signalling in the treatment of SLE.
Methods Eight patients with active SLE were treated with 1 million IU IL-2. Phophrylation of S6 ribosomal protein (S6RP), AKT and pSTAT5 were measured before and after the first 2 week of low-dose rhIL-2 administration. C57BL/6 mice (male, 8–12 weeks old) were intraperitoneally immunised with SRB and followed by administration of different doses (low:10 000 IU and hight:3 00 000 IU) of rhIL-2 or PBS from day 3 to day 9. The ratio of Th1, Th2, Tfh, Th17, Tfh and Treg as well as the level of S6RP, AKT and pSTAT5 were assayed by flow cytometry.
Results Low-dose IL-2 was efficient and well tolerated in active SLE, and was associated with expansion of Treg cells ($p<0.001$) and reductions of Tfh and Th17 cells ($p=<0.001$). No significant change of pS6RP and pAKT was observed. On the other hand, there was a signfciant induction of the activation of STAT5. In mouse studies, low-dose IL-2 inhibited the differentiation of Th17 cells and Tfh cells. Comparing with high dose IL-2 group, there was no significantly increased mTOR activity after low-dose IL-2 administration.
Conclusions Low-dose IL-2 might circumvent mTOR pathway and play a regulatory role in the T cells in lupus.

277 USEFULNESS OF SOLUBLE PD-1 IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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10.1136/lupus-2017-000215.277

Background and aims Programmed cell death protein 1 (PD-1/CD279) is a cell surface receptor that belongs to the extended CD28/CTLA-4 family and is expressed on T cells and pro-B cells. PD-1 plays an important role in down regulating the immune system by preventing the activation of T-cells. Soluble PD-1 (sPD-1), which is produced by the alternative splicing, can functionally block the regulatory effect of membrane-bound PD-1 on T cell activation. We aimed to retrospectively evaluate the usefulness of sPD-1 in patients with systemic lupus erythematosus (SLE).
Methods We measured the levels of sPD-1 by enzyme-linked immunosorbent assay (ELISA) kit in sera of patients with SLE (n=59) and systemic sclerosis, and healthy controls, and compared them. We also analysed the association between the levels of sPD-1 and clinical information in patients with SLE.
Results The levels of sPD-1 in SLE patients with SLEDAI-2K$\geq$6 were significantly higher than those in SLE patients with SLEDAI-2K<6, patients with systemic sclerosis, and healthy controls ($p<0.05$ in all comparisons), whereas there was no significant difference in other comparisons. In SLE patients, the levels of sPD-1 were moderately correlated with the titers of anti-ds DNA antibodies and SLEDAI-2K, and were moderately and inversely correlated with the levels of C3 and C4. In addition, the levels of sPD-1 were significantly higher in SLE patients with arthritis, mucosal ulcers, fever, leucopenia, or anaemia than those without ($p<0.05$ in all comparisons).
Conclusions The present study suggested that sPD-1 can serve as a new biomarker reflecting disease activity in patients with SLE.