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Oral Presentations

PL1 Cutting Edge of Lupus Research Plenary

PL1.01 & PO1.E.1

Retroelements as trigger of lupus
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Objectives: We tested the hypothesis that lupus disease is caused by, or contributed to, so-called endogenous retroelements—genomic segments of 'selfish' DNA. Forty percent of our genome consist of such retroelements, many of them active. As an indication that the retroelements may cause lupus disease, patients that lack the enzyme Trex1 suffer from systemic lupus erythematosus, chilblain lupus or Aicardi-Goutières syndrome—an inflammation of the brain, with lupus disease developing in the course of the disease. Trex1 is a DNA exonuclease that degrades retroelement cDNA. Results and Conclusions: To show that unrestricted retroelements contribute to autoimmune disease, we increased the concentration of endogenous retroviral cDNA in B/W mice and, thereby, exacerbated lupus disease. Conversely, to ameliorate autoimmune disease, we introduced human APOBEC3 (abbreviated A3) enzymes into mice that lack Trex1. The lack in Trex1 makes the mice inefficient in retroelement restriction; the single mouse APOBEC3 enzyme does not cover a wide range of retroelements, whereas the seven human A3 enzymes do. We will present data to show that human A3 enzymes are advantageous in Trex1-deficient mice with autoimmune disease. This indicates that retroelements are indeed a cause of the disease.

PL1.02 & PO1.E.2

Long-lived plasma cells adoptively transferred from NZB/W mice cause immune complex nephritis in immunodeficient Rag1-/- mice
Cheng, Qingsen1; Muntaz, Immaculate R.1; Hoyer, Bimba F.1; Radbruch, Andreas2; Hiepe, Falk1

Previously, we showed that long-lived plasma cells refractory to immunosuppression significantly contribute to autoantibody production in NZB/W mice used as a model of lupus nephritis. Since immunosuppressive and B cell depletion therapy affecting only short-lived plasmablasts and plasma cells can induce remission of the disease, the aim of this study was to elucidate the role of autoreactive long-lived plasma cells in the pathogenesis of lupus nephritis. For this purpose, CD138+ plasmablasts and plasma cells lacking B and T cells were isolated from spleens of >6 month-old NZB/W mice with high levels of anti-dsDNA antibodies and adoptively transferred to immunodeficient Rag1-/- mice (3 000 000 cells/mouse). Shortly after transfer, total IgG and IgM as well as IgG and IgM anti-dsDNA antibodies were detected in the recipient mice. The levels of these antibodies remained constant for 21 weeks after plasma cell transfer. Renal immunohistology showed immune complex nephritis with deposits of IgG, IgM and C3. The findings show that autoantibodies secreted by long-lived plasma cells can cause nephritis and suggest that long-lived plasma cells refractory to conventional immunosuppression and B cell depletion should be considered as candidate targets for future therapeutic strategies.

PL1.03 & PO1.E.3

Defective migration of tissue-resident dendritic cells: a novel pathogenesis of cutaneous lupus
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Objectives: Tissue-resident dendritic cells (DC) are believed to carry antigens from tissues to tissue-draining lymph nodes to induce immunity. We posit that this important function of DC plays a role in the pathogenesis of lupus. Using skin-resident DC as a model of tissue DC, we investigated the role of DC migration, and underlying mechanisms, in cutaneous lupus. Methods: We assessed skin-DC migration using ex vivo and in vivo assays in MRL/Mp-j-Fas-lpr (MRL-lpr) and MRL/Mp-j-Fas+/+ mice that develop lupus dermatitis. Skin harbors DCs that express langerin (Lang) and reside in epidermis and dermis, hereafter called Langerhans cells (LC), and Lang-negative DCs in dermis, called dermal DC. Subsequently, we generated MRL-lpr mice that express enhanced green-fluorescent-protein (EGFP) driven by a Lang promoter by introgressing the Lang-EGFP knock-in mutation from B6 background, which allows visualization of LC in vivo. We then tested whether modulating skin-DC migration affects lupus dermatitis. Results: LCs from MRL mice exhibited a profound defect in their ability to emigrate from the epidermis, to migrate through the dermal lymphatics, and to immigrate into skin-draining lymph-nodes (Eriksson and Singh, J Immunol Cutting Edge, 2008). Dermal DCs (Lang-negative), but not blood-derived DCs, also exhibited a similar defect in MRL strains. Since conventional DC migration assays require their activation, we subsequently used Lang-EGFP knock-in mice to assess LCs at steady-state. The proportion of LC (EGFP+ cells) was markedly lower in skin-draining lymph-nodes of MRL-lpr mice than in B6 mice, thus confirming what we found using inbred mice. Importantly, skin-DC migration defect precedes the onset of inflammation and correlates with the severity of dermatitis. Conversely, treatment with glycolipid α-galactosylceramide that binds CD1d restores skin-DC migration and ameliorates dermatitis, whereas deficiency of CD1d in MRL-lpr mice worsens dermatitis. Surprisingly, α-galactosylceramide-mediated increase in skin-DC migration does not require NKT-cells, but is associated with an expansion of epidermal γδ T-cells in a CD1d-dependent manner in MRL-lpr mice. Furthermore, CD1d and epidermal γδ T-cells play a physiological role in skin-DC migration, since the genetic deficiency of CD1d or γδ T-cells, but not of NKT-cells, in normal backgrounds reduces skin-DC migration. Finally, epidermal γδ T-cells are reduced in MRL mice as compared to control mice. Conclusions: We elucidate a novel mechanism, whereby CD1d-dependent epidermal γδ T-cells normally facilitate skin-DC migration from skin to skin-draining lymph nodes. This regulatory mechanism is disrupted in lupus dermatitis-prone mice, providing evidence for a novel pathogenetic mechanism of cutaneous lupus.
Background & Objectives: SLE is associated with increased risk of coronary heart disease (CHD) and is itself an independent risk factor for clinical and subclinical atherosclerosis. Dysfunctional circulating endothelial progenitor cells (EPCs) may contribute to abnormal endothelial repair and subsequent atherogenesis. Our aims were to determine whether EPC number and/or function are impaired in SLE patients and to examine potential mechanisms for abnormalities. Methods: Patients with SLE (ACR 1997 criteria) had a clinical assessment of disease activity (SLEDAI-2k) as a measure of early atherosclerosis. Peripheral blood mononuclear cells were isolated from patients and healthy controls. Cells were labelled with fluorescently-conjugated CD133 and CD34 antibodies and corresponding isotype controls (FITC or PE) to identify EPCs using flow cytometry. Formation of colony-forming units (CFU) following 7 days in culture was utilised to measure EPC function. Ageing phenotype of EPC-CFU was determined by extracting DNA and measuring relative telomere length using real-time quantitative PCR. Results: We studied 56 patients and 48 controls; median(IQR) age was 54(47, 58) and 46(30, 58) years respectively. The mean(SD) percentage of CD34+CD31+ EPCs in SLE was not different to controls [0.03(0.02)% vs. 0.02(0.02)%; P=NS]. We demonstrated a greater percentage of patients with undetectable EPCs in SLE patients compared with controls (27% vs. 18%, P=0.02). Vascular repair mechanisms may particularly affect the early stages of atherogenesis such as vascular stiffness. Improving the reparative capacity of EPCs may represent a novel therapeutic target to attenuate CHD risk in this population.

Objective: Systemic lupus erythematosus (SLE) is a highly heterogeneous disorder, characterized by differences in autoantibody profile, serum cytokines, and clinical manifestations. SLE-associated autoantibodies and high serum interferon alpha (IFN-α) are important heritable phenotypes in SLE which are correlated with each other, and play a role in disease pathogenesis. These two heritable risk factors are shared between ancestral backgrounds. The aim of the study was to detect genetic factors associated with autoantibody profiles and serum IFN-α in SLE. Methods: We undertook a case-case genome-wide association study of SLE patients stratified by ancestry and extremes of phenotype in 450 SLE patients and 522 controls using a multi-step screening approach based on novel metrics and expert database review. The seven loci were: leucine-rich repeat containing 20 (LRRRC20); protein phosphatase 1H (PPM1H); lysophosphatidic acid receptor 1 (LPAR1); ankyrin repeat and sterile alpha motif domain 1A (ANKS1A); protein tyrosine phosphatase, receptor type M (PTPRM); ephrin A5 (EFNA5); and V-set and immunoglobulin domain containing 2 (VISIG2). Results: SNPs in the LRRRC20, PPM1H, LPAR1, ANKS1A, and VSIG2 loci each demonstrated strong association with a particular serologic profile (all OR>2.2 and p<8x10-4). Each of these serologic profiles was associated with increased serum IFN-α. SNPs in both PTPRM and LRRRC20 were associated with increased serum IFN-α independent of serologic profile (p=3.2 x 10-6 and p= 5.0 x 10-3 respectively). None of the SNPs were strongly associated with SLE in case-control analysis, suggesting that the major impact of these variants will be upon subphenotypes in SLE. Conclusions: This study demonstrates the power of using serologic and cytokine subphenotypes to elucidate genetic factors involved in complex autoimmune disease. The distinct associations observed emphasize the heterogeneity of molecular pathogenesis in SLE, and the need for stratification by subphenotypes in genetic studies. We hypothesize that these genetic variants play a role in disease manifestations and severity in SLE.
and 386 controls. Serum IFN-α activity was measured using a functional assay. Results: Logistic regression of phenotypic variation in European-de
derived subjects resolved the IRF5 SLE-risk haplotype into allelic associations with particular autoantibodies [rs2004640T with anti-dsDNA (OR=2.41, p=3.3x10-14), rs10488631C with anti-Ro (OR=2.46, p=2.8x10-13), and a minor haplotype with anti-La (OR=1.96, p=0.035)]. Greater than 70% of the attributable risk of SLE due to IRF5 alleles was found in subjects who had these particular antibodies (40% of the European cohort). Anti-nRNP and anti-Sm autoantibodies were not associated with IRFS alleles in European ancestry. Meanwhile, in African-Americans the European-derived SLE-risk haplotype was present due to admixture and demonstrated a similar associa
tion with both SLE susceptibility and autoantibodies. Interestingly, the IRF5 promoter indel was present on African chromosomes and not in LD with other haplotype tagging SNPs. This promoter indel was a strong SLE risk factor in African-Americans (OR=2.16, p value=7.2x10-7), and was associated with anti-nRNP antibodies in African-Americans. In SLE patients of both ancestral backgrounds, elevations in IFN-α related to IRF5 risk alleles only occurred in those who had the particular autoantibody associated with that allele. This supports a model as follows: IRF5 gene variant + specific autoantibody = high IFN and risk of SLE. Conclusions: These data suggest that specific SLE-
associated autoantibodies cooperate with IRF5 variants to dysregulate IFN-α production, and consequently increase risk of SLE. These results demonstrate the power of phenotypic variability when informed by genomic origin to dis-
entangle complex genetic relationships.

Proinflammatory SLE neutrophils induce vascular damage and synthesize IFNs
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Obectives: While a potential role for neutrophils in lupus pathogenesis and organ damage was described decades ago, the exact role that this cell subset plays in SLE has not been well characterized. Neutrophil-specific genes are abundant in PBMC microarrays from lupus patients due to presence of low density granulocytes (LDGs) in mononuclear cell fractions. However, the functionality and pathogenicity of these LDGs have not been characterized. We developed a technique to purify LDGs from lupus PBMCs and assessed their phenotype, function and potential role in disease pathogenesis and vascular damage. Methods: LDGs, their autologous lupus neutrophils and healthy control neutrophils were compared in their microbialid and phagocytic ac-
citivities, generation of reactive oxygen species, activation status, L-selectin shedding, inflammatory cytokine profile and type 1 IFN expression and sig-
natures. Since SLE patients display evidence of accelerated endothelial cell apoptosis not coupled by proper vascular repair, we also assessed the capacity of LDGs to kill endothelial cells and their antiangiogenic potential. Results: LDGs were present in all SLE samples studied and high number of these cells in peripheral blood correlated with the presence of vasculitis, skin involve-
ment and arthritis. When compared to control neutrophils and their auto-
 logical normal-density lupus neutrophils, LDGs display an activated phenotype, secrete increased levels of type 1 IFNs, TNF-α and IFN-γ, but show impaired phagocytic potential. Importantly, LDGs induce significant endothelial cell cytotoxicity and synthesize sufficient levels of type I IFNs to disrupt the cap-
acity of endothelial progenitor cells to differentiate into mature endothelial cells. Further, LDG depletion restores the functional capacity of endothelial progenitor cells and abolishes type 1 IFN synthesis. There was no significant correlation between age, disease duration and/or use of immunosuppressive drugs or corticosteroid dose and the presence of LDGs. Conclusions: We have characterized in detail the phenotype of a low-density neutrophil subset which appears to be present in higher numbers in SLE patients with distinct clinical manifestations. LDGs have preserved neutrophil function overall but display impairments in phagocytic potential, have a proinflammatory phenotype and display pathogenic features, including the capacity to synthesize type 1 IFNs and induce vascular damage. As such, LDGs may play an important dual role in premature cardiovascular disease development in SLE by simultaneously mediating enhanced vascular damage while inhibiting vascular repair. The po-
tential role of these cells in lupus pathogenesis (in part mediated by enhanced type 1 IFN synthesis) and on organ damage warrants further investigation.

Chromatin-activated neutrophils represent a major source of interferon-α
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Objectives: Chromatin fragments, especially oligo-nucleosomes, represent major autoantigens SLE. Nucleosomes (DNA-histones complexes) are present in the circulation of patients due to an impaired chromatin clearance. Interferon-α (IFN-α) plays an important role in SLE development (known as the IFN-α signature). IFN-α concentrations are increased in SLE patients and favor the differentiation of monocytes in dendritic cells (DC). Although plasmacytoid DC (pDC) are known to secrete IFN-α, this cell type represents a minor cell population. Moreover, only a few lupus stimuli have been re-
ported to induce IFN-α secretion by pDC. We have therefore investigated whether other stimuli and cell types may be responsible for the strong IFN-α secretion observed in SLE patients. Methods: Chromatin was purified from calf thymus. Peripheral blood mononuclear cells (PBMC) and neutrophils (polymorphonuclear leukocytes, PMN) were freshly isolated from the blood of healthy individuals. The different cell types were characterized by flow cytometry. Cells were activated with different stimuli and IFN-α production was estimated by flow cytometry upon intracellular staining. IFN-α secre-
tion was confirmed by ELISA. PMN activation was verified by measuring CD66b up-regulation (flow cytometry) and IL-8 secretion (ELISA). Results: We show for the first time that isolated PMN secrete IFN-α upon activation. Using both flow cytometry and ELISA, nucleosomes and CpG-oligonucleo-
tides (CpG-ODN, a Toll-like receptor (TLR) 9 ligand) were the best IFN-α inducers, although R848 (a TLR8 ligand) triggered also IFN-α secretion. On the contrary, lipopolysaccharides (TLR4 ligand) usually did not induce IFN-α. When autologous PMN and PBMC were mixed, PMN-derived IFN-α produc-
tion was often more pronounced, suggesting that several cell types interplay. In co-cultures, pDC also produced IFN-α upon activation with CpG-ODN and nucleosomes but to a lower extent. Even monocytes produced IFN-α in response to some stimuli. Nucleosome-induced IFN-α production by PMN corre-
lated to IL-8 secretion and CD66b up-regulation. PMN isolated from healthy donors and SLE patients are currently being compared. Conclusions: This is the first report showing both that activated PMN can secrete IFN-α and identifying the stimuli involved. PMN are known to play a major role in in-
flammation and IFN-α-producing PMN may participate in SLE pathogenesis, especially upon migration into tissues, such as kidneys. Since PMN are 200 times more frequent than pDC in the blood, they may represent a major source of IFN-α in SLE. The increased concentrations of circulating chromatin in combination with the capacity of PMN to secrete IFN-α may partly favor the break of the peripheral tolerance in patients.
Systemic lupus erythematosus (SLE) is the prototypic autoimmune disease, and like most human diseases SLE is genetically complex. This means that multiple genes, as well as non-genetic factors, contribute to disease risk and outcome. Until recently, the genetic complexity of SLE has been a major obstacle to the identification of genetic risk factors. However, recent developments in molecular genetic and statistical methodology have led to the discovery of a large number of genes that influence SLE risk. In particular, the successful completion of several genome wide association studies (GWAS) has led to the identification of over 20 SLE risk genes, many of which overlap research in this area. TNF-induced NF-κB activity in an in vitro system, thus suggesting functional significance of this variant. Of interest, multiple other autoimmune diseases, including rheumatoid arthritis, Crohn’s disease, type 1 diabetes, psoriasis and Celiac sprue have now been associated with genetic variation in this region, however the pattern of genetic association across these diseases is quite complex. As with most of the other recently identified risk genes, much additional work, including DNA sequencing to identify potential causal variants and functional studies will be required to more fully elucidate the nature of the recently identified genetic associations. Given the phenotypic heterogeneity of SLE, additional work will also be required to refine genotype-phenotype associations, as genetic associations are key to differentiating clinical and serologic SLE subgroups. Extension of this work to other ethnic populations is also likely to be particularly informative. Finally, future work in this field will focus on other potential sources of heritability, including rare and structural variants that have not been adequately examined in recent GWAS and epigenetic modifications such as DNA methylation.

PL2.2 Lessons from genetics

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Systemic lupus erythematosus (SLE) is the prototypic autoimmune disease, and like most human diseases SLE is genetically complex. This means that multiple genes, as well as non-genetic factors, contribute to disease risk and outcome. Until recently, the genetic complexity of SLE has been a major obstacle to the identification of genetic risk factors. However, recent developments in molecular genetic and statistical methodology have led to the discovery of a large number of genes that influence SLE risk. In particular, the successful completion of several genome wide association studies (GWAS) has led to the identification of over 20 SLE risk genes, many of which overlap with recent findings in other autoimmune diseases. Many of the recently identified SLE risk genes function in the toll-like receptor and type I interferon signaling pathways, supporting the fundamental importance of these pathways in disease. One recently identified risk gene, tumor necrosis factor alpha-induced protein 3 (TNFAIP3), which encodes the A20 protein, nicely illustrates several features of work in this area. TNFAIP3 is a negative regulator of NF-κB, a key transcription factor in inflammatory responses. Multiple genetic variants have been associated with SLE risk, including a nonsynonymous SNP that results in an amino acid substitution at amino acid 127 in the OTU domain. This variant has been shown to decrease the ability of the A20 protein to inhibit TNF-induced NF-κB activity in an in vitro system, thus suggesting functional significance of this variant. Of interest, multiple other autoimmune diseases, including rheumatoid arthritis, Crohn’s disease, type 1 diabetes, psoriasis and Celiac sprue have now been associated with genetic variation in this region, however the pattern of genetic association across these diseases is quite complex. As with most of the other recently identified risk genes, much additional work, including DNA sequencing to identify potential causal variants and functional studies will be required to more fully elucidate the nature of the recently identified genetic associations. Given the phenotypic heterogeneity of SLE, additional work will also be required to refine genotype-phenotype associations, as genetic associations are key to differentiating clinical and serologic SLE subgroups. Extension of this work to other ethnic populations is also likely to be particularly informative. Finally, future work in this field will focus on other potential sources of heritability, including rare and structural variants that have not been adequately examined in recent GWAS and epigenetic modifications such as DNA methylation.

PL2.3 Where are we in SLE? Landmarks and futurescapes.
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Recent years have shown progress in treatment and understanding pathogenesis. Direct comparisons between the highly targeted MP inhibitor, mycophenolate mofetil (MMF) and the multi-targeted cyclophosphamide (Cy) show these treatments are at least equivalent, with similar rates of serious adverse events. Cyt has significantly lower response rates in African Americans. Pathogenesis studies show an broad array of genes, epigenetic changes, cells, activation pathways, cytokines and molecular targets. Beyond the roles of autoantibodies, adaptive T cells and B cells in causing SLE, we have identified importance of other cell systems. Thirty-to-40 genes increase risk for SLE or clinical subsets; newer work studies gene combinations and epigenetic changes that alter gene transcription, including DNA hypomethylation and the influence of miRNAs. The innate immune system expands autoimmune response to RNA- and DNA-containing lupus antigens. Cytokines made by innate immune cells, such as IFN-α and TNFα, are targets of novel therapeutic trials. Initial work confirming the upregulation of interferon-induced genes in some patients also showed signals for upregulated neutrophil genes; recent data suggests low density granulocytes (increased in SLE) may be the source and are toxic to endothelium, perhaps facilitating vascular damage. There is interest in targeting cytokines that drive B cell maturation into plasmablasts, such as BLyS and IL-6. Among the many pathways inappropriately activated or inactivated in T and B cells of SLE patients, some can be targeted by small molecules, including various kinases. Moving beyond searches for new, safer, more effective treatments are strategies to prevent damage. Can we change progression to scarring and atherosclerosis by altering proteins that cause scarring (hepatic growth factor, TGFbeta) or by interfering with oxidative damage to tissues, cells and lipids? There is an intense search for identifying new biomarkers in urine or in serum/plasma that can identify lupus flares when they first begin, such as monocyt chemoattractant protein (MCP-1) or mRNA for Foxp3. Two additional questions are of interest – 1) will most patients respond to targeting a single molecule – or will they require general immunosuppression followed by highly targeted therapies to maintain improvement? 2) can we prevent SLE? Would vaccines against viruses such as EBV prevent one of the environmental triggers, that in a genetically and environmentally susceptible person, result in SLE? At our next meeting, we are likely to have some answers.
CS1.1 Type I interferons and premature cardiovascular disease in SLE Kaplan, Mariana I. University of Michigan Medical School, Ann Arbor, MI, USA

SLE is characterized by strikingly higher rates of premature cardiovascular disease, with up to a 50-fold increase over matched controls. While immune dysregulation observed in SLE may play the dominant role in atherogenesis, the exact mechanisms leading to enhanced cardiovascular risk in lupus remain to be determined. Our group previously reported that individuals with SLE and no traditional cardiovascular risk factors display a striking imbalance between endothelial cell damage and repair manifested by an increase in the levels of circulating apoptotic endothelial cells which is not coupled by proper endothelial repair, as shown by a significant decrease in the numbers and function of bone marrow derived endothelial progenitor cells (EPCs) and circulating myeloid angiogenic cells (CACs). High levels of circulating apoptotic endothelial cells in SLE strongly correlate with endothelial dysfunction, a surrogate marker of future atherosclerosis development. Our group previously reported that IFN-α induces EPC/CAC apoptosis and skews myeloid lineage cells towards nonangiogenic phenotypes including dendritic cells (DCs). Importantly, neutralization of type I IFN pathways restores a normal function of phenotype and function of EPCs. All these observations support a potential role for type I IFNs in the development of premature atherosclerosis and altered vasculogenesis in SLE. Our group has recently described a neutrophil subset in SLE which is proinflammatory and has the capacity to synthesize type I interferons. These cells are toxic to the endothelium and interfere with proper vasculogenesis through type I IFN activity. Ongoing studies are determining the role that type I IFNs have in progression of atherosclerosis and vascular damage in human and murine lupus.

CS1.2 & PO1.E.14 Monocytes from SLE patients with carotid artery plaque and dysfunctional HDL contain upregulated PDGFRbeta that enhances monocyte chemotaxis Skaggs, Brian J.; Hahn, Bevra H.; Sahakian, Lori; Grossman, Jennifer; McMahon, Maureen University of California, Los Angeles, CA, USA

Objectives: Accelerated atherosclerosis is a major co-morbid condition of women with SLE. Monocytes are the main immune cell involved in atherosclerosis initiation and progression. The presence of dysfunctional HDL and monocytes has been proposed to modulate atherosclerosis susceptibility, but the role of monocytes in accelerated atherosclerosis, might directly influence monocyte gene expression and function. Methods: Peripheral blood monocytes were isolated from 54 SLE patients. Subjects were stratified into three groups: 1) carotid artery plaque+piHDL+, 2) plaque+piHDL- and 3) plaque-piHDL- (n=18/group). Transcript levels of 84 atherosclerosis-specific genes were examined by real-time PCR-based gene arrays. RNA from the human monocyte cell line THP-1 was isolated and examined by real-time PCR after direct, acute treatment with normal, anti-inflammatory HDL or piHDL that was isolated from SLE patients. Monocyte chemokinesis and TNFα/monocyte chemoattractant protein-1 (MCP-1) secretion were measured in THP-1 cells after piHDL/HDL treatment and inhibitors of piHDL and platelet-derived growth factor receptor beta (PDGFRbeta). Results: PDGFRbeta was upregulated in both primary monocytes from patients with plaque plus piHDL and in THP-1 monocytes acutely treated in vitro with piHDL compared to normal HDL. MCP-1 transcript levels were significantly upregulated in plaque-piHDL+ subjects versus the other two groups, suggesting the presence of piHDL might cause monocytes to migrate into the subendothelial space and initiate atherosclerosis. Monocyte chemokinesis was enhanced after treatment with piHDL versus normal HDL. Abnormal migration of piHDL-treated monocytes was restored to levels observed in cells treated with normal HDL after in vitro treatment with the PDGFR inhibitor imatinib or an apo-mimetic peptide (which is known to reverse piHDL function). Increased piHDL-mediated TNFalpha protein levels in treated monocytes were also reduced with both inhibitors. Conclusions: Dysfunctional piHDL directly influences expression of a small number of transcripts and proteins in primary monocytes and a human monocyte cell line. Inhibition of piHDL through reducing piHDL oxidation using an apo-mimetic peptide or blocking PDGFRbeta kinase activity with imatinib restored monocyte migration to normal levels. These experiments suggest piHDL influences monocyte biology and could directly promote accelerated atherosclerosis in SLE patients.

Effect of rosuvastatin on homocysteine, hsCRP and endothelial markers in systemic lupus erythematosus (SLE): a randomized controlled trial Mok, Chi Chi¹; Lai, Judy¹; Wang, Chun Kwok²; Lam, Cheuk Sum²; 1. Tuen Mun Hospital, Hong Kong; 2. Department of Chemical Pathology, Prince of Wales Hospital, Hong Kong

Objectives: To study the effect of rosuvastatin (crestor) therapy on homocysteine, hsCRP and biomarkers of endothelial activation / injury in patients with stable SLE with subclinical atherosclerosis Methods: Asymptomatic SLE patients who had abnormal coronary calcification (Agatston score ≥1) or normal carotid intima-media thickness (IMT) (≥0.8mm at any site) by Doppler ultrasound were randomized in a double-blind manner into 2 arms: (1) rosuvastatin (10mg/day); or (2) placebo (one tab/day) for 12 months. Levels of homocysteine, hsCRP, sVCAM-1, P-selectin and thrombomodulin were measured at baseline, month 6 and month 12. SLEDAI scores were assessed at 2-month intervals. Results: 138 SLE patients were invited for screening of atherosclerosis but 6 declined. Fifty patients did not have atherosclerosis, 2 were not eligible and 8 declined to participate. Finally 72 patients were enrolled (97% women). The mean age was 50.8±9.7 years and SLE duration was 11.8±7.1 years. At baseline, their mean Agatston score was 30.9±68 and mean carotid IMT was 0.67±0.13mm. The mean SLEDAI score was 1.6±1.7 and the SDI damage score was 1.3±1.4. The prevalence of traditional risk factors is as follows: smoking(10%), menopause(57%), diabetes mellitus(3%), hypertension(33%), LDL-cholesterol level >2.6mmol/L(44%) and waist-hip ratio >0.85(50%). Antiphospholipid antibodies were present in 35% of patients. 36 patients were randomized to each of the rosuvastatin and placebo arm of treatment. No statistically significant differences in clinical characteristics were evident at baseline. At month 12, a significant drop in total and LDL-cholesterol level was observed in the rosuvastatin group. A significant reduction in hsCRP level was observed in the rosuvastatin arm (p=0.02) but not in the placebo arm. The difference in hsCRP level between the rosuvastatin and placebo arms was significant at month 12 (p<0.03) after adjustment for baseline hsCRP values, risk factors and the mean SLEDAI score (AUC). A greater reduction in hsCRP level was observed in patients with baseline LDL of >2.6mmol/L treated with rosuvastatin. The drop in thrombomodulin level from baseline to month 12 was also significant in rosuvastatin-treated patients (p=0.04). However, serial changes in homocysteine, sVCAM-1 and P-selectin levels were not statistically significant. As the levels of thrombomodulin and sVCAM-1 correlated with disease activity, a subgroup analysis of those patients who had SLEDAI scores of ≥7 at month 0, 6, 12 revealed a significant drop in both thrombomodulin (p=0.001) and hsCRP (p=0.04) levels at month 12 in patients treated with rosuvastatin only. Rosuvastatin was well tolerated. Numerically more patients treated with rosuvastatin suffered from gastrointestinal upset and neurological symptoms. Conclusions: In SLE patients with subclinical atherosclerosis, low-dose rosuvastatin leads to a significant reduction in hsCRP level after 12 months’ therapy. Rosuvastatin also significantly reduces the level of thrombomodulin in patients with very low disease.
Antibodies towards high density lipoproteins inhibit the anti-inflammatory properties of the lipoproteins

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Anti-atherogenic properties of high density lipoproteins (HDL) are well recognized: they prevent the oxidative modification of LDL and its consequent uptake by monocytes and inhibit cytokine-induced adhesion molecule production. Anti-HDL (aHDL) antibodies have been reported in patients with systemic lupus erythematosus (SLE). Our group has previously showed the presence of these antibodies in two different cohorts as well as an association with decreased paraoxonase activity, increased biomarkers of endothelial dysfunction (nitric oxide, adhesion molecules: VCAM-1 and ICAM-1), reduced total plasma anti-oxidant capacity and an increase in disease-related damage and activity indices. This study aimed at confirming the capacity of aHDL antibodies, purified from SLE patients, to block the anti-inflammatory properties of HDL in vitro. aHDL antibodies were purified from serum of patients with SLE, by affinity chromatography. Human HDL were covalently coupled to CNBr-activated Sepharose 4B and assembled in column C10/10. Patients sera, selected according to the highest immunoreactivities observed in previous studies were injected through the column, and the antibodies directed towards HDL were retained. After elution with 0.1 M Glycine pH 2.4 and neutralization fractions collected were analyzed at 280 and confirmed by ELISA their capacity to bind to the HDL. Fractions containing immunoglobulins with binding activity were pooled and concentrate under vacuum. The production of vascular adhesion molecules (VCAM-1) by human umbilical vein endothelial cells (HUVECs) can be induced with tumour necrosis factor-alfa (TNF-alfa) and this can be prevented by pre-incubation with HDL. We investigated the effect of the purified antibodies on the expression of VCAM-1. Confluent monolayers of HUVECs were incubated with culture medium with 1% of serum (basal), HDL at a concentration 1.6mg/mL or HDL+aHDL antibody (10μg/mL or 50μg/mL) for 16 h at 37°C in 5% CO2. After wash, cells were incubated for a further 4 h in the basal or stimulated state following the addition of TNF-alfa, 10ng/mL. Expression of VCAM-1 was assayed by flow cytometry using a monoclonal anti-human VCAM-1- Fluorescein antibody. Values are expressed relative to the samples that were incubated with 1% of serum. aHDL antibodies purified from SLE patients abrogate the inhibitory effect of HDL on VCAM-1 expression, in more than 90%, when compared with the control (non-specif human IgG) (p<0.0002, and p=0.0081 respectively). This study shows that aHDL antibodies isolated from patients with SLE can inhibit HDL-associated anti-inflammatory properties in vitro, and may contribute to the pathogenesis of atherosclerosis.

Objective: Increased oxidative stress is a major contributor to the pathogenesis of atherosclerosis (ATH). Patients with SLE also demonstrate high oxidative stress and an unexplained increase in ATH. Our group and others have previously reported that several biomarkers and demographic variables associated with increased oxidative stress, including pro-inflammatory HDL (piHDL), elevated leptin, homocysteine, and increased age, are individually associated with subclinical ATH in SLE. It is unknown, however, whether these biomarkers of oxidative stress can be combined into an oxidative risk profile that better predicts future progression of atherosclerosis. Here we hypothesize that baseline presence of high oxidative stress is associated with longitudinal accumulation of subclinical atherosclerosis. Methods: Female SLE subjects not taking statins were studied. B-mode and Doppler scanning of carotid arteries was performed at baseline and at 24-36 months. Antioxidant function of HDL was measured as the change in fluorescence intensity caused by oxidation of DCBH by LDL in the presence or absence of test HDL. Fluorescence in the absence of LDL was normalized to 1.0. Values greater than 1.0 after the addition of HDL indicated piHDL. Plasma leptin was measured by ELISA, and homocysteine was determined by HPLC in the UCL clinical lab. Results: Follow-up ultrasounds were completed on 129 SLE women. Overall, 28.9% (37) of SLE patients had new plaques. Factors associated with plaque progression on bivariate analysis included the baseline presence of plaque (p<0.001), increased age (p=0.001), piHDL (p<0.001), high leptin levels (p=0.001), and increased homocysteine (p=0.05). Although piHDL was the strongest predictor for plaque progression on multivariate analysis (OR 14.2 (95% C.I. 2.2 - 161.3,), with a negative predictive value of 93.3%, the positive predictive value was only 48%. We next used a random forests model to determine which variables were most predictive of plaque progression, and also the most significant cutoffs to dichotomize each variable. We determined the most significant predictors were age >48, piHDL, high leptin levels ≥ 34ng/dL, and high homocysteine (≥212). We then created an “oxidative stress” variable, with low oxidative stress defined as 0-1 predictors, and high stress defined as ≥2. The “high” oxidative stress variable still had a negative predictive value for plaque progression of 91%, but the positive predictive value was 61%. In multivariate analysis controlling for other cardiac risk factors and disease factors, patients with high oxidative stress had a 15.8 fold increased odds for plaque progression (95% CI 3.8-65.7), and 10.8 fold increased odds for IMT progression (95% CI 3.3-38.6). Conclusions: Formation of an oxidative stress profile that incorporates several biomarkers and demographic factors that increase oxidative stress may provide a more complete means to identify patients at risk for progression of atherosclerosis.

CS2 T Cells and Lupus

CS2.1

TH17 in lupus nephritis

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In patients with systemic lupus erythematosus (SLE), both CD4 positive and the expanded CD3+CD4+CD8- (double negative cells) produce the proinflammatory interleukin 17 (IL17). IL17-producing T cells infiltrate the kidneys and may contribute to tissue inflammation and injury. IL23-treated lymph node cells from MRL/lpr mice injected to RAG1-/- mice caused the appearance of autoimmunity and lupus nephritis. B6.1pr mice made deficient in IL23 receptor failed to develop autoimmunity and lupus nephritis. The above studies in humans and mice strongly suggest a role for IL17 in the expression of lupus nephritis and make it a rational target for treatment.

CS2.2 & PO1.K.1

Frequency and functional activity of Th17, Te17 and other T cell subsets in systemic lupus erythematosus

Henriques, Ana2 Ines, Luís1, 3 Couto, Maura1, 4 Pedroso, Susana2 Santos, Catarina2 Magalhaes, Marianao Santos, Paulo2 Velada, Isabel2 Almeida,
Abstracts of Oral Presentations

Anabela2 Carvalheiro, Tiago2 Lanareijera, Paula2 Morgado, Jose M.2 Pais, Maria L.2 da Silva, Jose Antonio P.1,4 Paiva, Arturo5,6

Objectives: To compare frequency and functional activity of peripheral blood (PB) T cell subsets, namely Th(c)17, Th(c)1 and Treg cells and the amount of type 2 cytokines mRNA in active and inactive Systemic Lupus Erythematosus (SLE) and healthy controls (NC).

Methods: We recruited SLE patients with active (SLEDAI=4) and inactive disease (SLEDAI=5) and healthy age- and gender-matched controls. One blood sample was collected from each subject. We performed a flow cytometry quantification of Th17, Th1, Tc1 and Treg cells and of intracellular expression of IL-2, TNF-alpha, IFN-gamma and IL-17 at single cell level in Th(c)1 and Th(c)1 cells subsets. Quantification of TGF-beta and FoxP3 mRNA in peripheral Treg and whole blood IL-4 and IL-10 mRNA expression was performed by real-time PCR.

Results: This study included 47 subjects (34 SLE patients, 15 with active and 19 with inactive disease and 13 NC). Compared to NC, SLE patients presented a trend for increased proportion of Th(c)17 cells, but with lower amounts of IL-17 per cell and also a decreased frequency of Treg, but with increased production of TGF-beta and FoxP3 mRNA. In active compared to inactive SLE, there was a marked decrease in the frequency of Th(c)1 cells, an increased proportion of type 2 cytokines mRNA and a distinct functional profile of Th(c)17 cells, consisting of a higher proportion of these cells producing TNF-alpha and IL-2 with a minor proportion secreting IFN-gamma.

Conclusions: Our findings suggest a functional disequilibrium of T cell subsets in SLE that may contribute to the inflammatory process and disease pathogenesis.

CS2.4 & PO1.1.K.3

Increased frequency of circulating CXCR5+CD57+CD4+ follicular T helper cells in patients with systemic lupus erythematosus

Zhang, Xin; Lyman, Justin S.; Zakem, Jerald; Choi, Yong; QuiNet, Robert

Objective: Production of high-affinity auto-antibodies is central to the pathogenesis of systemic lupus erythematosus (SLE). CXCR5+CD57+CD4+ follicular T helper cells (TFH) play an important role in the generation of high affinity autoantibody secreting, long-lived plasma cells in part through their production of IL-21. The TFH subset is expanded in sanroque mice exhibiting excess germinal center formation along with additional features of SLE, such as autoantibody production and renal disease. In humans, CXCR5+ T cells are found in the peripheral blood of healthy donors; however, these cells are in a resting state and lack the expression of costimulatory molecules. In this paper, we compared the frequency of CXCR5+CD57+CD4+ follicular T helper cells in the peripheral blood of SLE patients with that of healthy donors along with their expression of IL-21. We also compared IgG secretion and B cell proliferative responses from B cells cultured with and without IL-21 in order to confirm the important role of this cytokine in inducing B cell proliferation and antibody production.

Methods: Peripheral blood was collected from five patients with SLE defined by ACR criteria and five healthy controls. The distribution of T cell subsets was defined by the expression of surface markers (CD3, CD4, CD8, CD57, CXCR5) determined through flow cytometry. IL-21 expression on the T cell subsets was determined by intracellular staining and detected by four color flow cytometry. B cells were purified by a MACS column and cultured with IL-21 in the presence of CD40L or anti-Ig. B cell proliferation was determined by 3H-TdR uptake and the IgG secretion in the culture supernatant was examined by ELISA.

Results: Our study demonstrated a significantly increased population of CXCR5+CD57+CD4+ T cells in the peripheral blood of SLE patients compared to healthy controls (P<0.01). These CXCR5+CD57+CD4+ T cells also expressed the costimulatory molecule ICOS. Intracellular staining showed that the CXCR5+CD57+ cells expressed significant higher IL-21 than CXCR5-CD57-CD4+ T cells and CD8+ T cells. Recombinant IL-21 potently induced B cell proliferation and IgG secretion in vitro.

Conclusion: This is the first study that demonstrates circulating CXCR5+CD57+CD4+ T cells in SLE patients. This could reflect an excessive TFH response in SLE that would suggest increased circulating IL-21 secreting TFH are central to the B cell hyper-reactivity underlying the pathogenesis of SLE.

CS2.5 & PO1.1.K.4

Lack of PKC θ kinase activity in T cells induces a lupus-like disease

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Objective: Systemic lupus erythematosus (SLE) is an autoimmune disease caused by a mechanism not yet well understood. Our group has reported that T cell DNA hypomethylation causes gene dysregulation by overexpressing immune genes leading to autoreactivity and autoimmunity. T cells from lupus patients display impaired ERK phosphorylation that is proportional to disease activity and also show hypomethylation of the same promoter sequences.
demethylated by ERK inhibitors and Dnmt inhibitors. These results correlate with the findings that impaired ERK pathway signaling may contribute to human lupus by decreasing DNA methylation in lupus. We characterized the signaling defect in idiopathic and drug-induced lupus T cells, showing that impaired protein kinase C (PKC) δ is the molecule responsible for the decreased ERK pathway signaling. More recent work suggests that oxidative damage to PKC δ is causing the ERK pathway signaling defect. In this work we investigated whether a lack of PKC δ activity in T cells is sufficient to cause a lupus-like disease. Methods: A dominant negative PKCδ (generous gift from Dr. Yusa, NIH) was introduced into pTER2 vector. The transgenic mice were bred with a transgenic SJL strain containing a reverse tetracycline transactivator under the control of a CD2 promoter. With this tet-on system, it is possible to generate an inducible PKC δ defect in T cells. Gene expression was measured by RT-PCR, anti-dsDNA antibodies by ELISA, and signal transduction activation by Western Blot. Results: The dominant negative PKC δ was measured in the spleen of double transgenic animals with and without doxycycline in the drinking water to verify inducibility. Animals with significant amounts of dPKCδ only in spleen, lymph nodes and thymus after doxy treatment were used. T cells from mice treated with dox overexpressed the dsPKC δ and had decreased ERK phosphorylation. The effects of decreased PKCδ activity on Dnmt1 expression were also studied. The number of Dnmt1 transcripts was reduced compared with animals without Doxy (Mean±SE: 0.51±0.11 vs 4.31±0.93, n=3) and it was inversely related to the expression of methylation sensitive gene CD70 (2.33±0.4 vs 0.05±0.03, mean±SE, n=4, p<0.05), reproducing the abnormalities observed in lupus T cells. The doxy-treated animals also produced significant amounts of anti dsDNA compared to untreated mice (p= 0.028). Conclusions: The lack of PKCδ activity in T cells induces a lupus-like disease by modifying gene expression most likely through DNA demethylation, resulting in the production of anti-dsDNA antibodies, a hallmark in lupus disease.

CS3 Epigenetics and Lupus

CS3.1

MicroRNA – mediated immune dysregulation in human lupus

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Systemic Lupus Erythematosus is a complex autoimmune disease caused by genetic and epigenetic alterations. DNA methylation abnormalities play an important role in SLE disease processes. MicroRNAs (miRNAs) have been implicated as fine-tuning regulators controlling diverse biological processes at the level of posttranscriptional repression. Dysregulation of miRNAs has been described in various disease states, including human lupus. Whereas previous studies have shown miRNAs can regulate DNA methylation by targeting the DNA methylation machinery, the role of miRNAs in aberrant CD4+ T cell DNA hypomethylation of lupus is unclear. Here, by using high-throughput microRNA profiling, we identified that two miRNAs (miR-21 and miR-148a) overexpressed in CD4+ T cells from both lupus patients and lupus-prone MRL/lpr mice, which promote cell hypomethylation by repressing DNA methyltransferase 1 (DNMT1) expression. This in turn leads to the overexpression of autoimmune-associated methylation-sensitive genes such as CD70 and LFA-1 via promoter demethylation. Further experiments revealed that miR-21 indirectly down-regulated DNMT1 expression by targeting an important autoimmune gene RAS guanyl nucleotide-releasing protein 1 (RASGRP1), which mediated the Ras-MAPK pathway upstream of DNMT1; miR-148a directly down-regulated DNMT1 expression by targeting the protein coding region of its transcript. Additionally, inhibition of miR-21 and miR-148a expression in CD4+ T cells from lupus patients could increase DNMT1 expression and attenuate DNA hypomethylation. Together, our data demonstrated a critical functional link between miRNAs and the aberrant DNA hypomethylation in lupus CD4+ T cells and could help us to develop new therapeutic approaches for SLE in future.

CS3.2 & PO1.G.31

Lupus, DNA methylation and the environment

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Environmental agents interact with the immune system to cause lupus-like autoimmunity in genetically predisposed people. How the environment modifies the immune system to cause lupus though, is incompletely understood. A growing body of evidence indicates that one mechanism by which the environment can alter T cell gene expression and break tolerance is by inhibiting DNA methylation, an epigenetic mechanism regulating gene expression. Early work demonstrated that the lupus-inducing drugs procainamide and hydralazine induce autoimmunity by inhibiting replication of CD4+ T cell DNA methylation patterns during mitosis, causing aberrant overexpression of genes that convert normal T cells into autoreactive, pro-inflammatory, cytotoxic cells, and that normal CD4+ T cells treated with these drugs or other DNA methylation inhibitors cause a lupus-like disease in mice. Identical changes in CD4+ T cell DNA methylation and gene expression were found in T cells from lupus patients with active disease, suggesting that environmental agents could also contribute to the development of lupus-like diseases by inhibiting CD4+ T cell DNA methylation. Replication of DNA methylation patterns depends on DNA methyltransferase 1 (Dnmt1) enzyme activity and the methyl donor S-adenosylmethionine (SAM), and is inhibited by S-adenosylmethionine (SAH), indicating that exogenous agents decreasing Dnmt1 activity and SAM levels, or increasing SAH, will synergize to cause DNA demethylation. The incidence of lupus also increases with age, while T cell Dnmt1 levels decrease with age, and recent studies demonstrate that age-dependent decreases in Dnmt1 levels synergize with low folate or methionine levels, and increased homocysteine levels, to demethylate T cell DNA and cause overexpression of genes contributing to lupus. Dnmt1 levels are also regulated by the ERK pathway, and exogenous ERK pathway inhibitors including xenobiotics, UV light and oxidative stress, can all demethylate T cell DNA. Together, these studies suggest that UV light, oxidative stress, and environmental agents can alter T cell gene expression and promoting the development of lupus-like autoimmunity in genetically predisposed people.

CS3.3 & PO1.G.1

Whole genome methylation scan reveals both hypomethylated and hypermethylated genes in lupus CD4+ T cells

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Objectives: Inducing a T cell DNA methylation defect causes lupus-like autoimmunity in animal models. T cell DNA methylation defect correlates with disease activity in lupus patients. Herein, we perform a whole-genome methylation scan in CD4+ T cells to identify differentially methylated genes in lupus patients and determine the functional implication of differentially methylated loci upon gene expression. Methods: We used DNA immunoprecipitation with anti-5-methylcytidine antibody coupled with microarray hybridization to determine genome-wide DNA methylation patterns in CD4+ T cells from lupus patients and controls matched for age, sex and race. Input and immunoprecipitated DNA from each sample were differentially labeled and hybridized to arrays with ~385,000 probes, covering all UCSC-annotated CpG islands and promoter regions for all RefSeq genes. CD4+ T cell gene expression data from Gene Expression Omnibus (GEO) were utilized and normalized signal intensity expression values were analyzed to detect differences between lupus patients and controls. Pathway analysis and literature mining analysis were performed using Ingenuity and IRIDESCENT soft-
ware. Results: We identified genes differentially methylated within the 5kb upstream to 5kb downstream of the transription start site between CD4+ T cells from lupus patients and controls. We found 624 hypermethylated and 661 hypomethylated genes in lupus patients. Functional analysis revealed that top canonical pathways shared among hypomethylated genes include Cell Cycle Regulation, Wnt/β-catenin Signaling, Dendritic Cell Maturation, Graft-versus-Host Disease Signaling, IL-10 Signaling, and p38 MAPK Signaling. Shared canonical pathways among hypomethylated genes include IL-15 Production, Dendritic Cell Maturation, p38 MAPK Signaling, Graft-versus-Host Disease Signaling, FLT3 Signaling in Hematopoietic Progenitor Cells, and Interferon Signaling. Furthermore, many differentially methylated genes in lupus CD4+ T cells were also variably expressed, suggesting that the methylation changes observed, at least in a subset of genes, might be functionally relevant. Of the hypomethylated genes, 112 were found to be overexpressed, while 99 hypermethylated genes were underexpressed in lupus CD4+ T cells. Some disease-relevant hypermethylated genes in lupus CD4+ T cells include CASP3, BCL6, IACM1, and GZMB. On the other hand, the genes CASP2, FCRL3, STAT1, STAT2, TYK2, IL-18, and TNFSF13 were hypomethylated. Conclusions: We performed the first whole-genome methylation analysis in lupus CD4+ T cells and mapped differentially methylated regions across the genome. In addition to global T cell DNA hypomethylation that has been previously described in lupus, our data suggest that promoter and CpG Island hypermethylation in CD4+ T cells might also play a role in the pathogenesis of lupus.

CS3.4 & POI.1.G.2

X chromosome signatures associated with the microRNome of human peripheral blood CD4+ T cells; integrating the epimicroRNome into the molecular basis of human lupus

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Objectives: Hypomethylation of regulatory sequences correlates with active transcription. Sequences on the inactive X may cause gene overexpression upon demethylation. X chromosome inactivation has been implicated in female predisposition to autoimmunity. Here we have investigated the epigenetic regulation of X chromosome miRNAs in CD4+ T cells to identify the effect of miRNA on gender bias in autoimmunity. Method: Methylation sensitive DNA and miRNA from CD4+ T cells were identified by using experimentally demethylated in vitro system using DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-azaC). Transcript profiling for isolated RNA was performed by using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Genomic-wide qPCR miRNA expression profiling was performed using System Biosciences (SBH) QuantiMir™ RT Kit. Results from hypomethylated experiments were compared to expression profiles from active lupus patients to identify methylation sensitive genes differentially expressed in lupus patients. Most likely oppositely-correlated miRNA: RNA interactions were identified by RNA22 tool from IBM research. Results: When the differential expression between women and men were compared without T cell stimulation, 5-AzaC caused a total of 97 genes to differentially express between women and men of which 78 were female biased genes. However, PMA-ionomycin stimulation caused down regulation of 57 genes out of 170 differentially expressed genes in women. Using a factor of 2 fold as a cut-off, we identified a total of 104 miRNAs up-regulated in 5AzaC treated CD4+ T cells, of which 27 were female biased and 11 were male biased. The X chromosome encoded miRNAs; hsa-let-7f-2*, hsa-miR-503, hsa-miR-421, hsa-miR-188-3p, hsa-miR-513-a-3p and hsa-miR-374a were found to be expressed higher in women compared to men in which the DNA was hypomethylated. Furthermore hsa-let-7f-2*, hsa-miR-503 and hsa-miR-188-3p were highly expressed in active female lupus patients tested (n =4) compared to the healthy controls. Functional Annotation clustering of these miRNA targets revealed that the genes belonging to “response to stress” category being most affected. Conclusion: These results suggest that the miRNA overexpression due to DNA demethylation may contribute to the downregulation of genes observed in hypomethylated CD4+ T cells. Furthermore, the demethylated of X chromosome resulting in elevated levels of X chromosome miRNAs, may play a major role in the decreased mRNA levels in stimulated CD4+ T cells in women. This in turn may contribute to the gender differences of gene expression levels. Better understanding of the intertwined connection between epigenetics to miRNAs and gene expression could add a new dimension to the understanding of molecular mechanisms leading to gender bias in autoimmune diseases.

CS4 Outcomes in Lupus

CS4.1

Biomarkers for diagnosis and disease activity in SLE: are clinical associations in early translational studies valid?

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Objective: Biomarkers can aid diagnosis and assessment of disease activity, yet early translational studies of new biomarkers may be misleading if not designed to address information bias. We evaluated if translational research studies of potential biomarkers in systemic lupus erythematosus (SLE) incorporated design features important for valid clinical associations. Methods: We searched ten journals for translational research studies published in 2005-2009 that reported associations between new laboratory markers and the presence of disease (SLE versus controls) or measures of disease activity. We examined studies for the following design features: age-, sex- and race-matching between groups; control for the effects of treatment on the expression of the biomarker; inclusion of both patients with early and late disease, or both active and inactive disease; longitudinal or cross-sectional design; and use of validated measures. Results: Forty-eight studies examined potential biomarkers for diagnosis and 27 studies examined biomarkers for disease activity assessment. Lymphocyte markers, other cell surface markers, and gene expression profiles were the most commonly examined biomarkers. Age-matched and sex-matched groups were examined in 62% and 58% of studies of diagnosis, respectively. Although 70% of studies reported information on treatment, only 52% examined if treatment affected the biomarker. Only 33% of studies included both patients with early and late SLE. Among studies of biomarkers of disease activity, age-adjusted or sex-adjusted comparisons were included in 37% and 40% of studies, 59% examined if treatment affected the biomarker, and only 40% of studies included a longitudinal component. The proportion of studies including important study design features tended to be higher than comparable studies in rheumatoid arthritis (N = 67 for diagnosis; N = 9 for disease activity). Conclusion: Early translational studies of potential biomarkers in SLE often do not include study design features needed for proper interpretation of clinical associations. Attention to these features could reduce false-positive and false-negative associations.

CS4.2

Malignancy in SLE

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Persons with systemic lupus erythematosus (SLE) have an increased risk of certain types of malignancies. Of particular concern are hematologic cancers, especially non-Hodgkin lymphoma, where a three- to four-fold increased incidence is seen in SLE, compared to the general population. There is some evidence that immunosuppressive medications play a role in mediating this risk, although there appear to be other factors driving the association as well. There is some evidence that lupus disease activity may itself be a mediator of the association between SLE and lymphoma. In addition to hematologic cancer risk, lung cancer also is increased in SLE compared to the general population, and smoking may drive this risk in large part. Last but not least,
cervical dysplasia has been flagged as a concern for women with SLE. Some of these issues will be highlighted in Dr. Bernatsky’s presentation. The discussion will feature some novel initiatives of the Systemic Lupus International Collaborating Clinics (SLICC).

CS4.3 & PO2.K.1

Statins reduce disease activity scores (SLAM-R) and cumulative organ damage (SLICC) in SLE patients from a multi-center, multi-ethnic, US multi-institutional cohort. Aguilar-Velazquez, Renan A.1 Seif, Alan1 Papalardo , Elizabeth1 Doon, Elis1 Deng, Neha1 Alarcon, Gaciela2 Reveille, John D.3 McWin, Gerard M.2 Pierangeli, Silvia S.2 1. University of Texas Medical Branch, Galveston, TX, USA; 2. University of Alabama at Birmingham, Birmingham, AL, USA; 3. University of Texas Health Science Center, Houston, TX, USA

Purpose: Statins, in addition to lower cholesterol levels, have are cholesterol anti-thrombotic, anti-inflammatory and cardiovascular protective effects. Cardiovascular morbidity and accelerated atherosclerosis are important features of SLE. However, it is uncertain whether statins may have beneficial effects in patients with SLE. Objectives: To examine longitudinally the impact of statins on levels of biomarkers of disease (cytokines/chemokines) and disease activity (SLAM-R) and with damage accrual (SLICC-ACR Damage Index, SDI)on in patients with SLE Methods: Sera from 21 SLE patients (ACR criteria) from a multi-ethnic, multi-center cohort (LUMINA) were assessed at baseline and at a subsequent visit after treatment with statins was started. Patients that were on more than 10 mg prednisone/day, or on other immunosuppressive therapy were excluded (age range: 16-67; mean age: 44.6): 38% were African American, 9% were Caucasians, and 4% were Puerto Rican Hispanics . 86% were females and 14% were males. Levels of IL1b, IL-6, IL-8, IFN-α, IP-10, MCP-1, VEGF, sCD40L and TNF-α, were measured in serum using a Millipore Miliplex™ Multiplex Assay and titers of soluble (s) E-selectin (E-sel), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), IgG and IgM anticardiolipin antibodies (aCL) were detected by ELISA. High sensitive C reactive protein (hsCRP) was determined by nephelometry. Changes in the levels of the biomarkers after treatment were calculated and the effects of statins were tested using a signed rank test. Spearman correlations were used to compare changes in levels of biomarkers with changes in SLAM-R or SLICC scores. Results: SLAM-R and SLICC scores were significantly affected by statins (p values 0.0199 and 0.039, respectively). IL1b, IL6, IL8, TNF-α VEGF, IP-10, sCD40L and sVCAM-1 levels were reduced after treatment with statins by: 28%, 28%, 43%, 57%, 42%, 52% and 62% respectively but the reduction was not statistically significant. Importantly, the changes in IL6, after treatment with statins correlated with changes in SLICC scores (p value: 0.0362), hsCRP was increased in 50% of the subjects at baseline and reduced by 37% after treatment with statins. Conclusions: Significant number of SLE patients’ samples showed a decrease in levels of some cytokines and chemokines and of hsCRP after treatment with statins. Importantly, SLAM-R and SLICC scores were significantly reduced by statins and the changes in IL6 correlated with a significant decrease in organ damage accrual (SLICC).

CS4.4 & PO1.F.1

The Georgia Lupus Registry: a population-based estimate of the incidence of SLE in patients with chronic cutaneous lupus Drenkard, Cristina; Shenvi, Neeta; Easley, Kirk; Lim, S.Sam Emory University, Atlanta, GA, USA

Objectives: It is deemed that a small proportion of patients with chronic cutaneous lupus (CCLE) develop SLE. One population-based study found that 12% patients with different forms of cutaneous lupus progressed to SLE. We studied the incidence of SLE and demographic risk factors in patients with CCLE from a large population-based registry of lupus in Georgia. Methods:

The Georgia Lupus Registry is a population-based registry aimed to estimate the incidence and prevalence of SLE in Atlanta, GA. Multiple sources are used to find potential cases of SLE and cutaneous lupus. Nearly 250 demographic and clinical elements were abstracted from medical records. Sources included: 96% hospitals, 100% high yield rheumatologists, 36% high yield dermatology practices, and the major dermatopathology laboratory in the target area. CCLE was defined as lupus profundus, discoid, chilblain, or mucous lupus, and the outcome as SLE (≥4 ACR criteria or 3 ACR criteria and diagnosis of SLE by rheumatologist). The Kaplan-Meier method was used for the analysis. The risk-time was from the date of CCLE to the date of event, or to the censored end-point (12/ 31/2004). Results: Among 706 CCLE cases, 190 were prevalent and 24 had unknown dates of CCLE. 494 CCLE cases were analyzed (79% female, 73% blacks, 17% whites).

When compared by gender, incidence estimates at 2, 5 and 10 years were higher in females than males (F: 5.6, 17.1 and 33.7%; M: 1.2, 8.1, and 14.2%, respectively, p=0.01). No differences were found by race or age at CCLE.

Conclusions: This population-based study shows that there is considerable progression to SLE among patients with CCLE-onset. The cumulative incidence of SLE at 10 years was 30%. The incidence estimates were significantly higher among females, particularly since the few years after the diagnosis of CCLE. Prospective studies assessing disease-related risk factors and potential triggers, principally in females with CCLE, are needed to improve early recognition of patients at higher risk of SLE.

CS4.5 & PO2.D.36

An introduction on Chinese registry of systemic lupus erythematosus: preliminary data from CSTAR (Chinese SLE Treatment and Research group) Zeng, Xiaofeng Peking Union Medical College Hospital, Beijing, China

Introduction: CSTAR is the first Chinese on-line registry of lupus, which is supported by Chinese National Key Technology R&D Program. The data was collected from 106 centers, which covered 30 provinces in China. The aim is to clarify several epidemiologic aspects of SLE in China and establish a platform to provide resource for future studies . Methods: CSTAR started with a multicentre, consecutive and prospective design. The cohort that is developing and studying a large and growing cohort of uniformly evaluated individuals from Chinese populations to achieve the goals previously mentioned. The registered patients must fulfill four or more of the American College of Rheumatology criteria for the classification of SLE. All CSTAR centers use the same protocol-directed methods to provide uniform evaluations, which include demographic data, clinical history, laboratory and radiological examinations, disease activity evaluations (BILAG2004, SLEDAI and PGA). All data are collected by e-CRF combined with biospecimen collections. Results: Preliminary analyses of CSTAR data from 1344 baseline evaluations were available. The demographic data showed the mean age of this cohort was 32.7±12.3 yrs (5-77), which consisted of 1242(92.4%) female and 102(7.6%) male patients (female to male ratio, 12.2). The mean age at onset was 29.4yrs(1.4-76.1) with a diagnosis at the age of 30.6yrs(6-77). 78(5.8%) patients had family history of rheumatic diseases including 20(1.5%) cases with SLE and other 58(4.3%) ones with rheumatoid arthritis, primary Sjogrens’ syndrome or systemic sclerosis. 66(4.8%) abnormal pregnancies were recorded among 1375 experiences. The characteristics of the CSTAR cohort were...
summarized with comparison of others in Table 1. The leading manifestations at onset were hematological disorders 772 (57.4%), arthritis 748 (55.7%), malar rash 630 (46.9%), nephropathy 616 (45.8%) and fever 516 (38.4%), but rare ones might be neurological 60 (4.5%), cardiac 43 (3.2%) and gastrointestinal involvements 48 (3.6%). The profiles of major autoantibodies included 1310 (97.5%) antinuclear antibodies, 903 (67.2%) anti-dsDNA, 139 (10.3%) anti-Sm, 143 (10.6%) anti-ssRNA, 226 (16.8%) anti-ssA, 124 (9.2%) anti-ssB and 99 (7.4%) anti-rRNP. The cohorts were stratified by SLEDAI scores to stable group (5) with 349 (46%), mild active (5-9) with 361 (26.9%), moderate active (10-14) with 395 (29.4%) and severe active with 239 (17.8%). **Conclusion:** CSTAR cohort provided epidemiological data and phenotypes of Chinese patients with SLE, which would be a resource to depict morbidity and mortality with a long-term followup. Biospecimen also made further basic studies possible in the future with international collaborations.

### CST5.2 Autoimmunity and tolerance: tracking self-reactivity to its source

Zouali, Moncef
Lariboisière Hospital, and University Denis Diderot, Paris, France

Accumulating evidence indicates that B-lymphocytes are key players in innate and adaptive branches of immunity, and that impairment of some of their functions can lead to a variety of disorders in humans. In SLE, a number of B lymphocyte alterations have been recognized, leading to novel immunointervention strategies based on specific B lymphocyte targeting. Despite this therapeutic progress, the precise mechanisms that underlie loss of B cell tolerance to self-antigens in autoimmune disease remain under scrutiny. During early B cell development, the pre-B cell receptor, formed by the assembly of newly formed immunoglobulin heavy chains with the B lymphocyte-restricted lambda 5 and Vpre-B chains, plays a role in negative selection of self-reactive B cells. Reduced expression of pre-B receptors may underlie an altered pre-BCR-mediated negative selection checkpoint of autoantibody-producing B cells, leading to deficient B lymphocyte signaling and abnormal expression of B cell subsets. At later B cell developmental stages, clonal deletion or clonal anergy may be disturbed so as to contribute to autoreactivity formation. Additionally, we have found that alterations in receptor editing, a chief mechanism of B cell tolerance to self, can contribute to SLE through at least two different mechanisms. Whereas impaired secondary rearrangements may result in ineffective silencing of B cells, exacerbation of receptor editing can give rise to the emergence of autoreactive receptors from clones that were initially devoid of autoreactivity. Both alterations can promote the pathogenesis of autoimmune diseases by favoring the uncontrolled emergence of these autoreactive B cell clones. In addition to predisposing genes, we have found that epigenetic factors can have an impact on lymphocyte tolerance to self-antigens. Since epigenetic mechanisms can affect various biological properties — from the eye color of flower petals to the morphology of players — further investigation into the impact of epigenetics might provide us with unexpected clues that will help elucidating more fully the basis of deregulated tolerance in autoimmune disease.

### CST5.3 & PO11.1

### CS5.3 Pathological immune response in NZB/W mice is not responsive to IVIG-treatment

**Introduction:** Systemic lupus erythematosus (SLE) is the prototype of a systemic autoimmune disease characterized by the formation of autoantibodies directed, i.e. against double stranded DNA. Intravenous immunoglobulins are one of the therapeutic options in severe organ involvement. The treatment with specific natural polyclonal anti-dsDNA anti-idiotypic antibodies (IVIG-ID) resulted in reduction of anti-dsDNA antibody titers, prolongation of survival and improvement of renal pathology in NZB/W F1 mice, the mouse model for SLE. Recently, it has been shown that long-lived plasma cells resistant to immunosuppressive drugs contribute to maintenance of autoimmunity. **Objective:** to study the effect of IVIG-ID on both short-lived and long-lived plasma cells in NZB/W mice with full-blown disease as well as in young mice in the phase of the establishment of the long-lived plasma cell compartment. **Methods:** 6 weeks, clinical healthy and 7 months old NZB/W mice with full-blown disease were treated 1x/week for 3 weeks i.v. with 2 mg/kg bw/injection of IVIG-ID. BrdU-feeding was started 2 weeks prior to treatment. Mice were sacrificed at the end of week 3 of treatment. BrdU-incorporation was measured by flow cytometry. Antibody-secreting cells from spleen, bone marrow and kidneys were quantified according to their isotype and specificity using Elispot. **Results:** Absolute viable lymphocyte count was not affected by the treatment neither in spleen nor bone marrow in both mouse groups. Numbers of total IgG- and IgM-secreting plasma cells as well were not changed by...
the treatment regimen in the organs investigated. DNA-specific plasma cells in the spleen as well as in bone marrow were not reduced independent of their isotype. The frequency of long- and short-lived plasma cells was not changed either. In the spleen of old mice a mean of 46 ± 9 % of the plasma cells of controls was long-lived compared to 48 ± 21% in treated animals. In young mice 33±4 % of the plasma cells in the spleen were long-lived in treated mice compared to 37±5 % in untreated mice. In bone marrow no changes could be found either. 

**Conclusion:** Both preventive and therapeutic administration of IVIG-ID does not show a significant effect on plasma cells originating from spleen, inflamed kidney or bone marrow in NZB/W mice. Therefore, other mechanisms leading to reduction of serum anti-dsDNA antibody titers should be taken into consideration. This suggests that temporary reduction of anti-dsDNA antibody titers by IVIG-ID is not due to plasma cells depletion.

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**CS5.4 & PO1.1.2**

The effects of the anti-CD22 monoclonal antibody epratuzumab on peripheral B cells and immune responses in vivo and immunoglobulin production in vitro

**Brown, Derek; Crook, Kenneth; Shaw, Stefan; Bourne, Timothy; Foulkes, Roland; Rose, Geoffrey; Shock, Anthony**

**UCB, Slough, UK**

**Objectives:** Epratuzumab is a monoclonal antibody against CD22 currently being evaluated clinically in patients with Systemic Lupus Erythematosus (SLE). The aim of the current study in Cynomolgus monkeys was to investigate the effects of epratuzumab on circulating B cell numbers and on the immune response to the challenge antigens keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT). Additionally, the effect of epratuzumab on immunoglobulin production from human B cells in culture was assessed. 

**Methods:** In one study, Cynomolgus monkeys received four weekly doses of epratuzumab at 10, 60 or 160 mg/kg and peripheral blood CD20+ B cell numbers were enumerated by flow cytometry. In a second study, Cynomolgus monkeys received epratuzumab at three different dose levels (1X 60mg/kg, 1X 10mg/kg or 4X 60mg/kg), or saline. The primary immune response to administered KLH and the secondary immune response to TT were then monitored over time using ELISAs to measure anti-TT and anti-KLH titres in serum. Human peripheral blood mononuclear cells (PBMC) or purified B cells from human tonsils were cultured in vitro with a range of stimuli and the effect of epratuzumab on IgG and IgM production, assessed by ELISA, was monitored after 5 days in culture. 

**Results:** There was a 40-50% reduction in the numbers of circulating B cells in Cynomolgus monkeys after treatment with epratuzumab at all doses tested, which occurred within 24 hours of dosing. Animals treated with saline showed a primary anti-KLH response, with an immune response to administered KLH and the secondary immune response to TT were then monitored over time using ELISAs to measure anti-TT and anti-KLH titres in serum. Human peripheral blood mononuclear cells (PBMC) or purified B cells from human tonsils were cultured in vitro with a range of stimuli and the effect of epratuzumab on IgG and IgM production, assessed by ELISA, was monitored after 5 days in culture. 

**Conclusions:** Epratuzumab treatment caused a reduction in B cells but had no effect on the capacity to raise an antibody response to challenge antigens in Cynomolgus monkeys in vivo. The production of immunoglobulin by B cells in culture was also unaffected by epratuzumab. This might indicate that the efficacy of epratuzumab in SLE patients is unlikely to be accompanied by a gross effect on the capacity to generate an adaptive immune response.

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**CS6 New Drugs in Development**

**CS6.1 & PO2.E.1**

Belimumab, a BlyS-specific inhibitor, reduced disease activity, flares, and steroid use in patients with seropositive systemic lupus erythematosus (SLE): BLISS-52 study

**Navarra, Sandra1 Bae, Sang Cheol1 Hall, Stephen2 Guzman, Renato3 Gallagher, Albert0 Levy, Roger A.3 Jimenez, Renato4 Li, Edmund K.3 Thomas, Mathew5 Kim, Ho-Youn6 Suh, Chang-Hee6 León, Manuel7 Tanasescu, Coman7 Lan, Jiong-Liang7 Yu, Chia-Li8 Pineda, Lilia9 Zhong, Z.John17 Freimuth, William12 Petri, Michelle16 BLISS 52 Study Group.**

1. Human Genome Sciences, Inc, Rockville, MD, USA; 2. University of Santo Tomas Hospital, Manila, Philippines; 3. Hanyang University Hospital, Seoul, Korea; 4. Cabrinhi University, Malvern, VIC, Australia; 5. Saludcoop, Bogotá, Colombia; 6. Hospital Británico, Buenos Aires, Argentina; 7. Universidad do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; 8. Hospital Dr Gustavo Fricke, Vila do Mar, Chile; 9. Prince of Wales Hospital, Hong Kong, China; 10. Kerala Institute of Medical Sciences, Trivandrum, India; 11. Catholic University, St Mary’s Hospital, Seoul, Korea; 12. Ajou University Hospital, Suwon, Korea; 13. Instituto de Ginecologia y Reproduccion, Lima, Peru; 14. Spitalul Clinic Colentina, Bucharest, Romania; 15. Veterans General Hospital, Taichung, Taiwan; 16. National Taiwan University Hospital, Taipei, Taiwan; 17. HGS Inc., Rockville, MD, USA; 18. Johns Hopkins University, Baltimore, MD, USA

**Objective:** To assess the efficacy and safety of belimumab, a BlyS-specific inhibitor, in seropositive patients with moderate to severe SLE. 

**Methods:** BLISS-52 was a phase 3, 52-wk double-blind study including 865 seropositive (ANA ≥1:80 and/or anti-dsDNA ≥30 IU/mL) SLE patients with SELENA-SLEDAI (SS) ≥6 (stable standard of care [SOC] therapy ≥30 d) from Latin America, Asia-Pacific, and Eastern Europe. Patients were randomized to receive belimumab (1 or 10 mg/kg) or placebo in addition to SOC with dosing on day(0) 0, 14, 28, then q28d for 48 wk. Disease activity was assessed using SS, BILAG, and SS Flare Index (SFI). The primary endpoint was the SLE Responder Index (SRI) at wk 52, including improvement in SS (≥4-point decrease), no new BILAG 1 A/2 B flares, and no ≥0.3-point worsening in Physician’s Global Assessment (PGA).

**Results:** Baseline disease characteristics were similar across treatment groups, with mean values as follows: disease duration 5.3 y; SS 9.8; BILAG 1 A/B 58%; ANA+ 95%; anti-dsDNA+ 75% low C4 59%; proteinuria (>0.5 g/24 h) 25%; antimalarials 67%; prednisone-equivalent dose of steroid ≥7.5 mg/d 69%; immunosuppressants 42%. Significant improvements were observed in SRI response rates for the 1-mg/kg and 10-mg/kg belimumab dose groups at 51% (p≤0.0129) and 58% (p≤0.0006), respectively, vs placebo at 44%. Significant improvement was also observed in at least 1 belimumab treatment group in the following measurements: SS ≥4-point reduction; improvement or no >0.3-point worsening in PGA; reduction in steroid use; reduction in flare rates, and increase in time-to-first flare (Table 1). Rates of overall AEs, deaths, serious AEs, infections, and lab abnormalities were comparable between belimumab and placebo groups. Serious or severe infusion reactions were slightly higher with belimumab than with placebo. No malignancies were reported.

**Conclusions:** Belimumab significantly reduced SLE disease activity, flare rates, and steroid use, and increased time-to-first SLE flare in patients with seropositive SLE. The overall rate of AEs, including serious AEs and infections, were comparable in the belimumab and placebo groups. (NCT00424476)
Global Assessment (PGA) vs baseline. **Results:** Mean baseline disease characteristics across treatment groups were generally similar: SLE disease duration 7.5 y; antimalarial use 63%; steroid use 76%; prednisone-equivalent dose of steroid ≥7.5 mg/d 46%; immunosuppressant use 56%; proteinuria (>0.5 g/24 h) 16%; low C4 53%; SS 9.7; BILAG A/A2 B 64%; ANA+ 92%; anti-dsDNA+ 64%. SRI response rates were 41% (p=0.1041) in the 1-mg/kg and 43% (p=0.0207) in the 10-mg/kg belimumab dose groups (vs placebo 34%). Significant improvement was observed in at least 1 of the belimumab treatment groups for SS ≥4-point reduction; no >0.3-point worsening in PGA; reduction in severe flares; improvement in SF-36 Physical Component Score (PCS) and FACIT-Fatigue (Table 1). Statistically significant improvement in PGA or reduction in steroid use was not seen with belimumab treatment. Overall AEs, deaths, serious AEs, infections, and lab abnormalities were comparable in the belimumab and placebo groups. Serious or severe infusion reactions were modestly increased with belimumab compared with placebo. A total of 6 malignancies occurred, with similar proportions across all study groups. **Conclusion:** In the BLISS-76 study, belimumab significantly improved the SRI response rate as well as reduced SLE disease activity and severe SLE flare rates in patients with seropositive SLE. The overall rates of AEs, including serious AEs and infections, were comparable in the belimumab and placebo groups. (NCT00410384)

# Abstracts of Oral Presentations

**Background:** To assess the efficacy and safety of belimumab in patients with seropositive SLE. **Methods:** 819 seropositive (ANA ≥1:80 and/or anti-dsDNA ≥30 IU/mL) SLE patients with SELENA-SLEDAI (SS) ≥6 (on stable standard of care [SOC] therapy ≥30 d) were treated in this phase 3 76-wk double-blind international study of belimumab (1 or 10 mg/kg) plus SOC or placebo plus SOC on days 0, 14, 28, then q28 d for 72 wks. Efficacy analyses included SS, BILAG, and SS Flare Index (SFI). Primary endpoint was the wk 52 SLE Responder Index (SRI) response rate: improvement in SS (≥4-point decrease), no new BILAG A or B flares, and no >0.3-point worsening in Physician’s

### CS62 & PO2.E.2

**Belimumab, a BlyS-specific inhibitor, reduced disease activity and severe flares in patients with seropositive SLE: BLISS-76 study**

Furie, Richard A; 1 Gladman, Dafna D; 2 D’Cruz, David; 3 Zamani, Omid; 4 Wallace, Daniel; 5 van Vollenhoven, Ronald F; 6 & PO2.E.2 Tegzova, Dana; 7 Merrill, Simon; 8 Freimuth, William; 9 BLISS 76 Study Group, The Joan T; 9 Schwarting, Andreas; 10 Clarke, Ann E; 11 Doria, Andrea; 12 Sanchez-Guerrero, Jorge; 13 Chatham, W Winn; 14 Manzi, Susan; 15 Ginzler, Ellen; 16 SUNY-Downstate, Montreal, QC, Canada; 12. Policlinico University, Padova, Italy; 13. Universtitätsklinik, Mainz, Germany; 11. McGill University Health Centre, Paris, France; 9. OMRF, Oklahoma City, OK; 8. Institute of Rheumatology, UK; 6. University of California Los Angeles, Los Angeles, CA, USA; 7. Karolinska University, Stockholm, Sweden; 8. Institute of Rheumatology, Prague, Czech Republic; 9. OMRF, Oklahoma City, OK; 10. Karolinska University Hospital, Stockholm, Sweden; 11. McGill University Health Centre, Montreal, QC, Canada; 12. Policlinico University, Padova, Italy; 13. INCNMSZ, Mexico City, Mexico; 14. University of Alabama, Birmingham, AL, USA; 15. University of Pittsburgh, Pittsburgh, PA, USA; 16. SUNY-Downstate, Brooklyn, NY, USA; 17. Oklahoma Center for Arthritis Therapy & Research, Tulsa, OK, USA; 18. USC, Los Angeles, CA, USA; 19. HGS Inc., Rockville, MD, USA; 20. Johns Hopkins University, Baltimore, MD, USA

<table>
<thead>
<tr>
<th>Efficacy</th>
<th>Placebo n=275</th>
<th>Belimumab (1 mg/kg) n=288</th>
<th>Belimumab (10 mg/kg) n=273</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRI at wk 52*, n (%)</td>
<td>125 (44)</td>
<td>148 (51)</td>
<td>167 (58)</td>
</tr>
<tr>
<td>• SS ≥4-point reduction</td>
<td>132 (46)</td>
<td>153 (53)</td>
<td>169 (58)</td>
</tr>
<tr>
<td>• No PGA &gt;0.3-point worsening</td>
<td>199 (69)</td>
<td>227 (79)</td>
<td>231 (80)</td>
</tr>
<tr>
<td>• No new BILAG A / 2 B scores</td>
<td>210 (73)</td>
<td>227 (79)</td>
<td>236 (81)</td>
</tr>
<tr>
<td>% PGA improvement at wk 24, mean (SE)</td>
<td>22 (2.6)</td>
<td>30 (2.2)</td>
<td>37 (2.4)</td>
</tr>
<tr>
<td>Steroid reduction from ≥7.5 mg/d by ≥50%, n (%)</td>
<td>23 (12)</td>
<td>42 (21)</td>
<td>38 (19)</td>
</tr>
<tr>
<td>Steroid increase from ≤7.5 mg/d to &gt;7.5 mg/d at wk 52, n (%)</td>
<td>34 (16)</td>
<td>25 (30)</td>
<td>17 (20)</td>
</tr>
<tr>
<td>SFI flare, % (hazard ratio [HR])</td>
<td>80 / 84</td>
<td>71 (0.75) / 126</td>
<td>71 (0.76) / 119</td>
</tr>
<tr>
<td>Median time to first flare, day</td>
<td>p=0.0026</td>
<td>p=0.0036</td>
<td></td>
</tr>
<tr>
<td>• Severe SFI flare</td>
<td>23</td>
<td>18 (0.76)</td>
<td>14 (0.57)</td>
</tr>
<tr>
<td>New BILAG A / B Flare, % (HR)</td>
<td>30</td>
<td>27 (0.89)</td>
<td>19 (0.58)</td>
</tr>
</tbody>
</table>

### Safety, n (%)

- AEs | 263 (92) | 264 (92) | 266 (92) |
- Serious AEs | 36 (13) | 47 (16) | 41 (14) |
- Infections | 183 (64) | 197 (68) | 194 (67) |
- Serious infections | 17 (6) | 22 (8) | 15 (5) |
- Infusion reactions | 40 (17) | 47 (16) | 48 (17) |
- Hypersensitivity | 6 (2) | 4 (1) | 2 (1) |
- Discontinuations | 61 (21) | 48 (17) | 49 (17) |
- Due to AEs | 19 (7) | 16 (6) | 15 (5) |

*Patients who withdrew from the study prior to wk 52 visit or who used protocol-prohibited medications were considered treatment failures.
†P-values were obtained from Cox proportional hazard model for time to first flare.

*Patients who withdrew from the study prior to wk 52 visit or who used protocol-prohibited medications were considered treatment failures.
†P-values were obtained from Cox proportional hazard model for time to first flare.
Epratuzumab demonstrates clinically meaningful improvements in patients with moderate to severe systemic lupus erythematosus (SLE): results from EMBLEM™, a phase IIb study

Wallace, Daniel J. 1 Kalunian, Kenneth 2 Petri, Michelle 3 Strand, Vibeke 4 Kilgallen, Brian 5 Barry, Anna 6 Gordon, Caroline 6
1. Cedars-Sinai Medical Center, Los Angeles, CA, USA; 2. UCSD School of Medicine, La Jolla, CA, USA; 3. The Johns Hopkins University, Baltimore, MD, USA; 4. University of Birmingham, Birmingham, UK; 5. UCB, Smyrna, GA, USA; 6. University of Birmingham, Birmingham, UK

Objectives: This 12-week, multicenter, phase IIb, randomized, double-blind, placebo-controlled study was designed to assess the efficacy and safety of epratuzumab in SLE and select a dose and regimen (NCT00624351).

Methods: Patients with moderate/severe SLE (≥1 BILAG 2004 A or ≥2 Bs) were randomized to 1 of 6 intravenous regimens: placebo (standard of care), cumulative doses (cd): 200, 800, 2400, or 3600mg of epratuzumab in equal, divided doses using 2 every other week (EOW) infusions or 2400mg cd as 4 equal infusions 1 week apart. A combined group of 2400mg cd (1200mg EOW plus 600mg weekly) was analyzed. Concomitant oral corticosteroids/immunosuppressors were to be stable before first infusion and during the study. Primary endpoint was a combined responder index of clinical disease activity at Week 12, defined as reduction of all baseline BILAG A to B/C/D and BILAG B to C/D in all body systems, no BILAG worsening in other organ systems, and no deterioration in SLEDAI or PGA, with no increase in corticosteroids/immunosuppressors over baseline.

Results: At baseline, in the entire population (N=227) mean age was 38.8 years, 94% were female, 78% Caucasian; with high disease activity (70% of ≥1 BILAG 2004 A or ≥2 Bs at baseline).

Results of epratuzumab in SLE.

<table>
<thead>
<tr>
<th>Body System</th>
<th>A/B (severe/moderate disease)</th>
<th>C (stable disease)</th>
<th>D (inactive disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>31 (54)</td>
<td>14 (24)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Epratuzumab 600mg CD</td>
<td>54 (84)</td>
<td>21 (34)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Epratuzumab 1200mg CD</td>
<td>64 (100)</td>
<td>36 (57)</td>
<td>9 (13)</td>
</tr>
</tbody>
</table>

Conclusions: Epratuzumab cd 2400mg demonstrated clinically meaningful improvements in disease activity in patients with moderate to severely active SLE at 12 weeks, with responder rates twice those of placebo. Results validate the combined index emphasizing BILAG and support phase III trials of epratuzumab in SLE.
Conclusions: Treatment with epratuzumab 600mg weekly during a 12-week cycle provided greater BILAG improvement over placebo in disease activity in all affected body systems. Efficacy was particularly prominent in cardio-respiratory and neuropsychiatric systems in which symptom improvements are often difficult to achieve. Within specific body systems, the majority had symptom reduction or absence of active disease after treatment. This analysis supports that epratuzumab may be an effective treatment for SLE.

CS6.5 & PO2.E.5
Safety and pharmacodynamic response with administration of single and repeat doses of rontalizumab in a phase I, placebo controlled, double blind, dose escalation study in SLE
McBride, Jacqueline M. 1 Wallace, Daniel J. 2 Morimoto, Alyssa Y. Zhao, Ningling 1 Abbas, Alex 1 Maciuca, Romeo 1 Jiang, Jenny 1 Drappa, Jorn 1 1. Genentech Inc, South San Francisco, CA, USA; 2. Cedars-Sinai Medical Center / David Geffen School of Medicine at UCLA, West Hollywood, CA, USA

Objectives: The expression of interferon regulated genes is elevated in patients with SLE and may be involved in the disease pathogenesis. The main objectives of this study were to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of rontalizumab, a humanized IgG1 monoclonal antibody that neutralizes human interferon alpha. Methods: This was a Phase I dose escalation study of single and repeat doses of rontalizumab or placebo in adults with mild/moderate SLE. PK and PD results demonstrated a dose-dependent decline in the expression of all seven IRGs in healthy donors. There was a rapid decline in expression of all seven IRGs in approximately 50% of patients as compared to normal controls. The reduction was sustained for one month following a single dose; a similar trend was observed during the repeat dose phase. The integrated gene expression reduction over time (AUEC) started to deviate from placebo at 1-3 mg/kg and appeared to reach maximum at 10 mg/kg. Conclusion: The safety profile and PK properties of rontalizumab support further investigation in larger trials. The PD results demonstrate a dose-dependent decline in the expression of IRGs consistent with the expected down-modulation of the IFN signaling pathway, suggesting the possibility of clinical benefit in SLE.

CS6.6 & PO2.E.6
Efficacy and safety of rituximab (RTX) in patients (Pts) with proliferative lupus nephritis (LN): results from randomized, double-blind phase III LUNAR study at week 52
Furie, Richard 1 Looney, John 2 Rovin, Brad 3 Latinis, Kevin M. 4 Appel, G7 Sanchez-Guerrero, Jorge 5 Fervenza, Fernando 1 Maciuca, Romeo D. 8 Brunetta, Paul 7 Zhang, David 7 Garg, Jay 7 1. NSSLHS, Lake Success, NY, USA; 2. Univ of Rochester, Rochester, NY, USA; 3. Ohio State, Columbus, OH, USA; 4. Univ. Kansas Medical Center,

Objectives: Small, uncontrolled studies suggested that RTX may be beneficial in LN. The efficacy and safety of RTX compared to placebo (Pbo) added to background mycophenolate mofetil (MMF) and corticosteroids in pts with proliferative LN were evaluated. Methods: Pts with class III/IV LN and UPCR >1 were randomized 1:1 to receive RTX (1000mg) or Pbo on days 1, 15, 168, and 182. The primary endpoint (EP), analyzed by a stratified Wilcoxon rank sum test, was the % of pts with complete (CRR) or partial renal responses (PRR). Results: The 72 pts randomized to each arm had similar baseline (BL) characteristics. Mean age at entry was ~51 yrs; ~90% were female; 28% were Black, 36% Hispanic, 31% White; and 67% had class IV LN. BL mean UPCR was 4.0 ±2.8, and serum creatinine was 1.0 ±0.5mg/dL. Mean daily MMF dose was 2.4±0.63g in Pbo and 2.7±0.41g in RTX. There were no statistically significant differences in the primary or clinical secondary EPs. Blacks and Hispanics randomized to RTX had greater responses compared to Pbo than Whites, but statistical significance was not reached. RTX had a greater effect on levels of anti-dsDNA and complement at Wk52 compared to Pbo. Peripher al CD19+ B-cells were depleted in all RTX pts. Serious adverse events (SAEs) and infectious SAEs were similar between groups. Neutropenia (4 vs 1), leukopenia (9 vs 3), and hypotension (9 vs 3) occurred more frequently in RTX. Two deaths (sepsis and pneumonitis) occurred in the RTX group. Conclusion: To date, LUNAR is the largest randomized, placebo-controlled trial to evaluate RTX as an intervention in LN. Although there were numerically more responders in the RTX group (57% vs 46%), the study did not show a statistically significant difference in primary or clinical secondary EPs. RTX had a significant effect on serologic markers, although the clinical significance of this is unclear. AEs and SAEs were similar in frequency between groups, with no new or unexpected safety signals.

Table: Efficacy EPs and Safety

<table>
<thead>
<tr>
<th></th>
<th>Pbo (N=72)</th>
<th>RTX (N=72)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRR</td>
<td>22 (30.6)</td>
<td>19 (26.4)</td>
<td>0.55</td>
</tr>
<tr>
<td>PRR</td>
<td>11 (15.3)</td>
<td>22 (30.6)</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Key Secondary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pts with BL UPCR&gt;3 reduced to UPCR&lt;1 at Wk 52</td>
<td>53.7</td>
<td>47.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Change from BL in anti-dsDNA Ab (%)</td>
<td>-50</td>
<td>-69</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Change from BL in C3 (mg/dL)</td>
<td>+25.9</td>
<td>+37.5</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td><strong>Exploratory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pts with BILAG Renal Domain Score C at Wk 52</td>
<td>28 (38.9)</td>
<td>39 (54.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>Overall response (CRR+PRR)</td>
<td>33 (45.8)</td>
<td>41 (56.9)</td>
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<tr>
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<td>14/20 (70)</td>
<td>0.20</td>
</tr>
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<td>10/19 (53)</td>
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<tr>
<td>Pts who started cyclo-phosphamide prior to Wk 52</td>
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*All p-values are 2-sided and not adjusted for multiplicity

Keywords: Lupus Nephritis; Rituximab; Safety; Efficacy; Randomized Controlled Trial; LUNAR
Background/Purpose: A high prevalence of vitamin D (vitD) deficiency has been previously described in patients with SLE, particularly AAs, with important implications for bone and immune system health. To test whether oral vitD repletion in patients with SLE is safe and well-tolerated, and determine the effective dose for replacement therapy, an open-label interventional study of vitD3 (cholecalciferol) was conducted. Methods: This was a 12 week open-label interventional study with 3 doses of vitD3: 800IU/day, 2000IU/day, or 4000IU/day (ClinicalTrials.gov: NCT00418587). Participants were recruited from the SLE in Gullah Health (SLEIGH) study of AAs from the Sea Islands of SC and GA, a population previously reported to have a 95% prevalence of low 25(OH)D<30ng/ml. Inclusion criteria included: SLE ($\geq$ ACR Criteria) with stable disease (no BILAG A or B in prior 4 weeks), stable prednisone $\geq$20mg/day in prior 4 weeks, baseline 25(OH)D of $\geq$20ng/ml, must be willing to discontinue other vitD supplements, have no hypercalcemia or hypercalcuria at screening, and no nephrolithiasis history. Total daily calcium and vitD intake was determined at baseline using the Block Food Frequency Questionnaire and serum 25(OH)D levels were determined by RAI (DiaSorin). Results were analyzed using descriptive statistics. Results: Out of 28 screened, 18 patients qualified and were enrolled into the study. At baseline, mean age was 43.7±12.2 years, BMI was 30.9±8.2 kg/m2, mean vitD intake was 133±121 IU/day, mean calcium intake was 635±351mg/day, mean prednisone dose was 4.3±3.7mg/day, and 93% were women. Six patients completed 12 weeks of 800IU/day, 6 completed 12 weeks of 2000IU/day, and 6 completed 12 weeks of 4000IU/day of vitD3. Compliance by pill counts was 98.5±12.7%, there were no dropouts and no treatment-related AEs. There were no disease flares or new anti-dsDNA antibody positivity. After 12 weeks, 67% of those taking 800IU/day, 83% of those taking 2000IU/day, and 67% of those taking 4000IU/day repleted to $\geq$30ng/ml. Even at 4000IU/day, only 33% reached a threshold of 40ng/ml after 12 weeks. Dose-dependent reductions in PTH were seen in all 3 dose groups (mean reduction 43.6pg/ml to 36.6pg/ml). Taking prednisone (50%) or hydroxochloroquine (64%) did not significantly influence 25(OH)D response, but hydroxychloroquine use did reduce the 1,25(OH)2D to 25(OH)D ratio from 2.0 to 1.4. Conclusions: Repletion of 25(OH)D levels with daily vitD3 at commonly used doses of 800-2000 IU daily as well as higher doses of 4000 IU daily was safe and well-tolerated among patients with SLE. Despite the observation that sunlight exposure can trigger disease flares, oral vitamin D repletion does not appear to cause activation of autoantibody production. At least 2000 IU/day of vitD3 is required to replete 25(OH)D to $\geq$30ng/ml and higher thresholds may be required for immune health.

**CS7.1** Pathogenic Mechanisms in Lupus

**Follicular helper T cells in immunity and autoimmunity**

Craft, Joseph E.; Poholek, A; Odegard, J; DiPlacido, L; Hernandez, S; Dong, M; Weinstein, J; Kim, S; Choi, J-Y; Sun, C

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Follicular helper T cells (Tfh), defined by expression of the surface markers CXC5R5, PD-1 and ICOS, are located in germinal centers where they provide help for B cell maturation through the secretion of IL-4 and IL-21. We have defined the transcriptional regulation of these cells, and the role of B cells in their induction in normal and autoimmune responses in mice, with ongoing studies devoted to understanding the control of their migration and development, as well as their characteristics and function in humans. These studies will be presented.


Neutrophil gelatinase associated lipocalin (NGAL) and its role in the pathogenesis of antibody mediated nephritis

Putterman, Chaim1 Pavar, Rahul1 Pitashny, Milena1 Gindea, Simona1 Levine, Benji1 Berger, Thorsten2 Mak, Tak W2
1. Albert Einstein College of Medicine, Bronx, NY, USA; 2. The Campbell Family Institute for Breast Cancer Research, Toronto, ON, Canada

Background: Anti-double stranded (ds)DNA antibodies are instrumental in the pathogenesis of lupus nephritis (LN), although the mechanism by which these antibodies contribute to kidney damage has yet to be determined conclusively. We had found that treatment of mesangial cells with pathogenic anti-DNA antibodies induced the expression of multiple proinflammatory mediators known to be involved in LN. One of the most highly induced genes was Neutrophil Gelatinase Associated Lipocalin (NGAL; AKA Lipocalin-2). NGAL has recently been shown to be upregulated in response to a variety of inflammatory, ischemic, and other insults to the kidney, and in fact is a sensitive and early urine biomarker in several types of human disease. Purpose: To determine whether NGAL is expressed in the context of lupus nephritis, and its role in the pathogenesis of renal injury induced by nephritogenic antibodies.

Methods: NGAL expression in serum, urine, and kidneys was analyzed in two murine lupus models (MRL/lpr and NZB x NZW F1 mice). Nephrotoxic serum nephritis (NTS) was induced by passive transfer of preformed rabbit anti-mouse glomerular antibodies to C57Bl/6 wild type and NGAL knockout mice. Results: MRL/lpr and NZB x NZW F1 lupus mice displayed higher kidney mRNA expression of NGAL than age-matched non-autoimmune BALB/c mice. In addition, serum NGAL was elevated in old as compared to young MRL/lpr (p<0.01), old MRL/+ (p<0.05), and old BALB/c mice (p<0.05). Importantly, NGAL kidney expression significantly correlated with anti-dsDNA antibodies titers (p<0.05). NGAL specific immunostaining was detected in kidneys of nephritic MRL/lpr and NZB x NZW F1 mice, but not in BALB/c mice. Similar elevations of serum and kidney NGAL were observed in non-autoimmune mice with induced nephrotoxic serum nephritis. To determine if NGAL upregulation is material in the pathways leading to renal injury, we compared the severity of renal damage in NGAL wild type and deficient mice following induction of NTS. We found that NGAL deficient mice had significantly attenuated proteinuria on days 7, 14, and 21 as compared to NGAL sufficient mice. In vitro, NGAL induced significant apoptosis and decreased proliferation of kidney cells. Conclusions: In vivo, nephritogenic antibodies upregulate NGAL, which then appears to be instrumental in the pathogenesis of antibody mediated nephritis. Whether the protection from nephritis observed in NGAL deficient mice is related to the proapoptotic effect of NGAL observed in vitro remains to be determined.

Pristane-induced lupus (PIL) in BALB/c mice: linking severe organ involvement with T-cell response

Summvoll, Georg H1 Leiss, Harald1 Huter, Eva N2 Savitskaya, Anastasiya1 Niederreiter, Birgit1 Steiner, Carl-Walter1 Steiner, Guenter1 Scheinecker, Clemens1 Smolen, Josef S1 Ulrich, Walter1
1. Dept. of Rheumatology, Medical University of Vienna, Vienna, Austria; 2. Dept. of Dermatology, University of Heidelberg, Heidelberg, Germany; 3. Dept. of Pathology, Hietzing Hospital, Vienna, Austria

Objective: PIL is a murine model of experimental lupus with antibodies (Abs) against nuclear antigens and various affections of internal organs. PIL can be observed in different mouse strains: We here characterize organ involvement in BALB/c, identify mice with severe renal lupus (nephritis-PIL) and try to link renal SLE with the T- and B-cell response in the respective animals.

Methods: Mice were injected i.p. with either 0.5ml of pristane or with PBS as control and sacrificed after 8 months. Histology was obtained from various tissues: kidney, lung, CNS, heart, liver, spleen, stomach and intestines (staining with HE and additional PAS stainings for kidney samples). Lymphocytes were isolated from intraperitoneal granulomas that represent the major site of inflammation and analyzed separately for each mouse (by FACS) to allow correlation with (renal) pathology. Splenic lymphocytes served as control population. We assessed frequencies of lymphocyte subtypes (CD4+, CD8+, and CD19+) and the percentages of CD4+ regulatory T cells (Treg) and CD4+ activated T effector cells (Teff) as well as their Th1, Th2 and Th17 phenotype (intracellular staining for FoxP3 and IFNγ, IL-4, IL-17, respectively ). In addition, anti-chemotactic and anti-histone serum-Abs were determined.

Results: All PIL, but no control developed lupus pneumonitis (pulmonary capillaritis). 28% of PIL mice developed proliferative lupus glomerulonephritis (corresponding to GN WHO III and IV in humans). Less prominent lymphocytic infiltration was also found in PIL spleens, livers and large intestines. The skin, heart and small intestines did not show signs of inflammation. We compared the T cell response in severely sick nephritis-PIL.
with PIL mice without renal involvement (moderate-PIL). In both groups, we found a higher frequency of activated Teff within the intraperitoneal granulomas (in both PIL groups >15% vs. <5% of Teff in healthy or PIL spleens, p<0.01%) and an increased Teff/Treg ratio. Granuloma lymphocytes of both PIL groups had a similarly prominent Th1 response (28.3 and 25.5%, respectively, p=0.004 vs. healthy control). Nephritis-PIL had an increased frequency of Th2 (42.4±24.7% vs. 22.3±11.6%, p=0.004) and, more prominent, of Th17 (35.1±27.2% vs. 15.3±8.6%, p=0.002) when compared to moderate-PIL. Both groups of PIL mice developed anti-chromatine- and anti-histone-Abs, but without difference in serum levels or temporal occurrence. Conclusion: PIL in BALB/c is characterized by pneumonitis, increased lymphocytic infiltrates in spleen, liver, large intestine, and, less frequent, by lupus glomerulonephritis. While, in line with the literature, PIL mice overall show an upregulated Th1 response, severely ill mice with lupus nephritis also exhibit an increased frequency of Th2 and Th17 cells. Thus, Th2 and particularly Th17 at the primary site of inflammation may be important in driving lupus GN in PIL mice.

CS8 Apoptosis and Lupus

CS8.1 The role of cell death in the generation of extracellular DNA
Pisetsky, David
Durham VA Hospital and Duke University Medical Center, Durham, NC, USA

DNA is a large polymeric macromolecule that plays a key role in the pathogenesis of systemic lupus erythematosus (SLE), serving as an autoantigen and autoadjuvant. This role reflects the intrinsic immunological activity of DNA which, depending on context and its participation in immune complexes with anti-DNA autoantibodies, can lead to the stimulation of nucleic acid sensor sensors. These sensors are located in the cytoplasm of cells and include both toll-like receptor (TLR) 9 as well as various non-TLR receptors. Stimulation of these sensors can lead to the activation of the innate immune system, including production of type 1 interferon, as well as B cell activation. Furthermore, immune complexes with DNA can deposit in the kidney to incite nephritis. For DNA to form immune complexes, it must leave its usual intracellular location, trafficking from the nucleus to the extracellular space. This process can occur during cell death, both apoptosis and necrosis, as shown in in vitro and in vivo models. In animal models, the expression of extracellular DNA occurs with the administration of apoptotic or necrotic cells or the treatment of mice with agents such as anti-Fas or hepatotoxins that can cause liver cell death. With the administration of dead cells, the extent of extracellular DNA is influenced by the presence of macrophages, prior inflammation as well as animal sex. Interestingly, in these models, the amount of extracellular DNA is greater in female mice than male mice, a finding perhaps related to the increased propensity of females for autoimmunity. In the extracellular space, DNA can exist in a free form as well as a particulate form called microparticles. Microparticles are small membrane-bound vesicles that exit from cells during activation or apoptosis and contain a variety of nuclear and cellular components. DNA, in the form of microparticles, is antigenically active, with particles capable of forming immune complexes. Together, these findings suggest that the extracellular release of DNA can be an important step in the pathogenesis of SLE, providing a source of antigen to stimulate autoantibody production as well as form immune complexes.

CS8.2 After apoptosis: inflammation and autoimmunity
Peng, YuFeng; Elkon, Keith B.
Departments of Medicine and Immunology, University of Washington, Seattle, WA, USA

It is now generally accepted that defective clearance of dying cells predisposes to SLE and, perhaps other systemic autoimmune disorders. One example of this association is the lupus like disease that develops in the absence of the apoptotic cell poison, MFGE-8. Precisely how an increase in dying cells leads to a break in tolerance is poorly understood. The identification of specific death associated molecular patterns (DAMPs) or ‘alarmins’ released from necrotic cells helps to explain induction of inflammation. Furthermore, intracellular sensors that recognize self RNA and DNA are increasingly been characterized. To evaluate how apoptotic cells are processed in the absence of opsonins, we examined intracellular trafficking of an apoptosis-associated antigen (OVA) by dendritic cells in mice deficient for MFGE-8. We observed that, in the absence of MFGE-8, apoptotic cell debris persisted longer in endosomes whereas in the presence of MFGE-8, apoptotic material trafficked to lysosomes. The change in intracellular trafficking was associated with an increased T cell response to apoptosis associated OVA by CD8 T cells indicating enhanced cross presentation. Furthermore, MFGE-8 deficient mice spontaneously developed a skin rash associated with infiltration of CD8+ T cells. MFGE-8 deficient RIP-OVA transgenic mice developed diabetes with higher frequency and greater rapidity at an earlier age following adoptive transfer of OT-1 T cells. These findings indicate that, in addition to the known role of MFGE-8 in promoting uptake of apoptotic cells by tingible body macrophages in germinal centers, this protein also influences the route of antigen processing in dendritic cells. In the absence of MFGE-8, antigen specific CD8+ T cells are excessively stimulated leading to loss of tolerance. As SLE patients have evidence of activated CD8+ T cells, opsonin deficiency may set up a positive feedback loop where autoreactive CD8+ T cells contribute to further generation of apoptotic antigens.

CS8.3 Clearance deficiency is involved in the etiology and pathogenesis of lupus
Herrmann, Martin

The inefficient clearance of dying cells can result in the accumulation of apoptotic cell remnants. This occurrence is considered an intrinsic defect that can cause the permanent presence of cellular debris responsible for the initiation of systemic autoimmunity in diseases such as systemic lupus erythematosus (SLE). If postapoptotic debris accumulates in germinal centers, activates complement and functions as a survival signal for B cells that accidentally had become autoreactive by somatic hypermutation, autoimmunity could arise (etiology). The accumulation of postapoptotic remnants and fragments derived from secondary necrotic cells remnants (SNEC) in the presence of autoantibodies against apoptotic cells or adaptor molecules obliges its pathological elimination and maintains autoinflammation. The autoimmunity occurring in SLE patients involves complex antigens that contain nucleic acids (virus mimetic). Complexes of autoantibodies, proteins and nucleic acids are likely to be mistaken by the immune system for opsonized viruses, resulting in the production of type I interferons, a hallmark of SLE (pathogenesis). The pathogenicity of autoantibodies is thought to strongly increase if autoantigens are accessible for immune-complex formation. The latter may be considered a binary pyrogen formed from less pro-inflammatory components. The accessibility of cognate autoantigens, in turn, is likely to be related to impaired or delayed clearance of apoptotic cells.
CS8.4

The TAM kinases in lupus and inflammation

Cohen, Philip1 Shao, Wenhai1 Zhen, Yuxuan1 Suh, Chang-Hee1 Su, Yin1 Hilliard, Brendan1 Li, Sophia1 Monestier, Marc1 Merrill, Joan3

To determine DNase1 activity levels in sera from healthy first-degree relatives of systemic lupus erythematosus patients Martinez-Valle1, Ferran1 Balada, Eva1 Solans-Laquè, Roser2 Vilardell-Tarrès, Miquel1 Ordi-Ros, Josep1

Objective: To determine DNase1 activity levels in sera from healthy first-degree relatives of patients with systemic lupus erythematosus (SLE). Methods: We evaluated 18 families with two or three members affected with SLE. All the SLE patients fulfilled at least four of the American College of Rheumatology criteria. In 13 families there were two SLE patients in each one, whereas 4 families included 3 SLE affected individuals in each and one family had 6 members affected. Overall, we collected 44 SLE patients, 168 healthy relatives and 106 were first-degree relatives (parents, brothers/sisters, and sons/daughters). An ethnically matched random healthy control population (102 blood donors) was also included in the study. The ratios men/women were similar in both groups (controls and healthy relatives) (1.07 versus 1.12). DNase1 activity was measured by an enzyme radial diffusion assay based on the DNA hydrolysis in an agar gel, and nucleosome levels were measured by a commercial ELISA kit. Data were analyzed by the statistical program SPSS 12.0. The TAM kinases (tyro 3, axl, and mer) are key regulators of innate immunity and serve as important receptors for binding and phagocytosis of apoptotic cells. Mice lacking one or more of these receptors develop lupus-like autoimmunity. We have investigated the role of these receptor tyrosine kinases and their ligands (Gas 6 and protein S) in autoimmune disease in mice and in humans. It is primarily tingible body macrophages that express mer in tissues and in inflammatory sites, and Gas 6 that serves as the principal ligand for macrophages. Macrophages from mer-deficient mice have enhanced expression of TNFα and IL-12, and are resistant to downregulation of cytokines by complexes of apoptotic cells and anti-inflammatory cytokines. Mer also is important in downregulating glomerular inflammation through its expression on mesangial cells. B cells activated through MHC II show increased mer expression, which is apparently involved in T-B interactions. Dendritic cell mer expression is crucial in determining their cytokine profile in the presence of apoptotic cells. Human expression of mer is minimal on resting mononuclear cells, yet these cells express axl constitutively. Tyro 3 expression increases upon B-cell activation in vitro. Gas 6 levels in lupus patients are similar to those seen in normal controls, while Protein S levels are reduced, particularly in patients with anti-phospholipid syndrome. These studies highlight the importance of the TAM ligands in experimental autoimmunity and in regulation of the immune response. The TAM ligands are potential targets for therapeutic intervention in immune and inflammatory diseases. Supported by grants from NIAID and NIDCR.

CS8.5 & PO1.A.1

DNase1 activity and circulating nucleosomes in healthy relatives of systemic lupus erythematosus patients

Martinez-Valle1, Ferran1 Balada, Eva1 Solans-Laquè, Roser2 Vilardell-Tarrès, Miquel1 Ordi-Ros, Josep1

To determine DNase1 activity levels in sera from healthy first-degree relatives of patients with systemic lupus erythematosus (SLE). Methods: We evaluated 18 families with two or three members affected with SLE. All the SLE patients fulfilled at least four of the American College of Rheumatology criteria. In 13 families there were two SLE patients in each one, whereas 4 families included 3 SLE affected individuals in each and one family had 6 members affected. Overall, we collected 44 SLE patients, 168 healthy relatives and 106 were first-degree relatives (parents, brothers/sisters, and sons/daughters). An ethnically matched random healthy control population (102 blood donors) was also included in the study. The ratios men/women were similar in both groups (controls and healthy relatives) (1.07 versus 1.12). DNase1 activity was measured by an enzyme radial diffusion assay based on the DNA hydrolysis in an agar gel, and nucleosome levels were measured by a commercial ELISA kit. Data were analyzed by the statistical program SPSS 12.0. The TAM kinases (tyro 3, axl, and mer) are key regulators of innate immunity and serve as important receptors for binding and phagocytosis of apoptotic cells. Mice lacking one or more of these receptors develop lupus-like autoimmunity. We have investigated the role of these receptor tyrosine kinases and their ligands (Gas 6 and protein S) in autoimmune disease in mice and in humans. It is primarily tingible body macrophages that express mer in tissues and in inflammatory sites, and Gas 6 that serves as the principal ligand for macrophages. Macrophages from mer-deficient mice have enhanced expression of TNFα and IL-12, and are resistant to downregulation of cytokines by complexes of apoptotic cells and anti-inflammatory cytokines. Mer also is important in downregulating glomerular inflammation through its expression on mesangial cells. B cells activated through MHC II show increased mer expression, which is apparently involved in T-B interactions. Dendritic cell mer expression is crucial in determining their cytokine profile in the presence of apoptotic cells. Human expression of mer is minimal on resting mononuclear cells, yet these cells express axl constitutively. Tyro 3 expression increases upon B-cell activation in vitro. Gas 6 levels in lupus patients are similar to those seen in normal controls, while Protein S levels are reduced, particularly in patients with anti-phospholipid syndrome. These studies highlight the importance of the TAM ligands in experimental autoimmunity and in regulation of the immune response. The TAM ligands are potential targets for therapeutic intervention in immune and inflammatory diseases. Supported by grants from NIAID and NIDCR.

CS9.1

Genes that make lupus possible

Harley, John B.

Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

Objectives: To determine DNase1 activity levels in sera from healthy first-degree relatives of SLE patients with systemic lupus erythematosus. Methods: We evaluated 18 families with two or three members affected with SLE. All the SLE patients fulfilled at least four of the American College of Rheumatology criteria. In 13 families there were two SLE patients in each one, whereas 4 families included 3 SLE affected individuals in each and one family had 6 members affected. Overall, we collected 44 SLE patients, 168 healthy relatives and 106 were first-degree relatives (parents, brothers/sisters, and sons/daughters). An ethnically matched random healthy control population (102 blood donors) was also included in the study. The ratios men/women were similar in both groups (controls and healthy relatives) (1.07 versus 1.12). DNase1 activity was measured by an enzyme radial diffusion assay based on the DNA hydrolysis in an agar gel, and nucleosome levels were measured by a commercial ELISA kit. Data were analyzed by the statistical program SPSS 12.0. The TAM kinases (tyro 3, axl, and mer) are key regulators of innate immunity and serve as important receptors for binding and phagocytosis of apoptotic cells. Mice lacking one or more of these receptors develop lupus-like autoimmunity. We have investigated the role of these receptor tyrosine kinases and their ligands (Gas 6 and protein S) in autoimmune disease in mice and in humans. It is primarily tingible body macrophages that express mer in tissues and in inflammatory sites, and Gas 6 that serves as the principal ligand for macrophages. Macrophages from mer-deficient mice have enhanced expression of TNFα and IL-12, and are resistant to downregulation of cytokines by complexes of apoptotic cells and anti-inflammatory cytokines. Mer also is important in downregulating glomerular inflammation through its expression on mesangial cells. B cells activated through MHC II show increased mer expression, which is apparently involved in T-B interactions. Dendritic cell mer expression is crucial in determining their cytokine profile in the presence of apoptotic cells. Human expression of mer is minimal on resting mononuclear cells, yet these cells express axl constitutively. Tyro 3 expression increases upon B-cell activation in vitro. Gas 6 levels in lupus patients are similar to those seen in normal controls, while Protein S levels are reduced, particularly in patients with anti-phospholipid syndrome. These studies highlight the importance of the TAM ligands in experimental autoimmunity and in regulation of the immune response. The TAM ligands are potential targets for therapeutic intervention in immune and inflammatory diseases. Supported by grants from NIAID and NIDCR.
**Abstracts of Oral Presentations**

**Objective:** Duplicated Tlr7 promotes lupus-like disease in male BXSB-Yaa mice, prompting us to evaluate TLR7 in SLE patients especially in males. Methods SNP genotyping was conducted using Illumina Infinium platform in the discovery panel, and Taqman in replication panels. Relative expression of TLR7 mRNA in PBMCs was measured by RT-qPCR. Allelic specific transcript analysis was performed by pyrosequencing. **Results:** Fine-mapping the 23-kb TLR7 region using 11 SNPs in 1,434 SLE cases of Eastern Asian descent vs. 1,591 EA controls showed association of 2 TLR7 SNPs with SLE (rs5935436 in the promoter, \( p = 1.8 \times 10^{-3} \); rs3853839 in the 3′UTR, \( p = 6.7 \times 10^{-4} \)). In this discovery panel, the association signal of rs3853839 was mainly found in Chinese (cases/controls: 563/522, \( p = 6.3 \times 10^{-6} \)) with a higher OR in males than females (OR=5.6 vs. 1.5), but not detected in Koreans (84/1,022, \( p = 0.32 \)). This association with SLE with a stronger male effect was replicated in both independent panels of Chinese (2,340/2,436, \( p = 2.7 \times 1.2 \), 9.0 \( \times 10^{-4} \)) and Japanese (560/913, OR = 3.5 vs. 1.2, \( p = 0.007 \)). Rs5935436 was not associated with SLE in replication panels. In the combined analysis of 4,334 cases and 4,940 controls, the G allele of rs3853839 was associated with SLE (\( p = 6.5 \times 10^{-10} \)), exhibiting a higher OR in males than females (OR = 6.7 vs. 1.1) and Japanese (560/913, OR = 3.5 vs. 1.2, \( p = 0.007 \)). Rs5935436 was associated with SLE in replication panels. In the combined analysis of 4,334 cases and 4,940 controls, the G allele of rs3853839 was associated with SLE (\( p = 6.5 \times 10^{-10} \)), exhibiting a higher OR in males than females (OR = 6.7 vs. 1.1) and Japanese (560/913, OR = 3.5 vs. 1.2, \( p = 0.007 \)). Rs5935436 was associated with SLE in replication panels. In the combined analysis of 4,334 cases and 4,940 controls, the G allele of rs3853839 was associated with SLE (\( p = 6.5 \times 10^{-10} \)), exhibiting a higher OR in males than females (OR = 6.7 vs. 1.1) and Japanese (560/913, OR = 3.5 vs. 1.2, \( p = 0.007 \)). Rs5935436 was not associated with SLE in replication panels. In the combined analysis of 4,334 cases and 4,940 controls, the G allele of rs3853839 was associated with SLE (\( p = 6.5 \times 10^{-10} \)), exhibiting a higher OR in males than females (OR = 6.7 vs. 1.1) and Japanese (560/913, OR = 3.5 vs. 1.2, \( p = 0.007 \)).

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**CS9.3**

**Genetics of lupus: what did we learn from the mouse?**

**Morel, Laurence**

**University of Florida, College of Medicine, Gainesville, FL, USA**

Great progress has been made in the field of SLE genetics in the past few years. Genome-wide association studies conducted on human SLE patients have provided over 20 validated susceptibility genes. In murine models of SLE, such as the NZM2410, MRL/lpr and BXSB-Yaa strains, studies that have been initiated over a decade ago as linkage analyses have started to identify susceptibility genes. In this context, this presentation will review the current status of genetic research in the NZM2410 model. So far, the susceptibility genes that have been identified affect B cells, T cells, and renal functions, and they correspond to known genes whose function had not been previously implicated in SLE or autoimmune pathogenesis. In addition, this presentation will show that spontaneous models of murine lupus constitute excellent models of the genetic architecture of human SLE. This notion has been greatly strengthened by the convergence of the functional pathways that are defective in both human and murine lupus. Within these pathways, variants in a number of genes have now been shown to be directly associated with lupus in both species. Consequently, murine models will continue to serve a pre-eminent role in lupus genetics research.

**CS9.4**

**Trans-ethnic association studies and the SLE-susceptibility locus TNFSF4**

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**Abstracts of Oral Presentations**

**CS10 T Regs in Lupus**

**CS10.1**

Characterization of regulatory T cells (Treg) and Treg subsets in patients with systemic lupus erythematosus (SLE)

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CD4+CD25+ Treg that specialize in the suppression of immune responses play a critical role in the regulation of peripheral immune tolerance. This has led to the hypothesis that either quantitative and/or qualitative deficiencies of Treg might be involved in the outbreak of overt autoimmune disease. However, as for SLE, but similar for other autoimmune diseases, data concerning Treg numbers and function are often conflicting. One of the reasons for controversial observations is certainly, at least in humans, the lack of a specific Treg marker molecule. Expression of CD25, but also of FOXP3, can be induced upon T cell activation and in principle this holds true for all Treg associated marker molecules such as CTLA-4, GITR, LAG-3 or CD127. In line with this, our own studies demonstrated decreased proportions of CD4+CD25+ T cells but increased proportions of CD4+FOXP3+ T cells in SLE patients with high disease activity, suggesting that the expression of FOXP3 on CD4+ T cells in SLE patients at least to some extent, reflects the activation of CD4+ T cells (1). In addition we observed a distinct CD4+FOXP3+ T cell population in SLE patients that lacked CD25 expression (CD4+CD25+FOXP3+). CD4+CD25+FOXP3+ T cells phenotypically resembled CD4+CD25+Foxp3+ Treg but were partially deficient in regard to their suppressive capacity in vitro. In summary analysis of T cells in patients with autoimmune diseases, such as in SLE, must be made with caution. Chronic inflammatory stimuli might create an environment that substantially affects Treg in regard to their developmental and regenerative potential.


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**Osteoscytectomy (n=116) with cardiac NL were identified from the U.S. Research Registry for Neonatal Lupus. Cases were genotyped using the Illumina 370K SNP platform and merged with 335 controls from the International Consortium on Systemic Lupus Erythematosus Genetics (SLEGEN). Standard quality control and admixture-adjusted tests of association were computed. Results: Outside the HLA region, a strong association was detected at 21q22, upstream from the transcriptional regulator ERG (rs743446, p=5.4E-06, OR = 2.40). Within the HLA, associated regions include PSORS1C1 (rs3130544, p = 1.94E-07, OR = 2.77) and a missense mutation (proline to serine) at TCF19 (rs7750641, p = 1.38E-07, OR = 2.79), at Class I; several variants in the MICB-NFκB1-LTA-TNF-LTB-AIF1 region at Class III (rs2230365, p=1.00E-03, OR=0.46; rs2857595, p=1.96E-09, OR = 2.37; rs3129892, p = 6.40E-06, OR =1.86; and rs3099844, p = 4.52E-10, OR = 3.34; and the C6orf10 locus at class II (rs3115553, p=2.69E-05, OR =1.81; rs6457536, pa=1.74E-05, OR =1.84; and rs7753597 (p = 1.35E-09, OR=3.30). These are consistent with our previous results (Clancy 2002). With the exception of the HLA region, no loci previously implicated in autoimmune diseases achieved genome-wide significance in the CHB children. Conclusion: These results suggest that genetic variation near ERG, PSORS1C1, LTA/TNF/LTB and C6orf10 in the fetus may promote an abnormal tissue response initiated by exposure to maternal autoantibodies. Identification of risk loci is an incremental step towards discovery of a fetal genetic component that contributes to the anti-SSA/Ro associated development of lifelong cardiac damage.

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**CS9.5 & PO1.G.3**

Genome-wide association study of cardiac manifestations of neonatal lupus identifies risk variants in the ERG, TCF19, C6orf10 and MICB-TNF-AIF1 region

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**Objectives:** Isolated congenital heart block (CHB) is highly associated with maternal anti-Ro/SSA antibodies. CHB carries a substantial mortality, approaching 30%, and morbidty, with over 60% of surviving affected children requiring lifelong pacemakers. To date, complete atrioventricular (AV) block is irreversible. This is a rare disease and factors beyond requisite maternal autoantibody are unknown. Data from twin studies and the 10-fold increased recurrence rate of cardiac neonatal lupus (NL) in subsequent pregnancies indicate a strong genetic contribution to risk. We posit that fetal genes influence the response to maternal autoantibodies. **Methods:** Children of European an-
T cells from patients with the autoimmune disease systemic lupus erythematosus (SLE) display a characteristic hyperactive phenotype. Furthermore, Foxp3 expression is increased within the CD4 population, which could also reflect increased T cell activation. Although CTLA-4 expression was increased in responder T cells, its expression was normal on the Foxp3+ population. However, CTLA-4 was unable to regulate responder T cell proliferation, lipid microdomain formation and phosphorylation of TCR-z following CD3/CD28 co-stimulation, in contrast to healthy responder T cells. Although lupus T cells responded in vitro to CD3/CD28 co-stimulation, there was no parallel increase in CTLA-4 expression, which would normally provide a break on T cell proliferation. These defects were associated with exclusion of CTLA-4 from lipid microdomains providing an anatomical basis for its loss of function. Collectively our data identify CTLA-4 dysfunction as a potential cause for abnormal T cell activation in patients with SLE, which could be targeted for therapy.

The origins and consequences of a regulatory T cell (Treg) disorder in systemic lupus erythematosus (SLE) are poorly understood. In the (NZBxNZW) F1 mouse model of lupus, we found that CD4+Foxp3+ Treg failed to maintain a competitive pool size in the peripheral lymphoid organs resulting in a progressive homeostatic imbalance of CD4+Foxp3+ Treg and CD4+Foxp3− conventional T cells (Tcon). In addition, Treg acquired phenotypic changes that are reminiscent of IL-2 deficiency concomitantly to a progressive decline in IL-2-producing Tcon and an increase in activated, IFN-gamma-producing effector Tcon. Nonetheless, Treg from lupus-prone mice were functionally intact and capable to influence the course of disease as shown by adoptive transfer of Treg into mice with already established disease. Systemic reduction of IL-2 levels early in disease promoted Tcon hyperactivity, induced the imbalance of Treg and effector Tcon, and strongly accelerated disease progression. In contrast, administration of IL-2 partially restored the balance of Treg and effector Tcon by promoting the homeostatic proliferation of endogenous Treg. IL-2 treatment of diseased mice also strongly impeded disease progression that was most efficient by application of a repetitive regimen. In summary, an acquired and self-amplifying disruption of the Treg-IL-2 axis contributed essentially to Tcon hyperactivity and the development of murine lupus. The reversibility of this homeostatic Treg disorder provides novel and promising approaches for the selective treatment of SLE (Hunrich et al., Proc Natl Acad Sci USA: Vol. 107, 2010).
patients in contrast to other organ manifestations was observed. In line with this, proportions of CD4+CD25-Foxp3+ T cells correlated with the extent of proteinuria. Conclusions: In summary we found a significant increase of CD4+CD25-Foxp3+ T cells in patients with SLE who suffer from glomerulonephritis suggesting their involvement in kidney pathology. Ongoing analysis of kidney biopsies have been designed to unravel their role in the development of glomerulonephritis in SLE patients.

CS11 Pediatric: Special Needs and Challenges for Children and adolescents with SLE: A Global Perspective

CS11.1
SLE in Children and Youth: The Global Challenge
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Systemic Lupus Erythematosus in children and youth poses unique challenges. First the diagnosis is often overlooked in this age group. Second, the manifestations differ from those in the adult population. Third, the management requires the expertise of physicians trained in both pediatrics and rheumatology. The problem of under-recognition of childhood SLE is probably worldwide, and requires attention to the problem in medical school curricula, and in the training programs in pediatrics, rheumatology and family medicine. Early exposure of medical students and pediatric residents to pediatric rheumatology clinics is needed. Even organizations which purport to represent patients with SLE sometimes neglect the child and youth with this disease. The clinical manifestations of SLE are often more severe in children and adolescents than in adults. This fact frequently influences the therapy recommended. Therapy including corticosteroids and cytotoxic drugs may have side-effect of particular importance to the child such as limitation of growth, and the accumulation of risk for long-term complications such as atherosclerosis and malignancy. Evidence principally from North America and Europe indicates that some ethnic and racial groups are at much higher risk for SLE than others. Unfortunately, similar data from Asia and Africa are lacking, and the worldwide burden of SLE can only be roughly estimated. The absence of such data limits the ability of physicians in the developing world to demonstrate the need for better education and access to newer, albeit more expensive treatment. A global collaborative effort to determine the burden of disease caused by SLE in children and adolescents could be an initial step leading to better care and healthier lives.

CS11.2
Clinical research in pediatric SLE: the APPLE experience
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There have been few clinical trials in pediatric SLE and treatment is largely based on research in adults. Through the Childhood Arthritis and Rheumatology Research Alliance (CARRA) and the Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial, the pediatric rheumatology community in the United States and Canada has collaborated on a complex multicenter prospective randomized controlled trial, which serves as a model for future clinical and translational research studies. Although it took three years, the APPLE study fully enrolled in 2006 with 221 children and adolescents with SLE. Follow up was completed in 2009, and the database was locked May 2010 with results available later this year. This flagship CARRA trial faced issues unique to the complex study design of APPLE (use of shared ultrasound machines), problems common to studies in SLE (how to capture SAE’s and successfully enroll minority subjects), as well as the more general difficulties performing trials in small, academic sites. There is large variability in the enrollment rate at different sites dependent on principal and site investigator involvement, adequate training of all research personnel, and site specific issues. Importantly, aggressive interventions initiated centrally either by networks such as CARRA or study principal investigators are successful in improving site performance. Experience and data gained from the APPLE trial has successfully been used to leverage funding for larger clinical research efforts moving forward, particularly the establishment of a CARRA wide registry for children and adolescents with SLE as well as other defined rheumatic diseases.

CS11.3
The UK pediatric lupus group
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Background: Progress in understanding rare pediatric autoimmune diseases such as juvenile-onset SLE (JSLE) are hampered by small patient numbers cared for in any one centre. To address this, a collaborative national cohort of patients was developed to include a comprehensive collection of clinical phenotypic data, related biobank and investigation of immune function and biomarkers in JSLE. Aims: To determine the demographics and longitudinal clinical characteristics of JSLE across the UK; develop a related biobank; investigate the immunopathogenesis of JSLE; facilitate international comparative studies and trials. Methods: Through the establishment of the UK JSLE Study Group, using multi-disciplinary consensus methodology, a comprehensive, prospective portfolio of clinical data collection was developed assessing: demographics, disease presentation, activity, damage and response to medication. Following full research ethical approval, the UK JSLE Cohort Study & Repository was established in August 2006 and is currently recruiting patients from 16 centres across the UK (all of the main JSLE centres). All patients with two or more of the ACR diagnostic criteria for SLE in patients 16 years or younger at the time of diagnosis are eligible for recruitment to this study, following informed consent. Results: To date, over 240 patients have been recruited. Clinical characteristics of this cohort will be presented in detail. All patients have been consented for collection of a related biobank for genetic and autoantibody profiling which is now being established. Initial investigation has explored the role of neutrophil apoptosis in the development of JSLE, demonstrating significant dysregulation of both pro- and anti-apoptotic mediators. Further work is focussing on surface expression of nuclear autoantigens and the role of toll-like-receptors in triggering the adaptive immune system leading to loss of tolerance and development of autoantibody production and the longitudinal investigation of renal biomarkers in lupus nephritis. This programme of translational research is integrated into a comprehensive portfolio of clinical studies / trials being developed by the UK Paediatric Rheumatology Clinical Studies Group. Conclusions: Through multi-centre, multi-disciplinary collaborative and consensus research methodology, a national Cohort Study & Repository of patients with JSLE has been is established with a comprehensive research agenda exploring the clinical characteristics, immunopathology and genetics of JSLE in the UK.

CS11.4
Special needs and challenges for children and adolescents with SLE: a global perspective
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The Indian Perspective – Introduction: The child with SLE in India has to contend with many serious issues that are unique to our part of the world. The needs and challenges exist in many areas and span a whole gamut of issues ranging from awareness of this condition, infectious complications that arise in a tropical climate, resources available to treat, patient retention and also
social taboos about disease and disability. I will discuss each of them briefly.

The challenges: (1) Physician and Public awareness. (i) Physician awareness. SLE in children is seen in about 1: 10000. It is thus a relatively rare disease and the prevalence is one tenth of juvenile arthritis. There are two reasons why this condition is not well recognized by physicians at an early stage of the disease. One: In India there still exists a huge burden of infectious diseases which is for several reasons: A tropical climate, poor preventive health and hygiene especially availability of safe drinking water and waste disposal, inequitable distribution of health care infrastructure, lack of awareness in the population and late self referral to available health care facilities. It is also an endemic area for tuberculosis and several vector borne diseases such as malaria and dengue. As a result, the burden of infectious diseases is high and so common place that SLE unless not an obvious presentation with a malar rash and nephritis is often overlooked and mistaken for an infectious disease. Two: There is a lack of post graduate training in Paediatric Rheumatology in India and there are only a handful of centres in metropolitan cities that cater exclusively to this population. Thus children with rheumatologic diseases are not recognized early and interestingly when they have joint pains are traditionally referred to the orthopaedic surgeons who are not tuned to recognizing these conditions. The child often goes from one medical care facility to the next seeking attention until the correct diagnosis is reached. The child with an explosive onset of disease who is very unwell and seeks care at a tertiary level centre is far more likely to be appropriately diagnosed and managed than the patient with indolent disease who has low grade fever and fatigue with mild cytopenia. (ii) Public awareness. Is virtually nonexistent, especially about complex immunoinflammatory conditions such as SLE. This is probably for two reasons: the adult literacy rate in India is about 61% and less in women and in rural areas. It is with literacy that one develops awareness and knowledge and can self refer and seek medical advice from an appropriate resource. Secondly; illness in the family especially in the girl child is kept a secret from the extended family and friends. The secrecy shrouding any illness especially a chronic one is an impediment to the patient with SLE as appropriate advice is not sought from elders or other knowledgeable persons in the family. (2) Social Taboos. Rudyard Kipling once famously remarked “East is East, and West is West, and never the twain shall meet”. This holds true for all the social taboos that surround illness in India and impact on the care given to the girl child. This gender inequality is slowly fading and not prevalent in large metropolitan cities in India, but is certainly seen in the tier II cities and in the rural India. The girl child is not usually given the same educational and social opportunities as her male counterpart, and may indeed be denied expensive and prolonged care that is to be provided at a centre far removed from the home –town that can offer care and support but not a cure. (3) Patient Retention and family fatigue. This is an area of concern both in India and the developed world. As the disease is a chronic one where the child or adolescent patient tends to have repeated flares, it is challenging to keep the child and the family engaged with the medical team. We are handicapped in that we do not have a trained pool of nurse specialists and social workers who can be useful team members and follow up and counsel the families who do not attend follow up appointments. (4) Alternative systems of medical care. There are several systems of medical care followed in India: Ayurvedic, Homeopathic, Unani, Yoga, Spiritual healing, etc. Several of these systems promise magical cures and patients who have persistent active disease are lured into these therapies often with disastrous results. Unfortunately there is no appropriate legislation that can prevent these practices. (5) The resources available to treat. (i) Financial. The disparities across the world are stark. The gross national income per capita in the USA is $ 44070, in Australia is $32830, in UK is $34800 and in India is $2460. The per capita government expenditure on health is also very low in India: a mere $ 21, in the USA is $3074 and in Australia is $2097. India’s total expenditure on health amounts to 1.1% of the gross domestic product (GDP), while its per capita total expenditure on health is $80 compared to an average of over $220 spent by many other developing countries. This expenditure far lags behind the industrialized world. Private expenditure on health as percentage of total expenditure on health is 80% in India, 55% in USA and 33% in Australia. Thus in India , there is certainly a major resource crunch, suboptimal money is spent by the government on health care , a fraction of the population is covered by the health insurance which in India does not support out patient care. This country is still grappling with polio eradication and tuberculosis therapy such that diagnosis and therapy of SLE is not on the radar as yet. (ii) The health care delivery system. India has a population of 1.2 billion people. To organize and deliver an affordable health care system that is equitable, efficient, progressive and user friendly is indeed a daunting task that we have not been able to achieve to date. The delivery of health care is fragmented: It is organized by both the public (Government) and the private sectors. Centres of excellence exist in both the private and public sector but there is no efficient system of referral or a defined catchment area for each centre. There are over 250 medical colleges in India but hardly any that have rheumatology services/care. Thus, it is the patient who has to make a tremendous effort to reach the correct health care provider. (iii) Medical personnel. As mentioned earlier there is a shortage of trained personnel in Paediatric Rheumatology in India and the child with SLE may get medical care from several different sources: An internist, a paediatrician , an adult rheumatologist or infrequently from a paediatric rheumatologist. The care given to the child is thus very variable and when the family are faced with a child who is persistently unwell they begin to “doctor shop” and move from one centre to the next. The long term care invariably suffers as different doctors only see one facet of the child’s illness and do not come to terms with the patient specific issues nor do they develop a bond and a trusting relationship with the child and family. (iv) Medications and regular investigations. All medications and the investigations needed to follow children with SLE are available in India; the main difficulty faced by the patient is the cost: Drugs such as mycophenolate and Intravenous Immunoglobulin and investigations such as C3C4 and DsDNA are costly and unaffordable by a vast majority of patients. A fraction of the patients are covered by medical insurance which is not well developed in India; most patients have to self pay. The Needs: To meet the above described challenges the lupus patient in India has several needs: (i) There should be an efficient well trained pool of Paediatric Rheumatologists available in nodal medical centres distributed across the country. To provide this service the undergraduate and post graduate training in paediatric rheumatology should be robust. (ii) The medical insurance facilities should be available for all children with chronic diseases and must include outpatient care. (iii) A Support structure such as an India Lupus Group and a well trained net worked pool of nurse specialists and social workers should be involved in the care of these children. They should be advocates of these young girls and should discuss social taboos, alternative systems of care and the need for regular follow up. (iv) There should be vocational and educational guidance along with a smooth transition to adult rheumatology care. (v) Many if not all our newly diagnosed patients should be recruited into international drug trials. (vi) The data base of these children should be maintained such that “India specific issues” can be well identified. Conclusion: It is with international collaborative efforts and advocacy for these children at home that we may aspire to fulfill the needs of our SLE patients and give them the best quality life that they deserve.

KS1 Lessons Learned from Animal Models

KS1.1 Deep sequencing reveals extensive functional variations in SLE susceptibility genes

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Genetic predisposition is a potent element in susceptibility to SLE. Previous studies by the International Consortium for Systemic Lupus Erythematosus (SLEGEN) have associated more than 20 genomic segments with susceptibility. These studies have localized causative genes into small genomic segments, but have not identified the precise genetic variations responsible for the functional changes that cause the disease. Towards elucidating the genetic lesions that are causative for SLE susceptibility, we have initiated deep sequencing studies of all of the genomic segments exhibiting suggestive or significant association with susceptibility to SLE. These studies are being performed using a targeted sequence enrichment strategy and the Illumina GAIIx next generation sequencer. We have completed sequencing on more than 95 individuals and our initial analysis of data from 32 Caucasian samples has

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revealed more than 2000 novel single nucleotide polymorphisms (SNP) or deletion/insertion variations (DIP) in 2.9 megabases derived from 25 genomic segments showing significant associations with SLE. Our ongoing analysis of the organization of these polymorphisms, utilizing median joining algorithms to network the SNP haplotypes formed in strong linkage disequilibrium with SNPs associated with SLE, has delineated multiple allele lineages with extensive functional variations in haplotypes that are associated with susceptibility to SLE. These results indicate that many of the SNPs currently used to identify susceptibility genes actually mark multiple lineages of alleles. Further, significant functional variations still exist among these alleles, which all carry markers that are strongly associated with SLE. As a result, we believe that our ongoing delineation of functional lesions in SLE-associated alleles will significantly improve our understanding of the functional changes that actually mediate disease susceptibility and ultimately increase the accuracy of relative risk estimates. The overall characteristics of the susceptibility alleles identified to date support the hypothesis that dysregulations of the adaptive and innate immune systems interact to mediate susceptibility to SLE in humans.

PL3 Lupus Epidemiology and Pathogenesis

PL3.1

Blood microarray analysis in SLE
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The past decade has seen an explosion in the use of DNA-based microarrays. These techniques permit to assess RNA abundance on a genome-wide scale and have been used to analyze the blood transcriptome in a wide range of human autoimmune diseases, including SLE. Microarray-based research is facing significant challenges with the analysis of datasets which contain noise, are difficult to interpret, and do not compare well across laboratories and platforms. We recently proposed a novel module-level microarray data mining strategy emphasizing the selection of coordinately expressed genes or transcriptional modules. Once these transcriptional determinants have been characterized, changes in gene expression between study groups can then be assessed on a module-by-module basis. This strategy allowed the identification of disease-specific leukocyte transcriptional fingerprints in patients with SLE. Importantly, we demonstrate that modular transcriptional data can be reproduced across microarray platforms and laboratories and is widely applicable to generate robust and interpretable module-level disease activity biomarkers. More quantitative and sensitive high throughput RNA profiling methods are starting to be available and will be discussed. These assays will make it possible in the foreseeable future for transcriptome analyses to become a routine test in the clinical setting.

PL3.2

What do studies of gene-environment interaction tell us?
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The explosion of genetic data and discoveries provides researchers with a myriad of data to be considered in complex diseases long believed to have genetic components interacting in some way with underlying non-genetic factors. As the list of genes potentially associated with systemic lupus erythematosus grows, understanding how to incorporate these results into epidemiologic studies is critical. In addition to discussing some recent gene-environment interaction (GxE) studies, we will consider GxE study design and the associated strengths and limitations. Two frequent approaches include case-control and case-only studies. In the former, control selection is important and carries a number of important assumptions. We will consider the trade-offs of general population controls, unaffected siblings, spouses, and other groups and how interpretation of results changes. Case-control designs are often chosen because they may require less resources. However, control selection is complicated and some may want to choose another design. The case-only design may be an efficient solution to estimate the association between exposure and genotype among cases, and removes the issues surrounding control selection. However, although we can estimate the relative interaction, we cannot assess the main effect of either the gene or environmental factors. Additionally we will discuss concerns of bias related to confounding and misclassification, which are sometimes dismissed in genetic studies and should not be downplayed in GxE studies. Lastly we will consider measures of multiplicative and additive interaction including the relative excess risk due to interaction, the synergy index, and the attributable proportion due to interaction.

CS12 Late Breaking Abstracts

CS12.1 & PO2.G.10

IRF5 is required for disease development in the FcrRIIB-/-Yaa mouse models of SLE
Yasuda, Kei; Richez, Christophe; Bonegio, Ramon G.; Watkins, Amanda A.; Aprahamian, Tamar; Busto, Patricia; Richards, Rocco J.; Liu, Chih Long; Cheung, Regina; Utz, Paul J.; Marshak-Rothstein, Ann; Rifkin, Ian R.
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Objectives: Polymorphisms in the transcription factor interferon regulatory factor 5 (IRF5) are strongly associated in human genetic studies with an increased risk of developing the autoimmune disease systemic lupus erythematosus (SLE). However, the biological role of IRF5 in lupus pathogenesis has
not previously been tested in an animal model. **Methods:** We crossed IRF5-/- mice with the Fc gamma receptor IIB-/- (FcγRIIIB-/-)Yaa and FcγRIIIB-/- lupus models to examine whether IRF5 is required for disease development in the models. One effect of IRF5 is to induce the production of the type I interferon (IFN), IFN-alpha, a cytokine implicated in lupus pathogenesis. To address the mechanism by which IRF5 promotes disease, we evaluated FcγRIIIB-/-Yaa mice lacking the type I IFN receptor subunit 1 and therefore unable to respond to any type I IFN. We examined disease manifestations. **Results:** We show that IRF5 is absolutely required for disease development in the FcγRIIIB-/-Yaa and FcγRIIIB-/- lupus models. In contrast to IRF5-sufficient FcγRIIIB-/-Yaa mice that developed severe disease, IRF5-deficient FcγRIIIB-/-Yaa mice do not develop lupus manifestations and have a phenotype comparable to non-autoimmune wild type C57BL/6 mice. Strikingly, full expression of IRF5 is required for the development of autoimmunity, as IRF5 heterozygous mice had dramatically reduced disease. Unlike the IRF5-deficient and IRF5-heterozygous FcγRIIIB-/-Yaa mice, type I IFN receptor subunit 1-deficient FcγRIIIB-/-Yaa mice maintained a substantial level of residual disease, demonstrating that the pathogenic effects of IRF5 are not primarily mediated through effects on type I IFN production. Furthermore, in FcγRIIIB-/- mice lacking Yaa, IRF5-deficiency also markedly reduced disease manifestations, indicating that the beneficial effects of IRF5 deficiency in FcγRIIIB-/- mice are not due only to inhibition of the enhanced TLR7 signaling associated with the Yaa mutation. **Conclusions:** We demonstrate that IRF5 plays an essential role in lupus pathogenesis in the FcγRIIIB-/-Yaa and FcγRIIIB-/- mouse models of SLE and that this is mediated through pathways beyond that of type I IFN production.

**CS12.2 & PO1.1.9**

**WASp deficient B cells play a critical, cell intrinsic role in triggering autoimmunity**

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Patients with the immunodeficiency, Wiskott-Aldrich syndrome (WAS), frequently develop systemic autoimmunity. The current study demonstrates that mutation of the WAS gene results in a B cell intrinsic break in tolerance. Whereas this defect leads to autoantibody production in WAS protein deficient (WASp-/-) mice without overt disease, chimeric mice in which only the B cell lineage lacks WASp exhibit severe systemic-lupus-(SLE)-like autoimmunity characterized by high affinity, class-switched autoantibodies, severe renal histopathology, and early mortality. While B cell negative selection was intact, WASp-/- mice exhibited evidence for altered peripheral tolerance beginning within the transitional compartment. In accord with this idea, WASp-/- B cells are hyper-responsive to B cell antigen receptor (BCR) engagement and exhibit impaired BCR internalization. Further, lambda-light chain usage is enriched in WASp-/- B cells beginning at a late transitional B cell stage and this cell population proliferates spontaneously in vivo, suggesting that WASp deficiency directly impacts B cell positive selection. Finally, BCRs specific for apoptotic cell determinants are enriched in WASp-/- mice and, in WASp-/- B cell chimera mice, B cells specific for a subset of such antigens are expanded and undergo T cell-dependent, class switch recombination. Our combined data provide a compelling explanation as to why a large proportion of WAS patients with mixed chimerism following stem cell transplantation develop severe, humoral autoimmunity. Our findings also highlight the primary role for altered peripheral B cell selection in initiation of SLE-like autoimmunity and provide insight into how B cell depletion therapies may operate under such conditions.

**CS12.3 & PO2.G.11**

**Interferon alpha (IFNα) inhibits C-reactive protein synthesis in human hepatocytes. Is the mechanism a decreased STAT3 phosphorylation?**

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**Objectives:** C-reactive protein (CRP) is an acute phase protein mainly produced by hepatocytes in response to interleukin (IL)-6 and IL-1β-triggered signalling via the transcription factors STAT3, C/EBPβ, and NFκB. Because of its rapid increase during inflammation it is widely used to monitor inflammatory activity in chronic diseases such as rheumatoid arthritis. CRP has immunomodulating properties with disease-modifying effects demonstrated in lupus-prone mice. However, despite extensive inflammation the serum levels of CRP typically remain low in disease flares of systemic lupus erythematosus (SLE) as well as in viral infections. Since SLE flares are characterized by an elevation of IFNα and/or upregulation of IFNα-regulated genes (the IFNα-signature) we have hypothesized that the lack of an adequate CRP response is due to IFNα, and we previously reported that the CRP-promoter activity in a transfected human hepatoma cell line is inhibited by IFNα. In the present study we investigated CRP synthesis in primary human hepatocytes and potential changes in intracellular protein phosphorylation to reveal the mechanisms that yield the inhibition. **Methods:** CRP secretion from freshly isolated primary human hepatocytes was measured by ELISA. Hepatoma G2 (HepG2) cells stably transfected with the 1-kb CRP-promoter and a luciferase reporter gene (generously provided by Dr. Jan Torzewski, Ulm, Germany) were used to study intracellular signalling proteins and transcription factors. The protein phosphorylation was quantified by a multiplex phosphoprotein detection assay. **Results:** IL-6-induced or IL-1β-induced CRP secretion from primary human hepatocytes was inhibited by 46-71% in IFNα treated cells (100 or 1000 IU/mL). CRP secretion induced by combined IL-1β and IL-6 stimulation was inhibited by 19.6-27.7%. A marked increase in STAT1 phosphorylation (122-281%) and a reduced STAT3 phosphorylation (17-35%) was observed in IFNα stimulated HepG2 cells after 5, 15, 30 and 360 minutes. No apparent change in phosphorylation of p38 MAPK or IκB was seen in IFNα treated cells. **Conclusions:** IFNα inhibits CRP secretion in primary human hepatocytes which may explain the modest CRP-response in disease flares of SLE but also in viral infections. We found that STAT1 phosphorylation increased, whereas STAT3 phosphorylation decreased, in IFNα treated cells. STAT1 and STAT3 are known to have counteracting effects and we therefore suggest a mechanism where IFNα-induced STAT1 phosphorylation reduces STAT3 phosphorylation and in this manner CRP-synthesis.

**CS12.4 & PO1.B.32**

**A gene expression score derived from interferon, plasma cell and neutrophil gene clusters is an informative biomarker of lupus flare**

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**Objectives:** Treatment of patients with systemic lupus erythematosus (SLE) aims to achieve clinical remission and avoid severe flare. Several indices have been designed to distinguish disease flare from remission but are not routinely used in clinical practice. Anti-dsDNA antibody titers are frequently used to assess flare in the course of patient management, but those antibodies are not present in all patients and often do not reflect disease activity. A biomarker that predicts or identifies lupus flare might provide a tool for more effective and timely medical intervention. **Methods:** Longitudinal PBMC and plasma samples were obtained over an average 6 visits (2-12) from 23 SLE patients and 5 healthy donors (HD). The duration of the study for individual patients varied from 197 to 812 days. Plasma levels of autoantibodies were evaluated using Multi-Analyte Profiling (MAP) technology (Rules-Based Medicine, Austin, TX). PBMC transcriptional profiles for each visit were established using Human Genome U133 Plus 2.0 Arrays. **Results:** Autoantibody profiling...
detected increased plasma levels of 14 specific autoantibodies, including anti-dsDNA, anti-Ro, anti-La, anti-RNP and anti-Smith in SLE patients compared to HD (p<0.05; fold change, FC, >1.5). The level of anti-dsDNA antibody paralleled the changes in the SLEDAI score in 22% of patients. One hundred sixty-nine microarray profiles were obtained from PBMC RNA isolated from the patients and control subjects. Data were subjected to K-mean clustering analysis (k=50, I=100). Among others, clusters characterized by plasma cell transcripts, type 1 interferon-inducible genes, and neutrophil transcripts were observed. Statistical analysis confirmed that those 3 gene clusters distinguished SLE patients from HD. A representative gene from each of the three clusters (CD36, IFIT3 and MMP9) was selected for further analysis as a flare score and reflected the mean of the relative expression values. The score distinguished SLE patients from HD (p<0.001; FC=5.6) and was significantly higher during severe lupus flares compared to remissions (p=0.002; FC=2.0). Analysis of individual patients showed that the flare score paralleled the SLEDAI score in 51% of patients. Conclusion: A score derived from expression levels of genes representing three important pathogenic mediators, plasma cells, interferon and neutrophils, was superior to anti-dsDNA titer as a biomarker of increased SLEDAI score in our study. Validation of this score as a marker of lupus flare may provide an informative tool for improved management of lupus patients and will stimulate an examination of the neutrophil subpopulation associated with disease activity.

CS12.5 & PO2.D.46
The long-term outcomes of leflunomide in patients with lupus nephritis
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Objective: To investigate the long-term outcomes of leflunomide in the treatment of lupus nephritis (LN) beyond six-month induction therapy in a multicentre, open-label extension of one phase III clinical trial. Methods: 108 patients with LN received leflunomide with a loading dose of 50 mg/day for 3 days, followed by 30 mg/day for six months in one phase III study. 56 patients voluntarily enrolled an open-label extension study and were maintained on leflunomide at dose of 20 mg/day, and the dose of prednisone was <10 mg/day. The primary endpoint was a composite of patient survival and renal survival. Renal parameters (24h-proteinuria and serum creatinine) were assessed. Results: A total of 56 patients (mean age 27.7±8.8 years) were treated with leflunomide for >6 months; 83.9% (47) of the patients were female. By conclusion of 6 months induction therapy, 85.4% reached clinical remission (complete remission rate was 34.5%, partial remission rate was 50.9%). The mean duration of leflunomide treatment was 47.0±26.7 months (range 9–88 months). During maintenance therapy, one patient died (of lupus relapse) and one patient developed chronic renal failure. The 84-month patient cumulative survival rate was 98%. The rate of proteinuria relapse-free survival was 94.1%, sustained doubling of serum creatinine rate was 2.5%. At the end of follow-up, 88.2% of the 56 patients achieved clinical remission (complete remission rate was 52.9%, partial remission rate was 35.3%), and the cumulative mortality is 0.004/patient-year (1/234.7 patient-year). Conclusions: Our data confirm that a maintenance regimen of leflunomide followed by induction therapy with leflunomide achieves long-term clinical results in control of LN.

CS12.6 & PO2.E.23
Aspreva Lupus Management Study maintenance results
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Objectives: The Aspreva Lupus Management Study (ALMS; protocol number: WX17801) was a large, multinational, multicenter, Phase III trial. The induction phase (data reported previously) compared the efficacy and safety of mycophenolate mofetil (MMF) with cyclophosphamide (IVC), both with corticosteroids, as treatment for active class III–V lupus nephritis (LN). This abstract focuses on the maintenance phase in which the efficacy and safety of MMF was compared with azathioprine in patients who had achieved a partial or complete response in the induction phase. Methods: In the maintenance phase of this prospective, double-blind, study, patients were re-randomized (Week 24/Month 0) 1:1 to receive either oral MMF (2 g/day) plus placebo or oral azathioprine (2 mg/kg/day) plus placebo. All patients received corticosteroids (maximum dose: 10 mg/day), with dose reduction per investigator judgment. Patients returned for assessment at Week 24/Month 0 plus 2 weeks, Month 1, Month 2, and every 3 calendar months thereafter until Month 36 or study termination. The primary efficacy outcome measure was treatment failure defined as any of the following: death, end-stage renal disease, sustained doubling of serum creatinine, or renal flare [proteinurea or nephritic]. Key secondary parameters included: time to event for each individual component of treatment failure; complete renal remission; combined renal and extra-renal remission; and comparisons of maintenance subgroups. Initial results and updates to this preliminary data will be presented. Results: Of 227 patients randomized (safety population) (North America, n=47; Europe, n=48; Asia, n=72; Latin America, n=40; 99 white; 23 black; 76 Asian; 29 other; 150 classified as non-Hispanic), 101 withdrew from the study and 126 completed. At baseline, mean [SD] age was 32.0 [10.71] years; 195 (85.9%) were female. Mean [SD] duration of LN was 3.4 [4.44] years. More patients had class IV (n=47) LN than class III (n=22), II/IV (n=7), IV/V (n=16), or V (n=35) disease. Measurement of laboratory parameters at baseline revealed the following: serum creatinine (low or normal, n=212; high, n=15); serum albumin (low, n=11; normal, n=212; high, n=4); urine protein (≤1 g/24 hrs, n=156; ≥1 g/24 hrs, n=71); urine protein/creatinine ratio (normal, n=35; high, n=177); serum C3 (low, n=98; normal, n=127; high, n=2) and C4 (low, n=60; normal, n=162; high, n=5); antibodies to double-stranded DNA (absent, n=64; present, n=163). Conclusions: ALMS, one of the largest studies conducted in LN to date, will provide evidence on the efficacy and safety of MMF compared to azathioprine as maintenance therapy.

CS12.7 & PO2.E.24
A randomized, double-blind, placebo-controlled study of splicoesomal peptide rigerimod in patients with systemic lupus erythematosus (SLE)
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Objectives: Rigerimod is a novel peptide medication that has putative immunomodulatory actions for patients with SLE. The effect of subcutaneous (sc) rigerimod on SLE disease activity was evaluated. Methods: Adults meeting ≥ 4 of the ACR criteria for SLE diagnosis, a clinical score ≥ 6 on the SLEDAI-2K, and no A on the BILAG index were enrolled. Patients were randomized to receive sc rigerimod 200 μg/wk, 200 μg/wk, or placebo/2 wk, plus standard of care (SoC) for 12 wks. SoC included antimalarials and oral corticosteroids (up to 80 mg/wk prednisone equivalent) at stable doses for ≥ 4 wks before study treatment. The primary efficacy measure was the SLE Responder Index (SRI) at wk 12 compared with baseline. Adverse events (AEs) were recorded throughout the study. Statistical significance was set at alpha = 0.025. Results: A total of 150 patients completed 12 wks of treatment and either the 24-wk follow-up visit or discontinued the study. 150 patients (female, 96%; mean age, 37.6 yr) received study medication (rigerimod 200 μg/wk, n=49; 200 μg/wk, n=52; placebo/2 wk, n=49). At wk 12, more patients achieved an SRI response with rigerimod 200 μg/wk (53.1%) vs the placebo group (36.2%; P=0.048). Similarly, 53.1% of the rigerimod 200 μg/wk group achieved a SLEDAI-2K response at wk 12 vs the placebo group (38.3%; P=0.0734). The rigerimod 200 μg/wk group showed a response rate of 45.1% on both measures vs the placebo group (36.2% and 38.3%, respectively; P=0.025). The most frequently reported AEs (≥ 5% patients) were urinary tract infection and injection site erythema. Most AEs were mild or moderate in intensity. Serious AEs (SAEs; n=7) included gastritis, soft tissue infection, herpes viral pneumonia, diverticulitis (n=1 each), and pneumonia (n=3); the investigator considered soft tissue infection and herpes viral pneu-
monia to be related to rigerimod 200 μg/4 wk and 200 μg/2 wk, respectively. One SAE, pneumonia, resulted in the death of 1 patient and was considered to be unrelated to rigerimod 200 μg/4 wk. **Conclusions:** Following 3 injections of rigerimod 200 μg/4 wk, patients with SLE showed reduced disease activity vs placebo as assessed by SRI and SLEDAI-2K. Rigerimod was generally well tolerated. Further evaluation of this agent is warranted in patients with SLE.

**CS12.8 & PO2.E.25**

**Long-term outcome of autologous hematopoietic stem cell transplantation (autoHSCT) using lymphoablative conditioning in recalcitrant systemic lupus erythematosus patients**

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**Objectives:** Despite recent improvements in mortality and morbidity of SLE patients with major organ involvement, treatment failure and relapse continue to affect significant majority of patients. We conducted a pilot study to test if intensive lymphoablative followed by autoHSCT can result in sustained, complete, treatment-free remission in severe, recalcitrant SLE and to determine whether this approach fundamentally changes abnormal immune response.

**Methods:** Patients were enrolled based on active SLE despite prior treatment with IV cyclophosphamide (CYC). Of the 8 patients treated, 2 had transverse myelitis, 1 retinal vasculitis and 5 WHO Class IV nephritis. Stem cell mobilization regimen consisted of 2,000 mg/m2 CYC, 750 mg/m2 rituximab (RTX) and G-CSF. Conditioning regimen consisted of 750 mg/m2 RTX, 4.8 g/m2 CYC and 120 mg/m2 fludarabine, followed by CD34+ selected stem cell infusion and G-CSF. All immunosuppressive medications and hydroxychloroquine were discontinued at the start of mobilization and steroids were rapidly tapered off after the transplant. Clinical response was evaluated by organ specific response criteria. Disease activity indices (SLEDAI and SLAM) were used to assess overall lupus activity. The primary endpoint was complete response (CR) at 24 months defined as no lupus activity and no treatment for lupus (including HCQ and steroids). **Results:** Among the 8 patients, there were 2 early deaths (one from diffuse alveolar damage, one from mycobacterial meningoencephalitis). One patient had lupus flare (retinal vasculitis, one from mycobacterial meningoencephalitis). One patient had lupus flare (retinal vasculitis responding to corticosteroids) 6 months post-transplant. Five patients were successfully tapered off corticosteroids, achieved CR criteria within 6 months of transplant and SLEDAI scores of zero. One of these patients flared 18 months post-transplant, whereas 4 continue to be in CR for 4 (n=2) to 5 years (n=2). All four of these patients continue to have negative ds-DNA antibody and normal complement levels since 6 months after HSCT. The reconstituted immune system showed a significant shift from a phenotype dominated by memory and activated effector T and B cells at baseline to a predominantly naïve phenotype post-transplant.

**Conclusions:** Our data indicate that lymphoablative autoHSCT leads to sustained (up to 5 years) clinical and serologic remission, without the use of any maintenance therapy in a subset of otherwise recalcitrant SLE patients. This clinical benefit is associated with marked normalization of the immune repertoire. Reducing to the intensity of conditioning and/or exclusion of patients with multiple organ dysfunction may decrease short term toxicity and would make this approach an acceptable alternative for the treatment of severe SLE.

**CS13 Lupus and the Environment**

**CS13.1 Environmental risk factors for lupus in post-menopausal women: results from the women’s health initiative**

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**Purpose:** Previous studies suggest a possible association of farming occupation and agricultural pesticide mixing with systemic lupus erythematosus (SLE), but such exposures are uncommon in the population and risk associated with personal and residential insecticide use is unknown. **Methods:** Using data from the Women’s Health Initiative Observational Study cohort (n=76,861, post-menopausal, age 50-79 years), we examined self-reported lifetime personal insecticide use, residential workplace application by others, and farm history in relation to risk of incident SLE, confirmed by use of disease modifying anti-rheumatic drugs at year 3 of follow-up. Newly reported SLE cases (n=35) were compared to women in the cohort who did not develop SLE or rheumatoid arthritis. Hazard ratios (adj. HR) and 95% confidence intervals (CI) were estimated in multivariable models adjusting for age, race, region, education, occupation, smoking, reproductive factors, asthma, other autoimmune diseases, co-morbidities and farm history. **Results:** SLE risk was associated with more frequent and longer duration of personal insecticide use, and was highest in those reporting very high or high cumulative personal use (0.12% of exposed developed SLE) compared to those reporting never or minimal use (0.02%; adj. HR= 2.96, 95%CI 0.76, 11.51; p for trend=0.046). Increased SLE risk was also seen for women reporting longer term application by others (p for trend=0.016). Longer duration farm history was associated with SLE risk after adjusting for age, but the association was diminished after adjusting for covariates. **Conclusions:** These findings suggest direct personal and indirect residential insecticide exposures may be related to SLE risk in post-menopausal women, and provide rationale for replication studies in other populations and investigation of specific insecticides.

**CS13.2 Epstein-Barr Virus and early events in lupus autoimmunity**

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Epstein-Barr Virus (EBV) has emerged as a compelling potential trigger of human SLE autoimmunity in susceptible hosts. However, little is known about the involvement of EBV at early, even pre-clinical, time points. For EBV to play important roles in initiating molecular mimicry with autoantigens or instigating interferon-induced pathways in susceptible hosts, then EBV infection should precede clinical SLE and lupus autoimmunity. Utilizing the United States Department of Defense Serum Repository, 130 cases with stored samples from before SLE diagnosis and 520 matched controls were identified and tested for seroconversion against herpesviruses and for standard lupus autoantibodies. EBV-VCA seropositivity was strongly associated with SLE before diagnosis (p=0.0019) as was the presence of antibodies against EBNA-1 (p=0.0017), whereas no relationship was seen with other Herpes viruses. Antibodies to EBNA-1 preceded autoantibody development in 35 patients, while autoantibodies preceded anti-EBNA-1 in only 1 case. Univariate analysis showed an increase in EBV antibody titers in patients compared to controls using all available samples (mean 3.47 for patients, 2.73 for controls, p<0.0001). The significant increase in titers was most prominent and statistically significant in the two years prior to diagnosis with the
patient mean ISR 3.89 and the control mean ISR 2.89 (P=0.0001). CMV titers were also significantly increased in patients overall (p=0.0025) and in the two years before diagnosis (p=0.0115). When all herpes viruses were included in the conditional logistic regression model for titers 2 years prior to diagnosis, only anti-EBV-VCA titers were significant. Antibodies against EBV-VCA and EBNA-1 are associated with SLE prior to disease onset. SLE patients in this cohort had significantly higher titers of anti-EBV-VCA and anti-CMV than controls, but this trend was not observed with HSV1 or HSV2. These data support the hypothesis that EBV plays a role in the early pathogenesis of human SLE. During this lecture we will also discuss data regarding the temporal evolution of the anti-EBNA-1 response in relation to onset and progression of lupus autoimmunity, as well as data showing unique EBV responses in unaffected blood relatives of lupus patients. We will also present and discuss data regarding the influence of EBV infection on lupus patient B cells compared to matched controls.

CS13.3
The US Africa lupus gradient hypothesis revisited: genetics versus environment
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According to the gradient hypothesis, lupus is rare in West Africa, while being common in African Americans. Potential genetic versus environmental causes for this proposed discrepancy include genetic admixture, infection exposures and vitamin D. One problem with the gradient hypothesis is that the assessments of disease prevalence in Africa were performed in African countries that were not major participants in the slave trade. Health care systems in the West African countries that were part of the slave trade are inadequate to assess lupus prevalence. The Gullah people of the Sea Islands of South Carolina and Georgia are unique in their genetic homogeneity and lack of genetic admixture. Ancestors of the Gullah are known to have originated primarily from Sierra Leone. As it is still impossible to perform an adequate epidemiologic study in Sierra Leone, we assessed serum autoantibody profiles, EBV serologies and 25OH vitamin D levels on over 185 sera from unaffected Gullah females and 71 sera from age matched asymptomatic females from Bo, Sierra Leone. ANA positivity was significantly more common in the Gullah sera compared to Sierra Leone sera, while vitamin D levels were significantly higher in the Sierra Leone sera. These data suggest autoimmunity is more common in African American Gullah than young women in Sierra Leone. Autoantibody spectra, EBV serologies and GWAS studies are in progress to further delineate autoimmunity and genetic similarities/differences between women in Sierra Leone and the Sea Islands of South Carolina.

CS13.4 & PO2.B.1
Seasonal variation in the incidence of disease flares in systemic lupus erythematosus (SLE): relationship with weather parameters and ultraviolet light intensity
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Objectives: To examine the seasonal variation in disease flares of SLE with regard to individual organ systems and their relationship with weather parameters and environmental ultraviolet light intensity. Methods: SLE patients who were followed up in our clinic between 2000 and 2008 were studied. Details of disease flares, defined by the SELENA-SLE flare instrument, were retrieved from review of the electronic medical records. Disease activity scores during the flare episodes were measured by the SELENA-SLEDAI. The monthly rates of disease flares (mild / moderate and severe) and of individual organ systems were calculated. Flares in five organ systems (cutaneous and musculoskeletal, serositis, hematologic flare, renal and neuropsychiatric) were defined by using the individual components of SLEDAI and the SELENA-SLE flare instrument. The rate of SLE flares was correlated by Pearson’s correlation with a number of weather parameters which included mean and maximum temperature, relative humidity, total rainfall, duration of sunshine and mean ultraviolet light intensity index each month as released by the Royal Observatory of Hong Kong. Results: 452 SLE patients were studied. There were a total of 425 mild / moderate SLE flares (0.87/100 patient-months) and 314 severe flares (0.64/100 patient-months) recorded. There were a total of 300 cutaneous and musculoskeletal flares (0.61/100 patient-months), 51 serositis flares (0.11/100 patient-months), 196 hematologic flares (0.41/100 patient-months), 196 renal flares (0.41/100 patient-months) and 49 neuropsychiatric flares (0.10/100 patient-months). The monthly rate of severe SLE flare was lowest in June and highest in January and the difference was statistically significant (p=0.042). Renal flare was significantly more frequent in the months January to March compared to June (p=0.041, 0.048, 0.043, respectively). The monthly rates of severe lupus flare and renal flare were negatively associated with the mean daily temperature (r=-0.73, p<0.01; r=-0.68, p=0.015, respectively), mean daily maximum temperature (r=-0.72, p<0.01; r=-0.67, p=0.016), total monthly rainfall (r=-0.73, p<0.01; r=-0.75, p<0.01) and mean ultraviolet light intensity index (r=-0.63, p=0.03; r=-0.69, p=0.012). The monthly total duration of sunshine was associated positively with cutaneous and musculoskeletal flares (r=0.62, p=0.03), but negatively with neuropsychiatric flare (r=-0.65, p=0.021). Conclusions: Seasonal variation in lupus flares in different organ system exists. Skin and joint lupus flares were more frequent in periods of more prolonged sunshine but were not associated with environmental ultraviolet light intensity. Severe lupus flare and renal lupus more commonly occurred during the winter months which were associated with lower temperature, humidity and ultraviolet light intensity.

CS14 Clinical Experience

CS14.1 & PO2.D.5
An analysis of the metabolic syndrome (MetS) phenotype in UK patients with SLE
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Objectives: The metabolic syndrome (MetS) is a clustering of metabolic abnormalities reflecting increased adiposity and insulin resistance and is associated with an increased risk of CHD in the general population. Whilst studies assessed MetS in SLE, it may not be as strongly associated with measures of adiposity than in population studies. We aimed to assess whether patients with SLE have a different phenotype of the MetS by investigating the prevalence of MetS and each of its criteria in a large lupus cohort. Methods: 200 Caucasian women with SLE and 100 healthy controls from the North West of England were studied. MetS was defined using the 2009 Consensus Statement from the International Diabetes Federation. Adiposity, assessed using both waist circumference (WC) and body mass index (BMI), was compared using Wilcoxon’s rank sum test. Age-adjusted odds ratios (OR) were generated for the prevalence of both MetS and each criterion in cases and controls. Results: Cases were older than controls (median (IQR) age 53 (46-59) years vs. 48 (42-56) years p<0.05). The overall prevalence of MetS in SLE was 29% compared to 18% in controls (Adjusted Odds Ratio = 2.17, 95% CI, 1.13, 4.19). In an age-adjusted analysis, measures of adiposity did not differ between groups. When examining individual criteria for MetS, SLE patients were significantly more likely to have hypertension and low HDL than controls (see Table).
Conclusions: In our cohort MetS is more prevalent in SLE than controls. This difference is not related to differences in adiposity. This suggests a different phenotype of MetS in SLE which may be related to chronic inflammation rather than to obesity and/or the metabolic effects of corticosteroids. Attention to the individual criteria as well as the whole syndrome may be needed to fully understand the impact of MetS to prognosis in SLE.

CS14.2 & PO2.J.1
Plasma exchange as rescue therapy for critical systemic lupus erythematosus: one center experience
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Objectives: To analyze the role of plasma exchange as a rescuing therapy for critical systemic lupus erythematosus (SLE) patients. Methods: A retrospective review was conducted to evaluate the patients with SLE undergoing rescuing plasma exchange (RPE) due to critical manifestations such as diffuse alveolar hemorrhage (DAH), neuro lupus, cutaneous antiphospholipid syndrome (CAPS), thrombotic thrombocytopenic purpura (TTP), and cryoglobulinemia between January 1988 and December 2009. The primary outcome detection contained all cause mortality and SLE disease activity index (SLEDAI) scores one month after RPE. The secondary outcome measurement included complications such as infection and hemolysis, cost effectiveness of plasma exchange and the change of autoantibody titers one month after RPE. Results: The study population comprised twenty-nine patients with SLE. The mean time for the duration of the disease was eighty-two months, from the diagnosis of SLE to the first RPE (range from one week to 200 months). The most common adverse effects were infection and weight gain. Five women developed amenorrhea during the treatment, but menses returned after its withdrawal. Seven patients reported menses return after RPE. Seven patients developed amenorrhea during the treatment, but menses returned after its withdrawal. Seven patients reported menses return after RPE. Conclusion: Plasma exchange is a safe and effective treatment for different manifestations of systemic disease or the scarring alopecia.

References:
1. The first RPE was done at median 19.6 months (2-76) after RPE. All patients achieved complete response after the drug was reintroduced. No response occurred in 3 patients (6%). The duration of thalidomide therapy was of 21±19.74 months (2-76). The most common adverse effects were sedation, constipation and weight gain. Five women developed amenorrhea during the treatment, but menses returned after its withdrawal. Seven patients reported symptoms of paresthesia, but only in three of them polynuropathy was confirmed by EMG. One patient, heavy smoker and without antiphospholipid antibodies, had a stroke. None of the three patients with antiphospholipid antibodies developed a thrombotic event. Thalidomide did not improve the systemic disease or the scarring alopecia. Conclusions: Low dose thalidomide is a safe and effective treatment for different manifestations of cutaneous lupus refractory to conventional therapy. In view of the high rate of relapses after treatment discontinuation, a long-term maintenance dose might be required. The rapid response achieved and the safe profile support that thalidomide, alone or in combination, might be used as initial therapy to avoid sequelae.

CS14.3 & PO2.K.2
Efficacy and safety of long-term use of thalidomide for refractory cutaneous lupus
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Objectives: Cutaneous manifestations of lupus erythematosus are characterized by a great heterogeneity of clinical manifestations that usually have a chronic and relapsing course. Although is not a life-threatening condition, the lack of a rapid improvement can lead to a significant permanent scarring and disfigurating lesions. Thalidomide is increasingly being shown to be effective for the treatment cutaneous disease refractory to conventional management. The aim is to prospectively evaluate the clinical efficacy and safety of long-term treatment with low-dose of thalidomide in a cohort of 50 patients with refractory cutaneous lupus disease. Methods: From 1992 to 2009, fifty consecutive patients with refractory disease (23 with discoid lupus erythematosus (DLE), 12 with subacute cutaneous lupus (S克莱), 4 with profoundus lupus, 7 with acute cutaneous lupus, 1 with lupus tumidus, and 3 with a non-specific rash) were treated with thalidomide. Initial treatment was started at 100 mg daily. If the cutaneous lesions vanished, the dose was lowered to 50-25 mg daily as maintenance therapy. Patients were followed up for a mean of 82±63 months (12-204). Complete response was defined as a total resolution of the cutaneous lesions. Partial response was considered when at least >50% of the improvement was achieved. Patients were followed up periodically and were assessed for the development of neuropathy and other adverse effects. Contraception was initiated in women of childbearing age. Results: Forty-seven patients (94%) achieved complete or partial response with thalidomide therapy. Complete response occurred in forty-one patients (82%). Time to remission was as quick as to 10.7±5.30 weeks (4-28). There was an elevated rate of relapses (65.8%), usually 8-16 weeks after thalidomide’s withdrawal or reduction. All patients achieved complete response after the drug was reintroduced. No response occurred in 3 patients (6%). The duration of thalidomide therapy was of 21±19.74 months (2-76). The most common adverse effects were sedation, constipation and weight gain. Five women developed amenorrhea during the treatment, but menses returned after its withdrawal. Seven patients reported symptoms of paresthesia, but only in three of them polynuropathy was confirmed by EMG. One patient, heavy smoker and without antiphospholipid antibodies, had a stroke. None of the three patients with antiphospholipid antibodies developed a thrombotic event. Thalidomide did not improve the systemic disease or the scarring alopecia. Conclusions: Low dose thalidomide is a safe and effective treatment for different manifestations of cutaneous lupus refractory to conventional therapy. In view of the high rate of relapses after treatment discontinuation, a long-term maintenance dose might be required. The rapid response achieved and the safe profile support that thalidomide, alone or in combination, might be used as initial therapy to avoid sequelae.

CS14.4 & PO2.K.2
Perceived stress in female patients with systemic lupus erythematosus (SLE)
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Objectives: To determine in women with Systemic Lupus Erythematosus (SLE) whether there is an association between patients’ perceived stress level and: a) disease activity; b) quality of life; and c) self-reported comor-
bid conditions. **Method:** Patients meeting four or more American College of Rheumatology classification criteria for SLE were enrolled in Canada. Disease activity was assessed using the SLE-Disease Activity Index (SLE-DAI), with SLEDAI ≥7 considered active disease. The 10-point Perceived Stress Scale (PSS) was used to assess stress levels, where PSS >20 (population mean ±1 standard deviation) was considered high quality. Consideration was given when high stress was used using the mental (MCS) and physical (PCS) component scores of the Medical Outcomes Short Form (SF-36). Comorbit conditions were reported on the Self-Administered Comorbidity Questionnaire and a standardized medical history form. **Results:** 156 female patients with SLE, aged (mean [SD]) 39.4 (14.7) years with SLE duration of 11.2 (9.6) years were included in the analysis. The majority of patients were Caucasian (44.9%), followed by Asian (24.4%) and African American (19.2%), and 44.9% had active disease at enrolment. Mean PSS for the group was 17.3 (6.8), which was higher than the female population norm of 13.7 (6.6). 29.4% of patients had PSS >20. Total PSS was higher for those with active disease compared to those with inactive disease [18.7 (6.7) vs 16.2 (6.6), p=0.023]. There was also a significant inverse correlation between PSS and disease duration (r = -0.17, p=0.03), MCS (r = -0.50, p=0.0001) and PCS (r = -0.67, p=0.0001). There was no association between PSS and: age, renal status, education level, marital status or having supplementary insurance coverage. Among many comorbidities reported, those with depression [16.7 (6.3) vs 19.7 (8.0), p=0.045] and diabetes mellitus [17.0 (6.8) vs 21.3 (4.5), p=0.031] had significantly higher PSS than those without these conditions. There was also a trend towards higher PSS in those with heart disease [17.0 (6.7) vs 19.9 (6.8), p=0.16] and arthritis [16.6 (6.7) vs 18.3 (6.8), p=0.13] compared to those without. **Conclusions:** Female patients with active SLE have higher perceived stress regardless of age, education, private insurance coverage, marital status or renal status. Presence of comorbid illnesses contributes to the higher PSS in these patients. Management of active SLE and other comorbidity conditions may decrease stress level in this patient population.

**CS145 & PO21.2**

Antimalarials have a protective effect against the development of renal disease in Latin American SLE patients

**Pons-Estel , Guillermo J.1,2,3 Alarcón, Graciela S.2,4 Hachuel, Leticia 1 Boggio, Gabriela 1 Wojdyla, Daniel3 Pascual-Ramos, Virginia4 Soriano, Enrique R.2 Saurit, Verónica 1 Cavalcanti, Fernando S.5 Guzmán, Renato A.5 Guibert-Toledano, Marlene5 Saenz de la Puebla, Maria J.6 Amigo, Mary-Carmen 1 Alva-Linares, Magaly 5 Esteva-Spinetti, Maria H.7 Pons-Estel, Bernardo A.5**

1. Facultad de Ciencias Económicas y Estadística, Universidad Nacional de Rosario, Rosario, Argentina; 2. Departments of Medicine (Division of Clinical Immunology and Rheumatology). The University of Alabama at Birmingham, Birmingham, AL, USA; 3. Servicio de Enfermedades Autoinmunes. Hospital Universitario Clínica, Barcelona, Spain; 4. Department of Epidemiology, Schools of Medicine and Public Health. The University of Alabama at Birmingham, Birmingham, AL, USA; 5. On behalf of Grupo LatinoAmericano De Estudio del Lupus (GLADEL), Rosario, Argentina; 6. Hospital Provincial de Rosario (Servicio de Reumatologia), Universidad Nacional de Rosario, Rosario, Argentina

Background and **Purpose:** Antimalarials (AM) have been shown to have numerous beneficial effects in lupus [diminished probability of flares, protective survival effect, longer time-to-damage accrual (specifically renal damage) and an increased probability of remission in mycophenolate mofetil-treated membranous nephritis]. The aim of this study was to determine if they also have a protective effect on the occurrence of renal disease (RD). **Methods:** SLE patients from GLADEL, a multi-ethnic, multinational Latin American cohort with a recent SLE diagnosis (≤2 years) have been recruited and followed-up longitudinally. For these analyses, characteristics of those patients with and without RD (persistent proteinuria and/or cellular casts (ACR criterion)) in a 1:2 proportion (nested case-control study design) were compared. Variables significant in these univariable analyses and other relevant variables were then entered in multivariable conditional logistic regression analyses; AM were found to have a protective effect in this analysis (OR: 0.37; 95%CI 0.23-0.60). Then to adjust for confounding a new model was performed with variables selected (p ≤0.10 ) from the comparison between AM-takers and non-takers among cases and controls combined (Table 1). **Results:** Of the 795 GLADEL cohort patients included in this study, 265 (33.3%) developed RD and 425 (53.5%) were AM users. Multivariable analyses’ results are presented in Table 2. **Conclusion:** After adjusting for possible confounding factors, we have demonstrated for the first time a clear protective effect of AM in the development of RD occurrence in SLE patients from this Latin American cohort.

**Table 1.** Socioeconomic-demographic, cumulative clinical, serologic and treatment characteristics in SLE patients with and without renal disease (combined) as a function of antimalarials use.

<table>
<thead>
<tr>
<th>Features</th>
<th>Anti-malarial Use</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=425)</td>
<td>No (n=370)</td>
</tr>
<tr>
<td>Gender, Female, %</td>
<td>90.8</td>
<td>91.4</td>
</tr>
<tr>
<td>Age at disease onset, years*</td>
<td>27.9</td>
<td>29.1</td>
</tr>
<tr>
<td>Age at diagnosis, years*</td>
<td>29.4</td>
<td>30.6</td>
</tr>
<tr>
<td>Delay in diagnosis, months*</td>
<td>19.0</td>
<td>18.2</td>
</tr>
<tr>
<td>Ethnic group, %</td>
<td>40.4</td>
<td>39.6</td>
</tr>
<tr>
<td>Caucasian (183/158)</td>
<td>43.1</td>
<td>42.7</td>
</tr>
<tr>
<td>Mestizo (187/162)</td>
<td>44.0</td>
<td>43.8</td>
</tr>
<tr>
<td>African-Latin American (55/50)</td>
<td>12.9</td>
<td>13.5</td>
</tr>
<tr>
<td>Residence, rural, %</td>
<td>7.6</td>
<td>6.6</td>
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<tr>
<td>Socioeconomic Status, %</td>
<td>12.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Upper/upper-middle</td>
<td>12.2</td>
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</tr>
<tr>
<td>Middle</td>
<td>28.0</td>
<td>30.9</td>
</tr>
<tr>
<td>Lower-middle/lower</td>
<td>59.9</td>
<td>59.2</td>
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<td>Education, years, %</td>
<td>30.1</td>
<td>31.4</td>
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<tr>
<td>0-7</td>
<td>45.9</td>
<td>40.5</td>
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<tr>
<td>8-12</td>
<td>24.0</td>
<td>28.1</td>
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<tr>
<td>More than 12</td>
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<td>Medical insurance, %</td>
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<td>18.5</td>
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<tr>
<td>Without coverage</td>
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<td>19.6</td>
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<tr>
<td>Partial coverage</td>
<td>61.9</td>
<td>62.0</td>
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<tr>
<td>Fall coverage</td>
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<tr>
<td>Diabetes, %</td>
<td>12.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>26.5</td>
<td>28.7</td>
</tr>
<tr>
<td>ACR Criterion, %</td>
<td>16.9</td>
<td>14.6</td>
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<tr>
<td>Malar rash</td>
<td>46.9</td>
<td>62.8</td>
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<tr>
<td>Discoid rash</td>
<td>63.5</td>
<td>41.8</td>
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<tr>
<td>Photosensitivity</td>
<td>43.5</td>
<td>18.9</td>
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<tr>
<td>Oral Ulcers</td>
<td>43.5</td>
<td>18.9</td>
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<tr>
<td>Arthritis</td>
<td>85.9</td>
<td>41.1</td>
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<tr>
<td>Serositis</td>
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<td>Neurologic disorder</td>
<td>10.8</td>
<td>3.8</td>
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<tr>
<td>Hematologic disorder</td>
<td>68.5</td>
<td>35.7</td>
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<tr>
<td>Immunologic disorder</td>
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<td>26.5</td>
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<td>Antinuclear antibodies</td>
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<td>Medications, %</td>
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<td>NSAIDs</td>
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</tr>
<tr>
<td>Azathioprine use</td>
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<td>6.5</td>
</tr>
<tr>
<td>Glucocorticoid dose, pulse</td>
<td>30.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Glucocorticoid dose, oral †</td>
<td>33.4</td>
<td>22.6</td>
</tr>
</tbody>
</table>

* Mean values for each group; † weighted dose

Abstracts of Oral Presentations
Table 2. Protective effect of antimalarials in RD among patients with SLE by multivariable analyses adjusting for confounders related to their use (dependent variable: RD).

<table>
<thead>
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<th>Features</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
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<td>Ethnic group,</td>
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<tr>
<td>Caucasian Reference</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mestizo</td>
<td>1.35</td>
<td>0.87-2.11</td>
<td>0.1829</td>
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<td>African-Latin American</td>
<td>0.74</td>
<td>0.38-1.45</td>
<td>0.3849</td>
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<td>Age at disease onset, years</td>
<td>0.98</td>
<td>0.97-1.00</td>
<td>0.448</td>
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<td>Socioeconomic Status</td>
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<td>Upper/upper</td>
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<td>0.22-0.95</td>
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<td>Middle</td>
<td>0.93</td>
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<tr>
<td>Lower middle/lower</td>
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<tr>
<td>Diabetes</td>
<td>2.64</td>
<td>0.31-22.78</td>
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<td>Hypertension</td>
<td>3.01</td>
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<td>ACR Criterion</td>
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<td>Malar rash</td>
<td>1.55</td>
<td>0.95-2.53</td>
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<td>Discoid rash</td>
<td>0.66</td>
<td>0.34-1.26</td>
<td>0.2028</td>
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<td>Photosensitivity</td>
<td>0.75</td>
<td>0.46-1.21</td>
<td>0.2314</td>
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<td>Oral Ulcers</td>
<td>2.18</td>
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<td>Arthritis</td>
<td>4.77</td>
<td>2.68-8.51</td>
<td>&lt;0.0001</td>
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<td>Serositis</td>
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<td>1.42-4.11</td>
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<td>Neurologic disorder</td>
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<td>Hematologic disorder</td>
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<td>Antimalarials</td>
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<td>0.25-0.70</td>
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<td>Azathioprine use</td>
<td>1.59</td>
<td>0.84-3.00</td>
<td>0.1535</td>
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<tr>
<td>Glucocorticoid, oral dose</td>
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<tr>
<td>Low (&lt;20 mg)</td>
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<td>0.81-3.13</td>
<td>0.1745</td>
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<tr>
<td>Medium (≥20 to &lt;60 mg)</td>
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<td>1.22-4.50</td>
<td>0.0102</td>
</tr>
<tr>
<td>High (≥60 mg)</td>
<td>1.57</td>
<td>0.76-3.25</td>
<td>0.2228</td>
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</table>

CS14.6 & PO2.D.2

Prolonged serologically active clinically quiescent (SACQ) systemic lupus erythematosus (SLE): clinical and serologic features

Steiman, Amanda J.; Gladman, Dafna D.; Ibañez, Dominique; Urowitz, Murray B.

Toronto Western Hospital, Toronto, ON, Canada

Objectives: Some patients with SLE are clinically quiescent despite persistent serologic activity, and thus present a clinical dilemma. We aimed to determine the frequency of SACQ and its outcome in a large cohort of SLE patients followed prospectively at a single centre. Methods: Patients followed in the Lupus Clinic between July 1970 and April 2008 with visits no more than 18 months apart were identified. SACQ was defined as at least a two year sustained period without clinical activity and with persistent serologic activity (increased anti-dsDNA antibody by Farr assay and/or hypocomplementemia at each clinic visit), during which patients could be taking antimalarials, but not steroids or immunosuppressives. The characteristics of patients with a SACQ period and its features were analyzed. Anti-dsDNA levels were categorized as normal (≤7), low (8-20), moderately (21-50), or highly (>50) elevated. Results: A total of 359 patients had a SACQ period (median 159 weeks). In patients who flared they do so after median 3 years. Thus prudent therapy would be close observation to discern which SACQ patients will ultimately flare.

CS15 Pregnancy and Lupus

CS15.1

Mediators and mechanisms of pregnancy complications in patients with anti-phospholipid antibodies and patients with lupus: the PROMISSE study

Salmon, Jane

Hospital for Special Surgery, New York, NY, USA

Pregnancy complications in women with the antiphospholipid syndrome (APS) and/or SLE include recurrent miscarriage, preeclampsia, placental insufficiency, and intrauterine growth restriction (IUGR). The mechanisms leading to placental and fetal injury in vivo are incompletely understood and treatment remains sub-optimal. We have identified complement as an early effector in pregnancy loss and/or IUGR associated with placental inflammation in a mouse model of APS and shown that complement activation causes the release of anti-angiogenic factors and abnormal placental development. The PROMISSE Study (Predictors of pRegnancy Outcome: bioMarkers In antiphospholipid antibody Syndrome and Systemic lupus Erythematosus) is a first-time effort to translate our novel findings in mice to humans and determine if alterations in complement regulation of angiogenic factors products predict pregnancy complications in patients with antiphospholipid (aPL) antibodies and/or SLE. In the first 7 years of this prospective, observational study of pregnant patients grouped and analyzed according to the presence or absence of aPL antibodies and preexisting SLE, we have enrolled over 550 pregnant patients in 8 centers, obtained detailed medical and obstetrical information monthly, and serially collected plasma, serum, DNA, RNA, and urine. Preliminary data from this study will be presented. Identification of biomarkers that predict poor pregnancy outcome in these patients will elucidate mechanisms of disease, define targets for treating patients, and generate clinically applicable indicators to permit initiation of interventional trials in patients at greatest risk for pregnancy complications.

CS15.2

Neonatal lupus: updates on pathogenesis, risk and reward

Buyon, Jill P.

Department of Medicine, Division of Rheumatology, NYU Langone School of Medicine, New York, NY, USA

One of the strongest clinical associations with autoantibodies directed to components of the SSA/Ro-SSB/La ribonucleoprotein complex is the development of congenital heart block (CHB) in an offspring, an alarming prospect facing 2% of primigravid mothers with these reactivities. Other abnormalities affecting the skin, liver, and blood elements are associated with anti-Ro/La antibodies in the maternal and fetal circulation and are now grouped under the overall heading of Neonatal Lupus Syndromes (NLS), Neonatal...
Lupus Erythematosus (SLE) or simply Neonatal Lupus (NL). NL was so termed because the cutaneous lesions of the neonate resembled those seen in SLE. The name is misleading and often a cause of undue concern because the neonate does not have SLE and often neither does the mother. Accumulated evidence suggests that anti-Ro/La antibodies are necessary but insufficient for fetal disease. Basic and clinical research is heavily vested in identifying fetal and environmental factors which convert disease susceptibility to overt expression. The pathogenesis of disease is likely complex and several models have been proposed. One focuses on a pathogenic antibody recognizing Ro52p200 and another postulates autoantibody perturbation of calcium channel electrogenesis via reactivity of anti-Ro with L type calcium channels. A third considers Ro/La as a means by which the normally inaccessible Ro/ La antigens can be trafficked to the cell membrane. Apoptosis is a selective process of physiological cell deletion in embryogenesis and normal tissue turnover and plays an important role in shaping morphological and functional maturity. It is generally accepted that apoptotic cells are rapidly removed to obviate any inflammatory sequelae. Compatible with the need for efficient clearance, human fetal cardiocytes are capable of engulfing apoptotic cardiocytes. This novel physiologic function may account for the general absence of apoptosis noted on evaluation of hearts from electively terminated fetuses. However, histologic studies of hearts from fetuses dying with CHB have identified exaggerated apoptosis, suggesting a potential defect in clearance. In vitro experiments reveal that antibodies to Ro/La inhibit cardiac uptake of apoptotic cardiocytes, thus explaining the histological findings. The consequence of persistent “opsonized cardiocytes” is to divert uptake to infiltrating macrophages which results in release of proinflammatory and profibrogenic cytokines culminating in transdifferentiation of cardiac fibroblasts and subsequent replacement of healthy conducting tissue with scar. The ssRNA component of the immune complex on apoptotic cardiocytes may stimulate macrophage TLR7/8 receptors. Data from the Research Registry for Neonatal Lupus provides practical information regarding counseling of mothers. Half of the women who are asymptomatic at the birth of an affected child will experience progression to clinical autoimmunity, ranging from minor rheumatic symptoms to overt SS or SLE. For women who have had a previous child with CHB, the risk of recurrence in a subsequent pregnancy is 18%. For those who have had a child with rash, the risk of CHB in a subsequent pregnancy is 13%. With regard to fetal monitoring a disturbing observation which has emerged from current research efforts is the rapidity of disease progression with advanced heart block and life threatening cardiomyopathy observed less than 2 weeks from normal sinus rhythm. Once third degree block is unequivocally identified, sustained reversal has never been achieved, despite dexamethasone. Accordingly, strategies aimed at preventing disease before irreparable scarring ensues, assume high priority. Two studies have concluded that the use of IVIG at replacement doses (400mg/kg given at 12,15,18,21, and 24 weeks of gestation) does not prevent the recurrence of CHB. Consistent with experimental evidence implicating TLR in the pathogenesis, a retrospective study regarding the use of hydroxychloroquine in preventing CHB has provided encouraging results. The clinical significance and treatment of PR prolongation in utero (first degree heart block) is still unclear but most investigators favor close echocardiographic surveillance. Although significant advances have been made, continued studies both at the bench and bedside are needed, including an understanding of the fetal and maternal genetic contributions.

Abstracts of Oral Presentations

CS15.3 & PO2.0.1

Placental C4d as an indicator of antiphospholipid antibody mediated fetal loss

Cohen, Danielle1 le Sesse, Saskia1 Goemaere, Natascha1 Scherjon, Nico1 de Heer, Emilie1 Braijn, Jan Anthonie1 Bajema, Ingeborg1

1. Leiden University Medical Center, Leiden, Netherlands; 2. Stichting Pathan, Rotterdam, Netherlands

Objectives: Recurrent miscarriage and intrauterine fetal death occur 20 to 40 times more often in patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome than in healthy pregnant women. During trophoblast differentiation, direct binding of antiphospholipid antibodies to trophoblast cells activates the complement cascade and interferes with trophoblast cell invasion and maturation. We investigated whether deposition of C4d, a marker of classical complement activation, is related to fetal outcome in placentas of patients with SLE and/or antiphospholipid syndrome. Methods: We studied 86 placentas of 83 patients by staining them with BI-RC4d polyclonal anti-C4d antibody, and scoring them semiquantitatively (no deposition, focal deposition, diffuse deposition). The patients were subdivided into a case group of 21 patients with SLE and/or antiphospholipid syndrome, a first control group of 40 patients with pregnancies that resulted in live births, and a second control group consisting of 22 patients with pregnancies that resulted in intrauterine fetal death caused by chromosome abnormalities. Results: There was a strong association between diffuse perivillous placental C4d deposition and antiphospholipid syndrome (p < 0.001), as well as between diffuse C4d positivity and intrauterine fetal death (p < 0.0005). C4d staining was never positive in placentas from patients with normal live births. Conclusions: C4d is present in placentas of patients with SLE and/or antiphospholipid syndrome, and is strongly associated with negative fetal outcome. The excessive deposition in some of our cases may be regarded as witness of a very strong antibody-mediated immune response from which inhibitory mechanisms failed, resulting in intra-uterine fetal death. Further prospective studies need to confirm if C4d in a previous miscarriage can be considered as a biomarker of a future complicated pregnancy.

CS15.4 & PO2.0.2

Markers of cardiovascular function in women with systemic lupus erythematosus (SLE) in pregnancy

Chirico, Debora1 Crocker, Ian P.1 Bruce, Ian 2; Baker, Philip N.1 Tower, Clare L1

1. Manchester Maternal and Fetal Health Research Centre, Manchester, UK; 2. Epidemiology Unit, University of Manchester, Manchester, UK

Objectives: SLE is associated with significant pregnancy complications, in particular pre-eclampsia, and pregnant women with SLE experience a 20-fold higher maternal mortality. Both SLE and pre-eclampsia significantly increase the risks of subsequent cardiovascular disease (CVD). Transforming Growth Factor beta1 (TGFβ1) is an immunosuppressive growth factor involved in the maintenance of normal blood vessel structure. It is known to be reduced in women with SLE. We hypothesise that TGFβ1 dysregulation accelerates arterial stiffness in women with SLE and thus may predispose them to pregnancy complications. Methods: TGFβ1 activation index (AI) and arterial stiffness index (SI) were measured in pregnant women with SLE and in healthy pregnant women at 12, 20, 28 and 36 weeks and at a single time point in the non-pregnancy state. TGFβ1 was assessed by activation assay and SI by digital pulse wave analysis; a measure of systemic arterial stiffness. Results: There was no difference in TGFβ1 AI in healthy women compared with the non-pregnant state (Table). In contrast, TGFβ1 AI was significantly lower in both pregnant and non-pregnant women with SLE (table, n=4, p=0.05; and, n=8, p=0.01 respectively). There was a corresponding increase in SI in non pregnant women with SLE (table, n=9, p=0.06) and in late pregnancy (table n=3, p=0.04) compared with healthy controls. Discussion: Women with SLE have a lower TGFβ1 activation index and raised arterial stiffness. SLE patients may therefore be intrinsically prone to vascular complications of pregnancy by having early vascular stiffness and low TGFβ1 may contribute to the risk of pre-eclampsia and poor pregnancy outcomes seen in SLE.

<table>
<thead>
<tr>
<th>TGFβ1AI</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Healthy NP</td>
<td>1.67 (1.56-1.94)</td>
</tr>
<tr>
<td>Healthy P</td>
<td>1.85 (1.56-2.56)</td>
</tr>
<tr>
<td>SLE NP</td>
<td>1.20 (0.97-1.59)</td>
</tr>
<tr>
<td>SLE P</td>
<td>1.25 (0.75-1.67)</td>
</tr>
</tbody>
</table>

NP, non-pregnant; P, 12 weeks pregnant, * 36 weeks

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CS16 Pediatric: Outcomes that Matter for Children and Youth with SLE: Bone Health, Development and Quality of Life

CS16.1

Outcomes that matter for children and youth with SLE: bone health, development and quality of life. An overview

Eberhard, Anne

Albert Einstein College of Medicine, Schneider’s Hospital, New Hyde Park, NY, USA

Outcomes for pediatric systemic lupus erythematosus (SLE) patients have continued to improve. As access to pediatric rheumatologists improves, earlier diagnosis and more aggressive treatment has meant continuing improvement in the 5-year survival rates. Unfortunately mortality in the pediatric age group from SLE is still much higher in comparison to adults with SLE. In addition the medications themselves, especially steroid therapy continue to contribute to morbidity. Some of these issues will be discussed in greater detail during this session. Over the past few years care of the sick SLE patient has changed. Aggressive treatment or an induction phase consisting of high dose steroids and an immune suppressant has become the norm. This is then followed by a maintenance phase where provided the SLE is controlled, steroid therapy can be minimized and immune suppression adjusted. The aim being a long and lasting remission. It may even be possible to tailor therapy. Studies have shown that certain medications are better in particular subsets of SLE. Cyclophosphamide (CTX) for example seems less effective in black patients with SLE nephritis, and overall it has been reported that over 1/3 of patients fail to achieve a remission on CTX. In comparison it would appear that response to MMF is better in Asian patients with SLE than in other ethnic groups. Unfortunately there are few large scale studies in the pediatric patient with SLE. The APPLE (Atherosclerosis Prevention in Pediatric Lupus Erythematosus) study designed to assess atherosclerosis in pediatric SLE is the first multicenter North American trial to follow a cohort of selected pediatric SLE patients over a defined time period. Pediatric patients are still, unlikely to be included in any trials regarding SLE therapy. So treatment protocols are extracted and modified from adult data. However the ASPREVA Lupus Management Study (ALMS), a randomized controlled trial, included 24 pediatric SLE patients. The trial was designed to study the efficacy of CTX vs mycophenolate mofetil (MMF) as treatment for SLE nephritis. MMF failed to show superiority over CTX in this trial with serious adverse events being similar between the 2 groups. The time has come for pediatric SLE patients to be included in ongoing trials. Now rheumatologists have an increasing number of newer treatment options available in treating SLE. It is important not only to have evidenced based pediatric protocols for treatment of this chronic illness but proven treatment strategies for pediatric SLE as pediatric patients continue to be the sickest and most challenging to treat.

CS16.2

Bone health for children with SLE

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Children with SLE are at increased risk for the development of skeletal deficits including short stature, delayed skeletal maturation and reduced bone mineral density (BMD). Although fractures often do not occur until later in life, the potential effect of childhood and adolescent chronic illness one bone mineral accrual may prevent patients from reaching normal peak bone mass. SLE and its associated therapy may have numerous negative bone effects. These include chronic exposure to glucocorticoids, renal insufficiency, circulating inflammatory cytokines, vitamin D insufficiency, and reduced physical activity. We conducted a longitudinal cohort study to better characterize these risk factors. We aimed to measure bone density at multiple skeletal sites using several radiologic methods, and to characterize the contribution of patient-, disease- and host-related variables to BMD. We also were interested in characterizing the bone turnover balance and in exploring the role of inflammation in the development of bone deficits in children with SLE. 93 pediatric SLE subjects (mean age 15.5±3.2 yrs), 87% female, of mixed ethnicity (31% Asian, 11% African American, 26% Caucasian, 17% Hispanic, 15% other) underwent densitometry and clinical assessment. SLE disease duration ranged from 1.2-15.3 years (mean 7.5). Mean prednisone dose was 0.2 mg/kg/d (range 0.009 to 6.6 mg/kg/d) and average cumulative lifetime exposure was 72 mg/kg (range 3.4 to 2431 mg/kg). The SLE Disease Activity Index (SLEDAI) ranged from 0-24. When compared to 169 healthy controls, 23% of SLE subjects demonstrated spine BMD Z-scores below −1.5 SD, with a mean (SD) spine BMD Z-score of −0.69 ± 1.4 compared to 0.13±1.06 for controls (p<0.0001). As expected, SLEDAI correlated with prednisone dose (mg/kg/d) (R=0.47, p<0.0001). Vitamin D deficiency was observed in approximately 30% of patients. Both disease- and treatment-related factors may contribute to reduced BMD in children with inflammatory disease, such as SLE. Understanding these factors is important for risk assessment and the development of treatment strategies for children with SLE.

CS16.4

Outcomes that matter for children and youth with SLE: bone health, development and quality of life

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Systemic lupus erythematosus (SLE) in children is a chronic multi-system disease with wide ranging effects on their health-related quality of life (HRQOL). Psychosocial implications of SLE in children are evident in the life-disruptive responsibilities that patients and their families must assume including hospitalizations, multiple sub-specialty visits, frequent laboratory monitoring, and health care costs. SLE and activities related to caring for the disease impose a burden on children’s school attendance and performance. Pediatric studies have explored the ideal method of measuring the widespread impact of SLE on HRQOL and the ideal methods of measuring the same. The multidimensional aspect of HRQOL, heterogeneous nature of SLE, and the changing growth and development of children need to be taken into account while measuring HRQOL. A novel HRQOL measure, Simple Measure of the Impact of Lupus Erythematosus in Youngsters© (SMILEY©) has been developed and validated for use in children with SLE and parents. SMILEY© is currently undergoing cross-cultural validation in several countries across the world, and will be a useful adjunct to clinical trials and outcomes research.

CS17 The Interferon Pathway in Lupus

CS17.1

Interferon-regulated biomarkers of disease activity in SLE

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by unpredictable flares of disease activity and irreversible damage to multiple organ systems. Our earlier study showed that SLE patients carrying an interferon (IFN) gene expression signature in blood have elevated serum levels of IFN-regulated chemokines. These chemokines were associated with more severe and active disease and showed promise as SLE disease activity biomarkers. To validate the potential utility of serum chemokine levels as biomarkers for disease activity, we measured serum chemokine levels – CXCL10 (IP-10), CCL2 (MCP-1), and CCL19 (MIP-3B) – in an independent cohort of 267 SLE patients followed longitudinally over one year (1166 total visits). Serum chemokine levels correlated with current visit to
lupus activity (p=2x10^{-10}), rising at flare (p=1x10^{-3}) and decreasing as disease remitted (p=1x10^{-3}), and performed better than currently available laboratory tests. Chemokine levels measured at a single baseline visit in patients with mild or inactive disease (SLEDAI ≤4) were predictive of lupus flare (in any organ system) over the ensuing year (p=6x10^{-4}). We next validated these results in a replication cohort of 257 SLE patients, in which chemokine levels were again elevated in active vs. inactive SLE (p=2x10^{-40}) and predictive of future lupus flare (p=0.001). Monitoring serum chemokine levels in SLE may thus improve assessment of current disease activity, the prediction of future flare, and overall clinical decision-making.

**CS17.2**

**Molecular pathways associated with lupus flare**
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Altered function of many components of the immune system underlies the diverse clinical manifestations and variable course of disease in patients with SLE. While anti-double stranded DNA antibodies and complement levels have been used to monitor disease activity and guide therapy, these serologic markers are not universally present or informative. In order to gain insight into lupus pathogenesis and identify biomarkers of flare and targets for therapy, our laboratory has characterized the molecular pathways that contribute to the immunologic dysfunction, autoimmunity and inflammation that result in organ damage and disease in SLE. We have identified interferon-α (IFN) as an important trigger for a broad molecular pathway that is associated with increased disease activity and severity. To define the relationship of IFN pathway activation to disease flare and identify additional molecular pathways associated with flare, we and our collaborators have established and studied a lupus cohort followed longitudinally, approximately every 3 months, over 1 ½ to 2 years. Based on microarray data we have identified multiple gene clusters that define molecular pathways activated in lupus peripheral blood. In addition to the genes activated by IFNs, plasma cell and neutrophil signatures are differentially expressed in patients compared to healthy controls and fluctuate over time in relation to disease flares. We have designed a composite score derived from these gene clusters. Our studies in progress are investigating the hypothesis that quantification of plasma cell, neutrophil and IFN signatures is superior to the IFN signature or anti-dsDNA antibody alone as a marker of future or current flare.

**CS17.3 & PO2.G.5**

**Cytokine attribution of gene expression and histone H4 acetylation changes in SLE monocytes**
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**Objectives:** Monocytes in SLE have been described as having aberrant behavior in a number of assays. Epigenetic changes could contribute to molding of aberrant behaviors by modulating gene expression. Examining the epigenome in SLE is desirable because epigenetic changes can be durable and can dictate responses to subsequent stimuli. To understand the role of cytokines in driving changes to gene expression and the epigenetic landscape we utilized an unbiased approach. **Methods:** Gene expression and the post-translational modification of histone mark, H4 acetylation, were examined using arrays. The U133A 2.0 platform was used for the expression analyses and the GeneChip Human Promoter 1.0R array was used to define H4 acetylation (Affymetrix). We compared SLE monocyte gene expression and H4 acetylation with α-interferon, γ-interferon or IL-4-treated monocytes to understand which cytokine effects predominated in SLE monocytes. Transcription factor binding sites were identified and clustering analysis was used to understand the driving forces of the changes. DAVID was used to relate the findings to biological processes. **Results:** We found that γ-interferon and α-interferon both replicated a broad range of the gene expression changes seen in SLE monocytes. There was less evidence of interferon effects on H4 acetylation patterns. H4 acetylation in SLE monocytes was overall higher than in controls and there was less correlation of H4ac with cytokine-treated cells than when gene expression was compared. The H4 acetylation changes in SLE monocytes mapped in large part to transcription factor binding sites. When DAVID was used to compare biological processes induced by the cytokines with those induced by SLE, we found little overlap, suggesting that monocytes have been impacted by a complex interplay of cues. A set of chemokine genes had down-regulated expression and H4ac. **Conclusions:** There are several lines of evidence suggesting that monocytes have been molded by a complex set of exposures. Our cytokine attribution found that the interferon-responsive genes cluster was up-regulated 36.3% in SLE monocytes, thus leaving a significant gene set unexplained by interferon exposure. The association was even less robust for H4ac. The finding that H4 acetylation was globally increased and this increase appeared to map to TFBSs suggest a globally altered epigenome with a complex etiology. Therefore, monocytes are significantly impacted by both IFN and αIFN exposure, however, our data suggest that additional cytokines and other exposures contribute to the aberrant monocyte behavior observed in SLE patients.

**CS17.4 & PO2.G.1**

**What are the pathways by which IFN-α decreases vasculogenesis in SLE?**
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**Objectives:** SLE is characterized by accelerated vascular risk due to premature atherosclerosis which is not explained by traditional risk factors. Our group previously proposed that type I Interferons (IFNs) play a crucial role in premature vascular damage by altering the balance between endothelial cell apoptosis and vascular repair mediated by endothelial progenitor cells (EPCs) and myeloid circulating angiogenic cells (CACs). We have now characterized the putative pathways by which type I IFNs interfere with proper vascular repair in SLE. **Methods:** EPC/CACs from control and SLE patients were treated with IFN-α for 6 hours and corresponding changes in gene expression were analyzed with Affymetrix Human U133 Plus 2.0 Genechips. Expression levels of genes of interest were validated with real time PCR. Effects on EPC/CAC function were tested utilizing functional in vitro assays. Proliferation and apoptosis were determined by XTT assay and caspase3/7 activation, respectively. DC phenotype was assessed by FACS. In vivo angiogenesis and validation of putative markers at the protein level was assessed by immunohistochemistry staining of kidney sections from lupus nephritis biopsies and controls. **Results:** Microarray data analysis revealed alterations in various molecules associated to IL-1 mediated signaling and of vascular endothelial growth factor-A (VEGF-A). Downregulation of IL-1β, IL-1 receptor-1 (IL-1R1) and VEGF-A and upregulation of IL-1RN (IL-1R1 receptor antagonist) and IL-1R2 (decoy receptor) were observed. This indicates a global downregulation of IL-1β function induced by type I IFNs. These abnormalities were more marked in the IFN-treated lupus EPCs/CACs than in the IFN-treated control cells, suggesting that lupus cells were more sensitive to IFN-α effects. Results were confirmed at mRNA and protein level. Treating lupus EPCs/CACs with IL-1β resulted in a significant improvement in their capacity to differentiate into a mature endothelium, therefore abrogating the deleterious effects of IFN-α. These beneficial effects were mediated, at least in part, by an increase in lupus EPC/CAC proliferation, decrease in EPC/CAC apoptosis, and by preventing skewing of lupus EPC/CACs towards non-angiogenic pathways. Confirming that decreased angiogenesis was occurring in vivo in SLE through IL-1 pathway dysregulation, the glomerular and vascular compartments of biopsies from patients with lupus nephritis also showed increased IL-1RN, decreases in VEGF and overall de-
creased endothelial density, as assessed by CD31 expression, while control kidneys as well as kidneys from other immune-mediated diseases with similar amount of renal tissue damage (ANCA-vasculitis) did not exhibit these abnormalities. **Conclusions:** These results indicate that type I IFNs mediate their antiangiogenic effects by downmodulating IL-1 mediated pathways and further suggest that strategies aimed at blocking type I IFN effects or its downstream pathways may abrogate premature vascular damage in SLE.

**CS17.5 7 PO2.G.2**

A genetic variant of TLR9 may impact interferon alpha levels in lupus

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**Objectives:** Genetic variants in proteins relevant to type I interferons (INF) are associated with risk for SLE. Toll-Like Receptors (TLR) 7 and 9 may regulate INF activation by DNA and RNA-associated autoantigens, and in some murine models obstructing TLR7 enhances the autoimmune potential of TLR7. We tested TLR polymorphisms in 221 lupus patients: (TLR7: rs179008, rs5935436, and rs835389. TLR9: rs187084, rs352139, and rs352140) hypothesizing that inherited variants in TLR might influence levels of interferon alpha in lupus patients. **Methods:** TLR polymorphisms were determined using real time PCR and published primers obtained from Applied Biosystems. An ELISA system for interferon alpha quantification was purchased from Bender Med Systems, and data calculated using their standards after confirmation of the dilution curve. Autoantibodies and other clinical information were obtained from medical records. **Results:** The frequency of each allele at these six common variant sites was similar in Caucasian, African, and Asian patients to previously published reports on these ethnic populations. In an exploratory multivariate model, two TLR9 polymorphisms (rs352139 and rs352140) were associated with anti-RNP antibody (p=0.031 and p=0.040 respectively) and rs352139 was marginally associated with anti-DNA antibody (p=0.067). These two polymorphisms tend to sort together, and further analysis suggested that the potential association with RNP antibodies was due primarily to the rs352140 polymorphism (CT). Positive RNP antibody impacted interferon alpha levels only in those patients homozygous for CC at this position (n=74) (median INF: 0.0 vs 46.2 pg/ml in RNP neg vs RNP+ pts with CC genotype, p=0.001). This difference was not seen in other genotypes. This same genotype also showed a marginal trend to increasing Ifn alpha levels in anti-dsDNA positive vs negative patients (median INF 0.0 vs 53.6, p=0.145). Interestingly, when interferon levels were compared in genotype subgroups of patients positive for anti-dsDNA the same rs352140 CC genotype was associated with significantly higher interferon alpha levels than was found in heterozygotes or those with two T alleles (median 53.6 vs 0, p=0.045). Further analyses of medications (including separate comparative analysis of patients on no meds, hydroxychloroquine and/or steroids) did not suggest major confounding effects on interferon levels. **Conclusions:** A polymorphism in TLR9 (rs352140 CC) may affect interferon alpha levels in those lupus patients with characteristic autoantibodies associated with TLR activation of the type 1 interferon pathway. These analyses were exploratory and would need to be confirmed in additional populations and prospective studies.

**CS17.6 & PO2.G.3**

**Inhibition of the lymphotixin-beta receptor pathway has unexpected effects on kidney pathology in the adenoaviral-IFNα BWF1 accelerated model of SLE**

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**Objective:** The lymphotixin-beta receptor (LTBR) pathway contributes to many important functions in the immune system, such as organization of lymphoid architecture and tertiary lymphoid structures, cellular positioning and trafficking through high endothelial venules (HEVs). Inhibiting the LTBR system with LTBR-Ig (baminercept) has been efficacious in established animal models of various autoimmune diseases and has undergone extensive clinical testing in rheumatoid arthritis. Here we questioned whether LTBR-Ig is efficacious in a murine model of lupus. **Methods:** Female (NZB×NZW) F1 (BWF1) mice were injected with adenovirus-IFNα at 9 weeks of age and LTBR-Ig was administered (10 mg/kg, twice a week) starting 3 weeks after the viral injection. **Results:** LTBR-Ig blockade had significant efficacy as evidenced by normalization of the serum and urine chemistry and the kidney histology showed reduced glomerular and tubular nephritis. Treatment led to a reduction of lymph node HEV, lymphophadonopathy, numbers of splenic activated/memory T cells and plasmacytoid DC. Serum titers of anti-DNA antibodies were not reduced by treatment, there was no shift to less pathogenic isotypes and no obvious loss of immune complex deposition in the glomeruli. For this reason, we hypothesized that downstream effector functions in the kidney were altered, however, such a dampening of local activity by LTBR-Ig was not foreseen by any of the known mechanisms of action. Quantitation of kidney RNA levels of many genes showed notably that macrophage markers were decreased (CD11b, CD14 and CD169) and, while the kidney levels of several chemokines were reduced (CXCL13, CCL2, CXCL9-10 and CCL17), CCL20 expression was dramatically eliminated. CCL20 is produced by epithelial cells and plays roles especially in the gut via CCR6 signaling in the attraction/retention of some DC subsets, Th17 cells and some B cells. **Conclusions:** Administration of LTBR-Ig was efficacious in this IFN accelerated BWF1 model. While many immunological parameters in the secondary lymphoid organs were affected, decreased kidney pathology may rely on local effects on monocyteic/DC involvement that is sustained by locally generated CCL20. We suspect that reduced chemokine production by LTBR-Ig may underlie some of this efficacy. CCL20 expression by epithelia in the gut has been previously linked to LTBR signaling. LTBR-Ig is an attractive agent for the treatment of SLE since it has actions at the immunological level as well as potentially on local monocyte/DC events in the kidney.

**CS17.7 & PO2.G.4**

Increased interferon-alpha activity is associated with increased autoantibody specificities and poor antigen-specific humoral immunity in systemic lupus erythematosus

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**Background:** Interferon-α (IFNα) has been identified as a key mediator in systemic lupus erythematosus (SLE) pathogenesis. IFNα is a type I IFN that has the ability to disrupt self tolerance by activating antigen-presenting cells containing self-antigen. Elevated IFNα activity is detected in many SLE patient samples, and these elevations have been shown to correlate with disease activity as well as multiple organ involvement. Our goal is to further understand the pathogenic role of IFNα in SLE humoral autoimmunity, as well as to investigate the effect of an antigen-specific challenge in SLE and whether increased IFNα activity in SLE impairs antigen specific responses. **Methods:** This study enrolled 72 SLE patients who met ACR criteria and matched controls. Detailed clinical and therapeutic information, as well as disease activity measures were obtained at baseline and 2, 6, and 12 weeks after influenza vaccination. Interferon humoral immune responses (native/denatured ELISA responses, relative affinities and hemagglutination inhibition) were measured. Serial samples were tested for interferon activity through a reporter cell assay which measures serum’s ability to upregulate three interferon inducible genes, MX1, PKR, and IFIT1. Lupus-associated autoantibodies (aAbs) (Ro, La, Sm, nRNP, ribosomal P, dsDNA, ANAs and phospholipid antibodies) were measured by ELISA and immunofluorescence. **Results Summary:** IFNα activity decreased in SLE patients 2 weeks after influenza vaccination compared to baseline levels (baseline mean = 6.4, 2 weeks post-vaccination mean = 5.9, p=0.0195, paired t-test). However, in the subset of patients whose disease activity scores increased after vaccination, this decrease in IFNα activity was
not seen. A correlation was seen between elevated baseline IFNα activity and poor humoral immune response to the influenza vaccine (p = 0.0126, r² = 0.22). Additionally, a significant association was seen between increased IFNα and total number of lupus-associated autoantibodies (IFNα < 1.0 [n=45], mean aAbs = 2.1, IFNα > 1.0 [n=27], mean aAbs = 3.4, p = 0.0003, unpaired t-test). Consistent with previous reports, we also saw a significant association between increased IFNα and disease activity (IFNα < 1.0 [n=45], mean SLEDAI = 7.6, IFNα > 1.0 [n=27], mean SLEDAI = 10.2, p = 0.0078, unpaired t-test).

Conclusions: A unique finding in this study was that IFNα decreases in SLE patients post-influenza vaccination. Increased baseline IFNα activity correlated with a poor humoral response to the influenza vaccine. Increased IFNα activity was also associated with an increased number of lupus-associated autoantibodies, as well as with increased disease activity.

CS18 Autoantibodies and Tissue Pathology

CS18.1 Innate immune mechanisms in experimental lupus
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Objectives: Chronic inflammation and the formation of ectopic lymphoid tissue are associated with many forms of autoimmunity. We have found that chronic inflammation in non-autoimmune prone mice following peritoneal injection of tetramethylpentadecane (TMPD) leads to the overproduction of Type I interferon (IFN-I), resulting in increased expression of IFN-I inducible genes as seen in many SLE patients. TMPD-treated mice develop lupus autoantibodies (anti-DNA, Sm, RNP, and others), inflammatory arthritis, and glomerulonephritis. The objective of these studies was to better understand how chronic inflammation precipitates lupus in mice.

Methods: Lupus was induced in BALB/c or C57BL/6 mice using TMPD and the critical immune pathways were identified using gene-targeted mice. Myeloid cells responsible for IFN-I production were purified and characterized by flow cytometry. Anti-RNP antibody-producing cells were identified using anti-U1A ELISPOT assays and serum autoantibodies were detected by ELISA. The role of ectopic lymphoid tissue in autoantibody production was investigated after transplantation into naive recipients.

Results: The induction of autoantibodies and nephritis by TMPD was abolished in mice deficient in the Type I interferon receptor. IFN-I in the TMPD-lupus model was derived largely from a population of myeloid cells with the phenotype Ly6C<hi>, Ly6G<lo>, CD11b<hi>, CD11c<lo>, B220<lo>, CD4<lo>, Sca1<lo>, most consistent with immature monocytes rather than plasmacytoid dendritic cells. IFN-I production and the development of lupus nephritis and autoantibodies in TMPD-treated mice was found to be mediated exclusively by the TLR7-MyD88 signaling pathway, and was independent of TLR9 and other pathways of IFN-I production mediated by TRIF, IPS-1, or TBK-1. We found that inflammation-induced chronic IFN-I production caused ectopic lymphoid tissue to become a site where antigen-specific B and T cells are activated and proliferate. In addition, large numbers of anti-RNP autoantibody producing plasma cells were found in the ectopic lymphoid tissue and were attracted/retained there by the chemokine SDF-1.

Conclusions: These studies suggest that chronic IFN-I production plays a direct role in the propagation of systemic autoimmunity, and will be reviewed.

CS18.3 Antiphospholipid antibodies and thrombophilia in the antiphospholipid syndrome
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The antiphospholipid syndrome (APS) is characterized by thrombosis in the presence of antiphospholipid antibodies (aPL). Tissue factor (TF), the major initiator of the coagulation system, is induced on monocytes activated by aPL, explaining in part the pathophysiology of this syndrome. Phosphatidylserine-dependent antiprothrombin antibodies (aPS/PT) are as prevalent as beta2-glycoprotein I dependent anticardiolipin antibodies (aCL/b2GPI) in patients with APS, and there were no difference in the clinical manifestations between patients with aCL/b2GPI and those with aPS/PT. Therefore, beta2-glycoprotein I and prothrombin are two major antigenic targets of aPL, and it is likely that aCL/b2GPI and aPS/PT share the pathophysiologic properties for thrombophilia. One of the enzymes responsible for this plasma membrane PL asymmetry is lipid scramblase 1 (LSCR1) which plays a major role in the transport of PS from the inner leaflet of cell membrane to the cell surface. The mechanisms how aCL/b2GPI and aPS/PT interact to procoagulant cells will be discussed. 231D was monoclonal aPS/PT, having a strong lupus anti-coagulant activity (Arthritis Rheum 60, 2457-67, 2009). The binding of 231D to monocyte cell line, RAW231.7, was investigated by flow-cytometry. 231D bound to RAW234.7 in the presence of prothrombin. RAW264.7 was treated with the monoclonal in the presence of prothrombin. The kinetics of several molecules after 231D exposure was investigated. TF mRNA expression was increased on RAW264.7 treated with 231D. The proteome profile showed phosphorylation of p38 MAPK, and confirmed by cell ELISA. Pre-treatment with INFa induced lipid scramblase-1 (LSCR1) and significantly enhanced TF mRNA induction by 231D. Those data showed that APS/PT activates procoagulant cells, similar to aCL/b2GPI induced procoagulant cell activation. The effect of INFa presumably leads to phosphatidylserine expression on cell surface and facilitate the accessibility of phospholipid-binding proteins, followed by further aPL interaction. Those phenomena would be relevant in vivo in APS patients.
B cell apotopes of Ro60 in lupus
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Ro60 is a major target of autoimmunity in SLE, neonatal lupus syndrome and primary Sjögren’s syndrome. The human B cell response to Ro60 targets multiple epitopes mapped traditionally by immunochemical techniques. A new approach has been to map apotopes (epitopes expressed on the surface of apoptotic cells) and intracellular epitopes by flow cytometry using apoptotic cells as a natural source of immunogen. This has proved superior to standard epitope mapping by identifying determinants on different configurations of native Ro60, leading to the discovery of new diagnostic markers and providing insight into the mechanisms by which different immunogenic forms of a systemic autoantigen may break immune tolerance. A pivotal immunodominant region of Ro60 spanning amino acids (aa) 193-236 has been identified which harbours either 1) an apotope that is associated with SLE with isolated anti-Ro60 responses, or 2) an intracellular epitope that is linked to SLE and primary SS with linked anti-Ro/La autoantibody sets. These determinants are virtually mutually exclusive, signifying the presence of two immunogenic forms of Ro60 autoantigen: a membrane-bound form on the surface of apoptotic cells, and a cytoplasmic form on the Ro/La RNP complex. The plasma protein beta-2-glycoprotein I (β2-GPI) binds to Ro60 on the surface of apoptotic cells and blocks opsonisation by anti-Ro60 autoantibodies. Plasmin cleavage of the hydrophobic loop of β2-GPI abrogates binding to apoptotic cells and reverses the protective effect of β2-GPI on anti-Ro60 IgG-antigenic cell immune complex formation. Thus stimulation of plasmin production may eliminate the protective effect of β2-GPI in maternal anti-Ro60 mediated congenital heart block. Studies are underway to map T-cell determinants in the immunodominant domain of Ro60; determine the in vivo role of the β2-GPI/ Ro60 interaction; and investigate the clonality of the various anti-Ro60 B cell responses.

Anti-heat shock shock protein 60 autoantibodies are associated with arterial vascular events in patients with anti-phospholipid antibodies
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Objectives: Anti-heat shock protein 60 autoantibodies (anti-HSP60) are associated with coronary artery disease and atherosclerosis, and are known to affect endothelial cells in vitro. However, their association with other vascular events (VE) remains unclear. In patients with systemic lupus erythematosus (SLE), elevated titers of anti-HSP60 have been associated with an increased risk of thrombosis when present with lupus anticoagulant antibodies. We have recently shown that anti-HSP60 promote thrombosis in a murine model of arterial injury. Based on these findings, we hypothesized that the presence of anti-HSP60 autoantibodies, alone or in combination with other thrombogenic risk factors (e.g., anti-phospholipid antibodies [aPL]), would be associated with an elevated risk of VE, in particular in patients with SLE. Methods: The study population was derived from the databases of three ongoing cohort studies: two SLE registries and one cohort of individuals with aPL. Only individuals with aPL testing performed on at least two occasions were included. aPL positivity was defined as: anti-cardiolipin (aCL) IgG/IgM >40 aPL units, and/or lupus anticoagulant (LA) positive, and/or anti-β2-glycoprotein I (anti-β2GPI) IgG/IgM positive, each on ≥2 occasions ≥ 12 weeks apart. Data from a total of 406 participants was captured and four groups were identified: (1) aPL-positive with VE (n=85); (2) aPL-positive without VE (n=83); (3) aPL-negative with VE (n=119); and (4) aPL-negative without VE (n=119). Arterial VE (VE-A) (n=123) or venous VE (VE-V) (n=97) were confirmed from medical records. Serum anti-HSP60 were determined by enzyme-linked immunosassay and values exceeding the 75th percentile of the healthy controls (n=25) were considered to be high-titer positive. Clinical and demographic variables captured included age, race, gender, family history of cardiovascular disease, smoking, SLE, hypertension, and diabetes mellitus. Results: Multivariate analyses revealed that total VE were associated solely with age or hypertension. However, analysis of the VE according to their origin showed an association of VE-A, but not VE-V, with anti-HSP60 (OR=2.326 [95% CI=1.157-4.673]). Furthermore, the concomitant presence of aPL with anti-HSP60 increased the risk of VE-A (OR=6.19 [95% CI=2.02-18.91]), but not VE-V (OR=1.09 [95% CI=0.36-3.28]). Finally, the presence of individual aPL (i.e., aCL, LA, or anti-β2GPI) with anti-HSP60 also increased the risk of VE-A, with the strongest association observed for aCL (OR=8.67 [95% CI=1.97-38.08]). Conclusions: Our results demonstrate that anti-HSP60 are associated with VE-A, and that the concomitant presence of aPL (particularly aCL) with anti-HSP60 further enhances the risk of these events.

CS19 Clinical Trials

CS19.1
Some lessons from RA trials to make lupus trials even better
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The science and art of designing and implementing randomized clinical trials (RCTs) has developed immensely over the past decades. In rheumatology, the most successful RCTs have been in rheumatoid arthritis (RA), where after some noted failures in the early 1990s, a large number of new treatments could demonstrate efficacy and safety sufficient to satisfy regulatory requirements and provide clinicians with useful initial knowledge of the new agent. Perhaps even more notably, many RCTs in RA – mostly investigator-initiated ones such as FinRACO, TICORA, BeST, SWEFOT and many others – have investigated not so much the specific agents as the optimal strategy to be employed when using these agents. In the case of SLE, fewer trials have been done and only very few have been successful. Although this may to some extent be due to the medications having, in fact, limited efficacy in SLE, it is also clear that clinical trial design and implementation in SLE may, to some extent, be less than ideal. Here, I will discuss some specific lessons from RA clinical trials with potential relevance for the developing field of RCTs in SLE.

CS19.2
Trials and tribulations: what comes next?
Wofsy, David
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A new era in the treatment of systemic lupus erythematosus (SLE) may be dawning. Twelve years after the first approval of biologic therapy for patients with rheumatoid arthritis, the positive results of two large trials of a novel biologic therapy for SLE have raised hopes that a new approach to treatment may be at hand. This encouraging news follows several disappointments in trials of other biologic therapies and provides a timely moment to reflect on where we stand, what we have learned, and what may lie ahead. For belimumab, the next set of questions are likely to revolve around establishing the clinical setting that best suits this agent. For rituximab, it will be important to establish whether the disappointing results of this approach to B-cell therapy reflect shortcomings in trial design, inadequate statistical power, or an unequivocal failure of the drug. For abatacept, the looming question is whether either of the two ongoing trials will constitute the first breakthrough in lupus nephritis.
Finally, for other novel therapies on the horizon, the recent successes will at the very least have set a new standard for comparison.

**CS19.3**

Outcome measures in SLE trials

Gordon, Caroline

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Most end-points used in clinical trials have not been validated in clinical trials but their use is based on other forms of validation. The BILAG index of disease activity and increasingly the improved revision, the BILAG 2004 index, are being used to measure disease activity and flare in outcome studies and trials alone or with other measures such as the SLEDAI. It is critical that investigators and monitors are trained to use the disease assessment methodology being used in a trial and that definitions in definitions between indices are understood and applied. Trials may use an adjudication committee to assess disease activity or flare at entry to trial or for assessing whether end-points are met. For future trials it will be important to reflect on the lessons learnt from past trials including the Abatacept and Epratuzumab studies as well as to consider new ways of measuring flare. The Abatacept and Epratuzumab trials used cumulative flare and composite landmark analyses respectively. End-points should be chosen that will reflect the most clinically important differences between treatment groups. Consideration should be given to novel trial designs that encompass comparison of conventional treatment strategies with new drug treatment combinations associated with less use of traditional immunosuppressive drugs.

**CS19.4**

Evolution of the SRI: are composite endpoints the future?

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Numerous challenges to drug development have long confronted the lupus community. Despite these obstacles, the last decade has witnessed unprecedented activity in lupus clinical trials. Unfortunately, most of these trials were met with disappointment. While the reasons for failure are multifactorial, ineffective trial design and inadequate response endpoints have contributed to the negative outcomes. The Systemic Lupus Responder Index (SRI) is a composite responder index that grew out of a failed phase II study of belimumab. It fulfilled the criteria put forth by the FDA in their draft guidance document and was approved by regulatory authorities as the primary endpoint for two large phase III studies of belimumab, BLISS-52 and BLISS-76. The SRI’s performance in these two successful studies supports the use of such a responder index in similarly-designed clinical trials.

**CS19.5 & PO2.F.9**

Flare assessment in systemic lupus erythematosus (SLE) patients treated with rituximab in the phase II/III EXPLORER trial

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Objectives: SLE patients (pts) with moderate-to-severe disease activity (≥1 BILAG A or ≥2 BILAG B domain scores) despite background immunosuppressives and corticosteroids, were randomized to placebo (PLA) or rituximab (RTX). Although differences in primary and secondary outcome measures were not observed, an exploratory analysis was performed to evaluate the impact of RTX on disease flares. Our objectives were to assess in those pts who achieved response whether RTX affected: 1) time to moderate or severe flares, 2) annualized flare rates, and 3) prednisone usage during flares. Methods: Pts who achieved response (BILAG C, D, E scores for all domains before wk52) on monthly BILAG assessments were included in this analysis. Flares, defined as increased disease activity following achievement of response, were stratified as follows: Severe flare: ≥1 BILAG A or >3 BILAG B domain scores; A flare: ≥1 new BILAG A domain scores; Moderate flare: 2 BILAG B domain scores. Kaplan-Meier estimates were used to assess time-to-flare. Annualized flare rates were calculated using number of flares per patient with a Poisson regression model. Only flares that occurred after the protocol-mandated prednisone taper at 12 wks were included in the analysis of prednisone use during flares. Results: Responses were achieved in 58/88 (66.0%) PLA-treated and 172/169 (75.1%) RTX-treated pts during the study. No difference was found between rituximab and placebo in preventing or delaying moderate to severe flares. However, when BILAG A flares alone were examined, rituximab reduced the risk of an A flare after achieving a response by 52 weeks (hazard ratio=0.612; p=0.0524) and lowered the annualized A flare rate (0.86 ± 1.47 (SD) vs 1.41 ± 2.14; p=0.038). Eighty-four of 169 (49.7%) rituximab-treated patients achieved low disease activity without subsequent A flares versus 31/88 (35.2%) patients in the placebo group (p=0.027). Prednisone rescue for A flares was similar in rituximab- (24%) and placebo-treated (14%) patients (p=0.204). Conclusions: No conclusions about rituximab efficacy can be drawn from this post hoc analysis. This exploratory analysis suggests that assessment of BILAG A flares may distinguish potential treatment effects with more sensitivity than BILAG B flares, which capture modest changes in disease activity. If confirmed in other studies, this observation may help in the design of more robust clinical trial protocols.

**CS20 World Wide Findings in Paediatric Lupus**

**CS20.1 & PO2.N.1**

Defining and measuring global flares in juvenile systemic lupus erythematosus

mina, rina; von scheven, emily; etherhard, anne; higgins, gloria; lapidus, sivia; eaton, jamie; schenberg, laura; ovel, karen; panaro, marilyn; olson, judyan; ying, jin; klein-gitelman; marisa; levy, deborah; giannini, edward; singer, nova; brunner, hermine

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Objective: To develop a definition of global flare in juvenile Systemic Lupus Erythematosus (jSLE, defined as SLE onset ≤ 16 years) and determine candidate criteria for measuring jSLE flares. Methods: Pediatric rheumatologists answered two Delphi questionnaires to achieve consensus on a common definition of jSLE global flare and identify variables for use in candidate flare criteria. The diagnostic accuracy of these candidate flare criteria was tested with data from jSLE patients (n=98; 623 visits total). Physician-rated change in the jSLE course (worsening yes/no) between visits served as the criterion. Results: The 1st Delphi survey was sent to 299 pediatric rheumatology members of CARRA, PANLAR and PRES (53% response rate), and the 2nd one had a response rate of 84%. There was 96% consensus that “a flare of disease is a measurable worsening of jSLE disease activity in at least one organ system, involving new or worse signs of disease that may be accompanied by new or worse SLE symptoms. Depending on the severity of
the flare, more intensive therapy may be required". Variables suggested for use in JSLE flare criteria were: physician-rated disease activity (V1), disease activity index score (V2), Child Health Questionnaire physical score (V3), patient well-being, protein/creatinine ratio, anti-dsDNA antibodies, ESR, and complement levels. Candidate flare definitions based on percent change of some or all of the JSLE variables were at most 53% sensitive and 97% specific with areas under the receiver operating characteristic curves (AUC) all < 0.67. Using multiple logistic regression, we derived several candidate flare criteria with complex algorithms but AUC as high as 0.92 (sensitivity > 85%; specificity > 85%). CART analysis suggested that V1, V2 and V3 suffice to identify JSLE flares (AUC = 0.81; sensitivity = 64%, specificity = 86%). Conclusions: Consensus has been reached on a common definition of global disease flare in JSLE and promising candidate flare criteria have been developed. Further assessment of ease-of-use and accuracy in a prospective study is needed.

CS20.2 & PO2.N.2

Psychiatric illness of systemic lupus erythematosus in childhood
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SickKids Hospital, Toronto, ON, Canada

**Purpose:** 1) To describe characteristic clinical, laboratory and imaging features; 2) To determine distinct entities in the spectrum of psychiatric disease of Juvenile SLE (JSLE) and 3) to report on treatment outcomes. **Methods:** Single centre cohort study of consecutive JSLE patients followed between 08/1985 and 12/2008. Patients were evaluated following standardized protocol. All patients were assessed by an experienced psychiatrist. Clinical features of psychiatric disease of Lupus were identified and classified according to ACR nomenclature except cognitive dysfunction. Cognitive dysfunction in this study defined as memory or attention deficits reported by patients/parents, affecting academic performance. Psychiatric **Outcomes:** 1) response- no psychiatric symptoms, stopped anti-psychotic medications and Prednisone ≤50mg daily dose for at least 3 months; 2) remission- no psychiatric symptoms, stopped anti-psychotic medications and Prednisone ≤30mg/day for at least 3 months; 3) relapse- recurrence of symptoms (after response) requiring 50% increase in dose of Prednisone, change of 2nd line immunosuppressive not due to adverse effects. **Results:** 447 JSLE patients were followed during the study period: 12% (53) developed psychiatric disease of JSLE; 87% (46) females, median follow-up from psychiatric Lupus diagnosis 2.0 years (0.5-6.8). Half (27/53) had psychiatric disease at diagnosis of JSLE. Median interval from first psychiatric symptom to diagnosis was 60 days (1-1460).Clinical features of psychiatric disease of JSLE: Psychosis-like symptoms seen in 75% (40) with hallucinations being predominant. Insight preserved in 70%. Novel symptom of visual distortions in 38% of those with psychosis. Clinically significant cognitive dysfunction present in 100%. No patient had isolated depression or anxiety. Specific investigations: 42 had Magnetic Resonance Imaging (MRI): 45% normal, 29% cerebral atrophy and 17% white matter changes. Lumbar puncture performed in 53% (28/53) at diagnosis: 29% had abnormally elevated total protein, 7% had elevated white cells. Treatment: Prednisone was started increased following protocol. 60% (24) of patients with psychosis required antipsychotic therapy. All 3 were treated with 2nd line agents: 85% (45) azathioprine, 55% (29) cyclophosphamide and 28% (14) mycophenolate. **Outcome:** 74% (39) responded, 25 attained remission (3 then relapsed), 6 relapsed, 8 improved but not attained remission. **Conclusion:** Psychosis and cognitive dysfunction were 2 distinct patterns found amongst children with Psychiatric Lupus. Psychosis of JSLE was different from adults with preservation of insight and unique feature of visual distortion. 76% responded to standard therapy.

CS20.3 & PO2.N.3

Performance of a new health-related quality of life (HRQOL) measure in pediatric lupus across five countries
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**Objective:** Simple Measure of Impact of Lupus Erythematosus in Youngsters© (SMILEY©) is a novel, brief, 24-item health-related quality of life (HRQOL) assessment tool for pediatric systemic lupus erythematosus (SLE). Responses are in the form of 5-faces scale for easy comprehension. SMILEY© is valid in US-English and has been translated and adapted to nineteen additional languages. Currently, we are conducting cross-cultural validation of SMILEY©. Our objective herein was to test preliminarily the performance of SMILEY© in different geographic populations. **Methods:** Children ≤18 years with SLE and parents completed the appropriate SMILEY© translation, as well as gold standard quality of life (QOL) and physical function scales. Demographic, medication and SLE-related data were obtained. We compared the means of age, child reports of SMILEY© total score, PedsQL™ generic module total score, global quality of life (QOL) rating (administered with SMILEY© using the same style of responses), Child Health Assessment Questionnaire (CHAQ) disability index, and Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI). Depending on the data distribution of the above variables, we used one-way ANOVA or the Kruskal-Wallis (KW) test. **Results:** Eighty-five children (73 girls, 86%) participated from five countries: Argentina (n=11), Brazil (n=33), Italy (n=16), Netherlands (n=10), and Spain (n=15). The mean disease duration was 36 months (1–150 months). The number of children with current/prior steroid use was 74 (97%, n=76), and disease modifying anti-rheumatic drugs (DMARDS) was 74 (95%, n=78). On preliminary analysis, the means/medians of child reports of the SMILEY©, global QOL rating, PedsQL™ generic module, CHAQ disability index, and SDI were similar across the five countries (Table 1 lists p values) and compatible with an assumption of no statistically significant difference. The cumulative means, standard deviation, range, and number of subjects are provided in Table 1. The only significant difference was found in age. The mean age in Brazil was 12 years, but otherwise was 15 years. **Conclusion:** SMILEY© performed uniformly across countries on preliminary analysis. The lower mean age in Brazil is likely related to referral. Given the small sample, the difference in age is not likely to be meaningful. We are actively enrolling from the above centers and from additional centers to expand our sample.

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Primary immunodeficiencies in juvenile systemic lupus erythematosus patients

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Objectives: To evaluate the frequency of complement and antibody primary immunodeficiencies (PIDs) in Juvenile Systemic Lupus Erythematosus (JSLE) patients and to compare lupus patients with and without PID regarding demographic data, clinical features, disease activity and damage, treatment and occurrence of infections. Methods: Seventy-two JSLE (ACR criteria) patients (1 to 16 yrs at diagnosis) were analyzed for early components of the classical complement pathway (C1q, C1r/C1s, C4, C2, C3) and immunoglobulin levels (IgG, IgA, IgM, IgE, and IgG2 subclass). Statistical analysis was carried out according to Fisher’s exact test, Mann-Whitney test and Backward Stepwise multivariate analysis. Results: Nineteen patients (26%) had underlying PID. Complement PIDs were: 5 cases of C2 deficiency and 2 of C4 deficiency (all with persistently very low values in the presence of normal levels of other complement components), and SLEDAI in 2 cases with complete C1q deficiency. All PID patients had normal C3 levels. Immunoglobulin deficiencies were: 4 cases with IgG2 deficiency (<20mg%), 3 with IgA deficiency (<7mg%), and 3 with IgM deficiency (<35mg%). One IgA deficient patient also presented C4 and C2 deficiencies. A clear gender bias was observed, since 54% of the boys (7/13) and 20% (12/59) of the girls presented an underlying PID (p=0.032; RR: 3.25; CI: 1.25–8.46). The 2 cases of infantile SLE (age at onset <2 years) were both males (one with C1q deficiency and other with IgM deficiency). A remarkably higher frequency of severe sepsis was observed in the PID group (31% vs 7.5%; p=0.017; RR: 4.2; CI: 1.32–13.2). Specific lupus clinical features (cutaneous, mucosal, neuro-psychiatric, cardiopulmonary, renal, hematological and articular manifestations and antiphospholipid syndrome) were uniformly alike in patients with and without PIDs. On the other hand, the median of the cumulative damage related to SLE (SLICC/ACR-DI) was significantly higher in immunodeficient subjects [1(0-5) vs 0 (0-3); p=0.0075]. Logistic regression showed that male gender (odds ratio=4.7; 95% CI=1.2–19.2; p=0.034) and SLICC/ACR-DI (odds ratio=2.5; 95% CI=1.13–4.8; p=0.007) were independent risk factors for PID (Nagelkerke R2=0.26). Conclusions: An exceedingly high frequency of antibody and complement deficiency was observed amongst JSLE patients, suggesting that these immunologic defects may contribute to the disease development. Our results command that these two groups of PIDs should be systematically investigated in early onset lupus.

Bone mineral density in childhood and adult onset systemic lupus erythematosus (cSLE and aSLE) may be partly explained by UGT genotype

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Introduction and Objectives: Mycophenolate mofetil (MMF) is a prodrug that is pre-systemically hydrolyzed by esterases to the biologically active moiety, mycophenolic acid (MPA). MPA is mainly metabolized by UDP-glucuronosyltransferases (UGTs) in the liver, intestine, and kidney into the inactive 7-O-glucuronide (MPAG) metabolite. Since genetic variants in UGT1A8, 1A9, and 2B7 have been proposed to explain the large variability of MPA exposure (PK) in transplant patients, the utility of pharmacogenetic (PG) testing is currently being investigated for individualizing MMF therapy. The relevance of these UGT polymorphisms to MPA PK in cSLE has not been addressed, but may be helpful in better understanding the MPA exposure-effect relationship in cSLE. In our ongoing pharmacokinetic-pharmacodynamic study, the contribution of UGT genotype in relation to MPA PK in cSLE was investigated in an exploratory fashion. Methods: Full 9-hour pharmacokinetic profiles were obtained from cSLE patients (n=18; F:M=17:1; age 10-30 years, 43% Caucasian, 57% African-American, 81% non-Hispanic) who were on stable MMF treatment. The MPA PK parameters dose-normalized area under the curve (AUC0-9h) (mean ± S.D.: 38.7 ± 19.8 mg*hr/L/gMPA, range: 14.9 - 95.4) and oral clearance (CL/F) were assessed and analyzed by non-comparitional analysis. Genomic DNA was extracted using standard procedures and genotyped for UGT1A8*5 (830G-A), UGT1A9*3 (98T-C), 1A9-2152T>C, 1A9-440C>T, 1A9-331T>C, 1A9-275T>A and UGT2B7-900A>G by TaqMan assay and direct sequencing. Results: Large inter-patient variability in AUC0-9h (mean ± S.D.: 38.7 ± 19.8 mg*hr/L/gMPA, range: 14.9 - 95.4) and oral clearance (CL/F) of MPA (mean ± S.D.: 27.9 ± 13.7 L/hr, range: 8.3 - 59.3) was observed. Patients with UGT1A9-*40T (331C) or UGT2B7-*900G appeared to have a trend toward lower CL/F and higher MPA exposure (AUC) than the wild-type of all evaluated single nucleotide polymorphisms (SNPs). Patients with UGT1A9-*275A showed relatively high CL/F over a wide range. No patients with the UGT1A9*3 genotype were found. In an exploratory fashion, wild-type and -275A-carriers were analyzed and showed significantly higher CL/F compared to the rest of the carriers of SNPs 1A9-*40T (331C) or 2B7-900G (p<0.05, Mann-Whitney’s U test). Conclusion: Our preliminary data suggest that UGT1A9 and UGT2B7 poly-
KS2 Pitfalls in the Development of New Lupus Drug

KS2.1

Pitfalls in the development of new lupus drugs
Kotzin, Brian L.; Chung, James B.
Medical Sciences, Amgen, Inc.,

The development of new medicines is facing unprecedented challenges as exemplified by increasing costs, lengthening timelines, and decreasing numbers of novel drug approvals. Efforts to develop new therapeutics for systemic lupus face additional challenges due to the heterogeneous nature of the disease and the lack of a well-defined path. This presentation will sort through the steps involved in lupus drug development with a focus on key pitfalls and gaps. Illustration of key challenges will involve examples from published results of therapeutics being tested in lupus and from selected aspects of Amgen’s lupus programs. Choosing drug candidates among the many compelling targets that are likely to influence lupus disease pathways represents a significant challenge, and the added value and predictive information from studies in mouse models of lupus is currently unclear. Biomarkers are essential in early-stage trials to quantify coverage of the target and the relevant biological pathway but they do not necessarily predict clinical effects. Insight into potential clinical benefit in early-stage trials has been difficult resulting in a reliance on relatively large phase 2 clinical trials to demonstrate the first evidence of clinical effect for novel agents. This gap with its associated major cost and delay to understanding impact on disease can be a major deterrent for companies considering lupus as the primary indication. Results from recent large clinical trials also emphasize the inherent challenges in designing later-phase clinical trials for agents that target a systemic multi-organ disease like lupus. Central to improving success is the ability to accelerate advancement of promising molecules and rapidly eliminate candidates with a low likelihood of success prior to conducting large clinical trials. Biomarkers that reliably predict clinical effect, alternative trial designs with narrowed organ-specific outcomes, or enhanced ability to measure general disease activity could greatly help span this gap. In summary, the opportunity to transform the therapy of lupus has never been greater, and surmounting key gaps will greatly enhance the possibility of success.

PL4 Lupus Epidemiology and Pathogenesis

PL4.1

How has murine lupus informed us about human lupus?
Wither, Joan
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The fundamental immunologic abnormality in lupus is the loss of tolerance to nuclear antigens. While the nature of the immune abnormalities that lead to this loss of tolerance in human SLE have proved elusive, study of mice with genetic deletions or transgenes that promote lupus has provided a conceptual basis for understanding the type of immune defects that lead to this breach of tolerance. In general these can be classified into three broad categories; 1) those that promote presentation of, or the response to, apoptotic debris in an immunogenic form; 2) those that affect B and/or T cell signaling resulting in abnormal stimulation of autoreactive lymphocytes; and 3) those that promote survival of autoreactive lymphocytes. In addition, genetic manipulations affecting the inflammatory response initiated by deposited immune complexes modulate the extent of end organ damage. While study of induced-mutant mice has proved an excellent approach for identification of potential immunopathogenic mechanisms in SLE, they represent an extreme situation which is rarely duplicated in human SLE, where multiple genes act in concert to produce disease. Consequently, study of susceptibility loci in mice that spontaneously develop lupus-like autoimmunity may be more reflective of human SLE. Identification of these loci and the immune mechanisms through which they act to promote lupus has been greatly aided by the study of congenic mouse strains. In these strains a homoyzogous interval containing one or a small cluster of susceptibility genes derived from a lupus-prone mouse strain, has been introgressed on a lupus-resistant mouse strain, usually C57BL/6, enabling the study of each genetic locus in isolation. There are now a number of congenic mouse strains that have been produced from a variety of different lupus-prone mouse strains. Investigation of these strains has provided insights into the types of cellular abnormalities that promote lupus and how they interact with each other to produce the global lupus phenotype. As increasing numbers of genetic polymorphisms are identified that confer an increased risk for human lupus, their proposed function falls into similar categories to those identified in murine lupus models. Thus, the insights obtained from study of murine models are likely to be highly relevant to human disease.

PL4.2

Treatment of lupus nephritis
Chan, Daniel Tak Mao
Department of Medicine, University of Hong Kong

Lupus nephritis is an important cause of renal failure. The severe complications that could result from disease or treatment are challenges to the clinician and the patient. At the same time, the treatment of lupus nephritis can be very rewarding, when one witnesses the reversal of severe disease and acute renal failure. The addition of cyclophosphamide to corticosteroid improved renal outcome, and this combination has become standard therapy since the 1980s. Acknowledging the many adverse effects of cyclophosphamide, the quest for new treatments continued. Over the past ten years there is an increasing trend to use the combination of corticosteroid and mycophenolate mofetil as induction immunosuppressive treatment for severe proliferative lupus nephritis, based on the data from clinical trials which showed that this treatment had at least comparable efficacy compared with conventional cyclophosphamide-based therapy. Recent data also suggest an impact of ethnicity on the comparative efficacy of immunosuppressive treatment and the propensity for adverse effects related to treatment. Against initial expectations, the results to date on biologic therapies that target specific molecules involved in the aberrant immunological or inflammatory responses in lupus have not been affirmative on their clinical efficacy in human lupus nephritis. This highlights the complexities of managing patients with lupus and our inadequate understanding of disease pathogenesis. Notwithstanding the setbacks, the vibrancy of both clinical and basic research in lupus nephritis justifies the optimism that treatment options will continue to increase, so that therapy can be tailored to suit the distinct characteristics of individual patients.

PL4.3

New therapeutic targets in SLE
Isenberg, David
Centre for Rheumatology, University College, London, UK

The last five years has seen the apparent dashing of several ‘biologic’ hopes in the treatment of SLE patients. A range of targeted therapies including abatacept, rituximab and LJP394 failed to meet their end points in double-blind controlled trials. However, good news has more recently emerged with the report that belimumab (an anti-BLYS monoclonal) and epratuzumab (an anti-CD22) have met their primary end points in clinical trials and, for good measure; rituximab has been shown to be effective in a vasculitis trial. There is a...
paramount need to optimize clinical trial design in SLE (1) and in particular the optimal method of capturing genuine flares (distinguishing them from ‘grumbling disease’) has proved difficult has yet to be established. Another issue is to avoid the ‘over indulgence’ of corticosteroids and concomitant therapies as this will inevitably blur the demonstration of any benefit by the therapy under investigation. With these important caveats, I think we can now realistically anticipate good news from a variety of on-going studies including trials of atacicept (which block the B cell activating factors BLYS and APRIL); antibodies to interferon α and a spliceosomal peptide P140. The exciting thing about the biologic approaches is that they target cells and molecules for which there is evidence that they are actually involved in the pathogenesis of SLE. Traditionally, drugs ranging from steroids to mycophenolate (via azathioprine, methotrexate, cyclophosphamide and cyclosporine) were used largely on the principle that they worked for other diseases (notably cancer) or post-organ transplant so ‘let’s try it out on a few lupus patients’. We are truly living in an age in which we are moving rapidly from therapeutic serendipity to (immuno-)logical sense!

PO1A Apoptosis

PO1.A.2

Cytopenias in systemic lupus erythematosus: the role of TRAIL
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Faculty of Medicine, University of Alexandria, Alexandria, Egypt

Objective: Several mechanisms have been identified for the pathogenesis of systemic lupus erythematosus (SLE) - associated cytopenias. Apoptosis of peripheral blood cells has been implicated in SLE-associated cytopenias. Accordingly, the aim of the present study was to determine the level of serum TRAIL (Tumour Necrosis Factor-Related Apoptosis Inducing Ligand) and surface expression of TRAIL on lymphocytes of newly diagnosed SLE with cytopenias and their clinical correlation. Methods: The study population consisted of 4 groups. Group I included 20 SLE patients presenting with cytopenias and diagnosed as having SLE according to the ACR criteria. Group II comprised 10 newly diagnosed SLE patients with cytopenias. Group III included 10 patients having cytopenias secondary to conditions other than SLE or malignancy. Group IV consisted of 10 age-sex-matched healthy volunteers. All subjects underwent a detailed interrogation and physical examination. Disease activity was assessed by SLEDAI. Serology included ANA, antidsDNA and bone marrow examination in patients with cytopenias. Serum soluble TRAIL level was assayed by ELISA and surface TRAIL expression on lymphocytes was measured by flow cytometry. Results: Both membrane-bound and soluble TRAIL were significantly higher in SLE patients. Percent lymphocyte-expressing TRAIL tended to be higher in Group I. Neutrophil counts correlated negatively with levels of TRAIL both serum and lymphocyte bound. Serum TRAIL was significantly higher in Group III than in controls. Lymphocyte-bound TRAIL correlated negatively with the level of antidsDNA. Levels of TRAIL tended to be lower in patients with higher SLEDAI. Bone marrow examination of SLE patients revealed that 4/20 patients had hypoplastic myelopoiesis. Myelopoiesis and erythropoiesis were depressed in 30% of cases. Conclusions: TRAIL in both membrane-bound and soluble form is markedly increased in SLE, seems to be disease-specific and may contribute to pathogenesis. TRAIL has a role in inhibiting erythropoiesis and thus may play a role in anemia-complicated SLE. TRAIL also mediates neutrophil apoptosis in SLE and may be partially responsible for neutropenia. Apoptosis of blood cells goes some way to explain cytopenias, offers an accessible model of pathophysiology of disease in the different body systems and provides hope for a new era of apoptosis-based therapies.

PO1.A.3

Apoptotic neutrophils, a potential source of toll-like receptor (TLR) ligands in juvenile systemic lupus erythematosus
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Background: Juvenile-onset Systemic Lupus Erythematosus (JSLE) is a severe multi-system autoimmune disease, characterised by production of auto-antibodies against nuclear material. We have demonstrated increased and dysregulated neutrophil apoptosis in JSLE [1]. The toll-like receptor (TLR) family are essential components of the innate immune system. TLRs 3, 7-9 recognise auto-antigens typical of SLE. Up-regulation of TLRs in adult-onset SLE correlates with disease activity [2]. We have also demonstrated up-regulation of these TLRs in JSLE B and T cells. In addition, apoptotic neutrophils can result in auto-antigen exposure [3]. Aim: To determine whether apoptotic neutrophils in JSLE can induce TLR activation measured by IFN-α expression. Method: PBMCs isolated from healthy controls were incubated with commercial agonists for TLRs 3, 7, 8 and 9 for 24 and 48 hours, followed by RNA extraction and qPCR quantification of IFN-α mRNA expression. Control neutrophils were incubated with control serum and JSLE serum for 2 hours to induce apoptosis, quantified using Annexin V staining and flow cytometry. Control PBMCs were then incubated with these apoptotic neutrophils (24 and 48 hours) and IFN-α mRNA expression measured. IFN-α mRNA expression was normalised (fold difference) to 18s mRNA expression. Results: PBMCs from JSLE patients exhibited a 2-fold increase in mRNA expression compared to control. Both membrane-bound and soluble TRAIL are increased markedly in PBMCs incubated with apoptotic neutrophils treated with JSLE serum (x19 fold) compared to control PBMCs with nothing added. IFN-α mRNA expression in PBMCs incubated with control serum did not change significantly from that occurring in un-stimulated PBMCs (x1.2). Discussion: We have demonstrated that stimulation of TLRs 3, 7 & 8 by their natural ligands on PBMCs leads to increased IFN-α expression. Induction of control neutrophils with JSLE serum resulted in increased apoptosis compared to control serum. IFN-α expression in PBMCs was most markedly increased in neutrophils undergoing apoptosis induced by JSLE-serum. This suggests that there may be factor(s) within JSLE serum that induce neutrophil apoptosis that may result in TLRs activation that induces an inflammatory response. References: 1. Middley et al. 2009. A&R; 60(8):2390, 2. Papadimitraki ED et al 2006. A&R; 54: 3601-3611 3. Middley et al. 2009 BISPAR.

PO1B Biomarkers

PO1.B.1

Elevated levels of cytokines and chemokines in patients with SLE in a multi-center, multi-ethnic, US multi-institutional cohort
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1. University of Texas Medical Branch, Galveston, TX, USA; 2. University of Alabama at Birmingham, Birmingham, AL, USA; 3. University of Texas Health Science Center, Houston, TX, USA

Systemic Lupus Erythematosus (SLE) and Antiphospholipid Syndrome (APS) are two closely related autoimmune and multisystemic diseases. Approximately 30-40% of SLE patients present with antiphospholipid (aPL) antibodies and 50% of those have APS. Previous studies have indicated that cytokines and chemokines can be associated with SLE activity. However, whether those biomarkers are elevated in serum/plasma of SLE or APS patients and whether they are associated with disease activity is not clearly understood. Objectives: To examine if the levels of cytokines/chemokines in patients with SLE in samples from a multicenter multi-ethnic, and US multi-institutional cohort (LUMINA) are elevated and correlate the levels of the biomarkers with disease activity (SLAM-R) and with damage accrual (SLICC-ACR Damage index). Methods: Fifty-six (56) sera, plasma from SLE patients (ACR criteria) was obtained from LUMINA. Patients that were on more than 10 mg prednisone/day, or on other immunosuppressive therapy were excluded from the study and were not on statins or on hydroxychloroquine. In the SLE group (age range 16-67): 55% were African American, 23% were Caucasians, 12.5% were Hispanics from Puerto Rico and 8.5% were Hispanics from Texas, 87% were
females and 13% were males. Thirty-two healthy donors (age range: 18-65; 85% females, 15% males), without evidence of autoimmune or inflammatory diseases were used as controls (age range:18-65). Levels of IL1b, IL-6, IL-8, IFN-α, IP-10, MCP-1, VEGF, TNF-α, VEGF were measured in serum using a Millipore Milliplex™ Multiplex Assay, titers of IgG and IgM anticiardio-lipin antibodies (aCL), sE-selectin, sVCAM-1 and sTF were detected by ELISA, and hsCRP by nephelometry. Cut-off values for the assays were determined using the 95th percentile of 32 controls. Nonparametric Kruskall-Wallis test was used to compare levels of biomarkers in SLE vs. controls and Spearman correlations were utilized to correlate levels of biomarkers with SLAM-R or SLICC scores. Results: aCL IgG, aCL IgM and hsCRP were elevated in 64%, 13% and 50% of the SLE subjects, respectively. IL-6, TNF-α, IFN-α, IP-10, sCD40L, sTF were significantly elevated in Lupus patients (>0.0001) The levels of IFN-α significantly correlated with SLAM-R scores (p=0.0546) and the levels of IL1-b and sVCAM-1 correlated with SLICC scores (p=0.0476 and 0.0009, respectively) in this group of SLE patients. Conclusions: Significant number of SLE samples had elevated levels of IgG aCL, hsCRP, IL-6, TNF-α, VEGF, IFN-α, IP-10, sTF, sCD40L and their titers were significantly different when compared to controls. In this group of selected SLE patients the levels of IFN-α correlated with disease activity (SLAM-R scores) and IL-1b and sCAM-1 levels were directly correlated with SLICC-ACR scores. This study underscores the importance of identifying biomarkers of disease that may help to predict disease activity and possibly to better address treatment of patients with SLE and APS

PO1.B.2

Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus

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1. The University of Hong Kong; 2. Imperial College London, London, UK; 3. The University of Glasgow, Glasgow, UK

Objectives: Interleukin (IL)-33 has recently been found to be the specific ligand of ST2, a IL-1 receptor family member that is selectively expressed on Th2 cells and mediates Th2 response. This study aims to measure serum levels of soluble ST2 (sST2) and IL-33 in patients with systemic lupus erythematosus (SLE) and to examine their association with disease activity. Methods: Seventy SLE patients were evaluated for disease activity determined by SLE disease activity index (SLEDAI), levels of anti-dsDNA antibody, C3 and C4. Fifty-seven patients were evaluated longitudinally on a second occasion. IL-33 and sST2 were measured by sandwich ELISA in the 127 SLE serum samples and compared to 28 age- and sex-matched healthy controls. IL-33 and sST2 were measured by sandwich ELISA in the 127 SLE serum samples and compared to 28 age- and sex-matched healthy controls.

Results: Serum sST2 level was significantly higher in active SLE patients (0.51±0.18ng/ml) compared to inactive patients (0.42±0.08ng/ml) (p=0.006) and normal controls (0.36±0.13ng/ml) (p=0.001). Patients who had active lupus nephritis (n=35) (0.52±0.21ng/ml) and those with nonrenal active exacerbations (n=32) (0.49±0.12ng/ml) had significantly higher serum sST2 compared to inactive patients (p=0.02 and p=0.03 respectively). Among patients with biopsy proven nephritis, those with pure Class IV nephritis (n=4) (0.46±0.04ng/ml) (p=0.006 by ANOVA), sST2 level correlated significantly with SLEDAI, anti-dsDNA antibody, prednisolone dosage and negatively with C3. Linear regression analysis showed that serum sST2 level was an independent predictive factor for modified SLEDAI excluding anti-dsDNA and complement score after controlling for age, sex, glomerular filtration rate and prednisolone dosage (regression coefficient: 8.5 95%CI 2.6-14.3) (p=0.005). There were significant changes in the serum sST2 level at the first and second evaluations among patients (n=13) with increased or decreased disease activity (0.53±0.20ng/ml and 0.41±0.09ng/ml for the occasions with higher and lower disease activity respectively) (p=0.02) with an effect size of sensitivity to change of d = 0.29 but not among those with stable disease (n=44) (0.46±0.12 vs 0.48±0.16 ng/ml) (p=0.34). Elevated serum IL-33 was comparable in frequency (4.3% vs 7.1%, p=0.62) and levels (p=0.53) between SLE patients and controls. Conclusions: Elevated serum sST2 level in SLE patients was found to correlate with parameters of disease activity, sensitive to change in levels of disease activity and was not influenced by age, sex and renal function, suggesting a potential role as surrogate marker of disease activity in SLE. The level of IL-33, its specific ligand, is only infrequently detected in SLE serum by ELISA.

PO1.B.3

Neutrophil gelatinase-associated lipocalin (NGAL) and TNF-like weak inducer of apoptosis (TWEAK) as disease activity urinary biomarkers in lupus nephritis (LN)
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Objectives: 1) To evaluate urinary NGAL (uNGAL) and TWEAK (uTWEAK) as biomarkers of active lupus nephritis (LN) in SLE patients. 2) To study the association of both biomarkers in patients with active LN, partial (PR) and complete (CR) response, non-renal flare and inactive SLE patients. 3) To calculate specificity/sensitivity ROC curves for these biomarkers to differentiate between active lupus nephritis, non-renal flare and ISLE patients. Methods: Five groups of patients and one healthy group control (n=35) were included in this cross-sectional study. Groups were the following: A) Patients with active LN (n=38) defined by an active renal sediment, urinary protein:creatinine ratio (uP/C) ≥ 0.5 and/or biopsy-proven renal disease; B) Patients with CR (n=29) defined by an uP/C<0.2 with an inactive sediment; C) Patients with PR (n=56) with an uP/C between 0.2-2.0; D) Patients with non-renal flare (n=23) defined by a SLEDAI<6; and E) Patients with inactive SLE (n=39) (SLEDAI<6). In all patients both uNGAL and uTWEAK levels were measured by ELISA kits according to the manufacturer’s instructions. Results: uNGAL (ng/mg creatinine) and uTWEAK (ng/mg creatinine) values were expressed as median, interquartile range (IQR). The values of both biomarkers were higher in ALN patients compared to the other groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>uNGAL (ng/Cr mg)</th>
<th>uTWEAK (pg/Cr mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active renal flare</td>
<td>0.409 (0.181-0.828)</td>
<td>0.78 (0.18-1.35)</td>
</tr>
<tr>
<td>Complete response</td>
<td>0.244 (0.120-0.394)</td>
<td>0.0 (0.0-0.47)</td>
</tr>
<tr>
<td>Partial response</td>
<td>0.26 (0.155-0.489)</td>
<td>0.36 (0.0-0.79)</td>
</tr>
<tr>
<td>Non-renal flare</td>
<td>0.190 (0.111-0.306)</td>
<td>0.0 (0.0-0.54)</td>
</tr>
<tr>
<td>Inactive SLE</td>
<td>0.14 (0.067-0.268)</td>
<td>0.40 (0.0-0.85)</td>
</tr>
<tr>
<td>Healthy control subjects</td>
<td>0.10 (0.055-0.224)</td>
<td>0 (0.0-0.52)</td>
</tr>
</tbody>
</table>

No significant differences in uNGAL values were found between active LN and patients with PR. On the other hand, we do not find statistically significant differences between uTWEAK levels of active LN and non-renal flare patients. For both biomarkers the differences between active LN and the rest of groups were statistically significant (p< 0.05). Therefore, the uNGAL levels were better to differentiated patients with active LN from non-renal flare patients, since AUC values of ROC profiles were equal to 0.73. UTWEEK showed better ROC profiles to differentiate active LN from CR patients with AUC values equal to 0.71. Conclusions: uNGAL seems to be a better biomarker for the diagnosis of active renal disease, whereas uTWEAK is better discerning patients with CR.
POI.B.4

Urinary MCP-1 as a potential biomarker in juvenile systemic lupus erythematosus

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Objective: Urinary Monocyte Chemotactic Protein-1 (uMCP-1) is a cytokine expressed in response to pro-inflammatory stimuli [1] and correlates with a poor renal prognosis [2],[3]. We aimed to determine whether uMCP-1 could be a suitable biomarker for monitoring disease activity in Juvenile-onset Systemic Lupus Erythematosus (JSLE).

Methods: Children (diagnosed <17 years) with JSLE and healthy controls were eligible for inclusion. Ethical approval and parental consent were obtained. Urine specimens (5-10mLs) were collected and infection excluded. Samples were spun and supernatants stored at -70°C. UMPc-1 concentration was quantified by enzyme-linked immunosorbent assay (ELISA) and standardised for urinary creatinine. Concentrations are expressed as mean values (± SEM).

Results: Seventeen patients with JSLE were recruited: 8 males; mean age (range) at sample collection 15.2 years (7.5-17.9); healthy controls: (n=9) 2 males, 13.8 years (10.3-15.8). Twenty two urine samples were collected. JSLE patients had a significantly higher concentration of uMCP-1 than healthy controls: JSLE: -3106 pg/mg Cr (± 637 pg/mg Cr); controls 1034 pg/mg Cr (± 151 pg/mg Cr); p=0.009. Longitudinal data noted; over a four week period, a 15 year old female with grade II lupus nephritis, uMCP-1 changed significantly in relation to urine albumin creatinine ratio (uAcr): (MCP-1: 2667, 7204, 3192 pg/mg Cr; uAcr: 2.0, 19.7, 2.4 mg/mmol). In contrast, a 13 year old female with no demonstrable renal involvement, uMCP-1 correlated with a fall in serum dsDNA concentration over time: uMCP-1: 14,279, 5918, 1051pg/mg Cr; dsDNA: 110, 49, 45 IU/ml.

Conclusion: Urinary MCP-1 concentrations were significantly higher in children with JSLE. UMPc-1 could have the potential to act as a biomarker of renal and/or overall disease activity in JSLE. Longitudinal assessment of this chemokine and determining its role in lupus nephritis is needed.

References
3. Wada, T., et al. MIP-1alpha and MCP-1 contribute to crescents and interstitial lesions in JSLE activity in LN. Our results indicate that uNGAL can be a useful tool in the management of the patients with LN.

POI.B.5

Neutrophil gelatinase-associated lipocalin (NGAL) as a urinary biomarker of disease activity and severity in lupus nephritis

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Objectives: To evaluate urinary (uNGAL) and serum (sNGAL) NGAL levels as a biomarker of active lupus nephritis (LN) in SLE patients. To study the association of uNGAL and sNGAL levels in patients with active LN, partial (PR) and complete (CR) response as well as in patients with non-renal flare and inactive SLE. To establish the relationship between uNGAL levels, the clinical and biological parameters in patients with active LN and to calcu- late the specificity/sensitivity of this biomarker for distinguishing active renal from non-renal lupus.

Methods: All patients fulfilled the ACR classification criteria for SLE. Active renal disease was defined by active renal sediment, a urinary protein:urine creatinine ratio (uPCR)≥0.5 and/or a biopsy-proven renal disease. Complete remission (CR) was defined by an uPCR<0.2 with an inactive sediment and partial remission (PR) by an uPCR between 0.2-0.7. A non-renal flare was defined by a SLEDAI>6 and inactive disease by a SLEDAI≤6. This cross-sectional study included 5 groups of patients and one healthy group control (n= 35). Groups are as follow: a group of active LN (n=38), a group of patients with PR (n=56), with CR (n=29), with non-renal flare (n=23) and inactive SLE (n=39). For each patient laboratory parameters (anti-dsDNA, C3, C4, FBC, serum creatinine (sCr) and albumin, estimated glomerular filtration rate (eGFR by Cockcroft-Gault equation) and uSLEDAI were measured. Both uNGAL and sNGAL levels were measured by ELISA kits according to the manufacturer’s instructions (Bioporto, Denmark). ROC curves were used to calculate the specificity/sensitivity.

Results: The uNGAL values were expressed as median (ng/mL), interquartile range (IQR). In patients with renal flare, uNGAL was significantly higher (47.25 (25.08- 88.65) than in those with CR (20.00 (17.10-47.50), PR (34.30 (15.63-61.10), non-renal flare (24.00 (16.30-41.60), inactive SLE (20.00 (12.50-37.80) and healthy control subjects (16.30 (9.14-28.70). Whereas, no significant differences in uNGAL values were found between active LN and patients with PR, the differences were statistically significant with the rest of groups (p<0.05).

On the other hand, the sNGAL levels only could differentiate the active LN from the inactive SLE group (p = 0.0084). The uNGAL values in patients with active LN correlated significantly with uSLEDAI (R = 0.35, p=0.001), and uPCR ratio (R = -0.23, p=0.001), eGFR (R = -0.28, p = 0.0151), and serum albumin (R = -0.23, p = 0.0018). The uNGAL levels showed ROC profiles, with AUC values equal to 0.69, reflecting an acceptable specificity and sensitivity for discriminating active LN from non-renal flare SLE patients.

Conclusions: uNGAL is a promising potential biomarker of activity and severity in LN. Our results indicate that uNGAL can be a useful tool in the management of the patients with LN.

POI.B.6

Hypoferritinemia is associated with serologic antiphospholipid syndrome in SLE patients

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Background and Aims: Hypoferritinemia may be a direct immunomodulator. The present study examines the association between hypoferritinemia and disease activity and organ involvement in a large cohort of lupus patients.

Methods: The concentration of ferritin was assessed in 272 serum samples from lupus patients utilizing the LIASON Ferritin automated immunoassay method (DiaSorin S.p.a, Saluggia-Italy). Hypoferritinemia was defined as >341.2 ng/dl in men, >104.2 in women younger than 45 years of age and >232.3 in women 45 years of age or older. Disease activity was defined as presence of SLEDAI>4 or ECLAM>2. Utilizing an ECLAM database, we com- pared elevated ferritin levels to manifestations grouped by organ involvement.

Results: Of 272 lupus patients, 89% were female, the median age was 37 years old, and disease duration was 10.6 ± 7.7 years. The mean ECLAM was 3.2 ± 2.0 and the mean SLEDAI was 2.6 ± 2.8. Hypoferritinemia was found in 18.6% of SLE patients. Compared to normoferritinemic subjects, those with hypoferritinemia were thrombocytopenic (15.4% vs. 33.3%, p=0.003), had elevated lupus anticoagulant (11.3% vs. 29%, p=0.01) and marginally higher antiphospholipid antibodies IgG (12.1±8.9 vs.15.3±15.9, p=0.096). ECLAM was significantly higher in hypoferritinemic subjects (2.91±8 vs. 4.3±2.5 units, p = 0.04) and SLEDAI was marginally higher in hypoferritinemic sub- jects (2.4±2.6 vs. 3.4±3.2 units, p=0.1). No association was found between hypoferritinemia and cutaneous, joint, hematologic, renal, or neuropsychi- atric manifestations of lupus.

Conclusion: Hypoferritinemia was associated
with thrombocytopenia, elevated lupus anticoagulant and anti-cardiolipin antibody titers and may be an early marker for secondary antiphospholipid syndrome in SLE patients.

PO1.B.7
A pilot study: telomere length as a biomarker for osteoporosis in women with systemic lupus erythematous (SLE)

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Objectives: Telomere shortening, a marker of cellular senescence, has been noted in the peripheral white blood cells (PBCs) of patients with low bone mineral density (BMD) in the general population. Patients with SLE are at an increased risk of low BMD. The aims of this study are to compare telomere length between patients with SLE and healthy controls and to test if telomere length is associated with BMD. Methods: A pilot study of 154 SLE patients and 152 controls were recruited from the parent study, SOLVABLE (Study of Lupus Vascular and Bone Long-term Endpoints). Data collected at the study visit included demographic information, osteoporosis risk factors, BMD, and buffy coats from PBCs were frozen at -80°C. Genomic DNA was isolated from PBCs using the QiAamp DNA Blood Mini Kit (QIAGEN). Telomere length was quantitated using real time quantitative polymerase chain reaction (PCR) as previously described by Cawthon et al, with further modifications using an oligonucleotide standard. BMD was measured by dual-energy X-ray absorptiometry (DEXA) and reported as hip and spine Z scores. Results: The average telomere lengths (kb) in PBCs in SLE patients compared to controls was 5.83 kb vs 6.22 kb (p=0.42). In both patients and controls, as expected telomere length shortened with increasing age, but a trend was noted in patients with SLE showing shorter telomere lengths than controls at an earlier age. When telomere length was grouped into quartiles, SLE patients in the lowest quartile were significantly younger than controls in the lowest quartile of telomere length (42.4 vs 49.1 p=0.04) using a pair-wise comparison. In patients with SLE and PI >0, there was a trend toward shorter telomere lengths in patients under 35 (5.04 kb vs 8.49 kb, p=0.33) and 35-44 years old when compared to controls (4.95 kb vs 6.16 kb, p=0.40). After controlling for age, there was a non-significant trend toward shorter telomere length in patients with PI=0 (OR 0.987, 95% CI 0.930-1.048). After controlling for age, patients in the lowest two quartiles of telomere length showed a non-significant trend of more plaque compared to patients in the upper two quartiles of telomere length (OR 1.062, 95% CI 0.53-2.13). Conclusions: Telomere length tended to be shorter in patients with SLE compared to controls. Patients with SLE and PI >0 trended toward shorter average telomere lengths at a younger age compared with controls. Further research is warranted to test whether telomere length can be used as a biomarker to identify patients with SLE at risk for osteoporosis.

PO1.B.8
A pilot study: telomere length as a biomarker for cardiovascular disease (CVD) in women with systemic lupus erythematous (SLE)

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Objectives: Telomere shortening, a marker of cellular senescence, has been noted in the peripheral white blood cells (PBCs) of patients with CVD in the general population. CVD is a major cause of morbidity and mortality and occurs at an earlier age in patients with SLE compared to the general population; and the risk of CVD in SLE is not explained by traditional risk factors. The aims of this study are to compare telomere length between patients with SLE and healthy controls and to test if telomere length is associated with premature subclinical CVD as measured by plaque index (PI). Methods: A pilot study of 154 SLE patients and 152 controls were recruited from the parent study, SOLVABLE (Study of Lupus Vascular and Bone Long-term Endpoints). Data collected at the study visit included demographic and CVD risk factors. PI was measured by B-mode carotid ultrasound. Buffco coats from PBCs were frozen at -80°C. Genomic DNA was isolated from PBCs using the QiAamp DNA Blood Mini Kit (QIAGEN). Telomere length was quantitated using real time quantitative polymerase chain reaction (PCR) as previously described by Cawthon et al, with further modifications using an oligonucleotide standard. Results: The average telomere lengths (kb) in PBCs in SLE patients compared to controls was 5.83 kb vs 6.22 kb (p=0.42). In both patients and controls, as expected telomere length shortened with increasing age, but a trend was noted in SLE patients showing shorter telomere lengths than controls at an earlier age. When telomere length was grouped into quartiles, SLE patients in the lowest quartile were significantly younger than controls in the lowest quartile of telomere length (42.4 vs 49.1 p=0.04) using a pair-wise comparison. In patients with SLE and PI >0, there was a trend toward shorter telomere lengths in patients under 35 (5.04 kb vs 8.49 kb, p=0.33) and 35-44 years old when compared to controls (4.95 kb vs 6.16 kb, p=0.40). After controlling for age, there was a non-significant trend toward shorter telomere length in patients with PI=0 (OR 0.987, 95% CI 0.930-1.048). After controlling for age, patients in the lowest two quartiles of telomere length showed a non-significant trend of more plaque compared to patients in the upper two quartiles of telomere length (OR 1.062, 95% CI 0.53-2.13). Conclusions: Telomere length tended to be shorter in patients with SLE compared to controls. Patients with SLE and PI >0 trended toward shorter average telomere lengths at a younger age compared with controls. Further research is warranted to test whether telomere length can be used as a biomarker to identify patients with SLE at risk for CVD.

PO1.B.9
Serum TNF-like weak inducer of apoptosis (TWEAK) is a potent soluble biomarker of disease activity in systemic lupus erythematous

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Department of Internal Medicine, Catholic University of Daegu School of Medicine, Daegu, Korea

Objective: Disease activity in systemic lupus erythematous (SLE) is frequently clinically and serologically determined by diverse traditional activity indices, such as SLE Disease Activity Index (SLEDAI), anti-dsDNA, complement proteins, and beta2-microglobulin (beta2-MG). Lupus nephritis (LN) contributes to increased mortality and morbidity in SLE. Recent studies revealed that TNF-like weak inducer of apoptosis (TWEAK), monococyte chemotactic protein-1 (MCP-1), and IFN-y-inducible protein 10 (IP-10) reflect well disease activity, especially in LN. We investigated association of TWEAK, MCP-1, and IP-10 with traditional disease activity indices including SLEDAI, anti-dsDNA, complement proteins, and beta2-MG in SLE and then determined roles of these biomarkers in the renal involvement of SLE.

Methods: Sixty-two female patients with SLE were consecutively enrolled for analysis of TWEAK, MCP-1, and IP-10 from serum and urine samples using ELISA. Laboratory parameters including anti-dsDNA, complements, urine protein:creatinine ratio, and beta2-MG were assessed. Clinical disease activity was determined by the SLEDAI. We also classified patients into 2 groups like these; active LN (n = 31) and non-LN (n = 31). Statistical analysis was performed using Spearman’s correlation coefficient analysis and Mann-Whitney U test.

Results: Serum TWEAK, IP-10, MCP-1 and urine beta2-MG correlated well with SLEDAI scores (p = 0.042, p = 0.042, p = 0.038, and p = 0.008, respectively). Also there were statistically significant correlation between serum TWEAK and anti-dsDNA antibodies (R = 0.303, p = 0.027) and inverse correlation between serum TWEAK and C3 (R = -0.253, p = 0.047) as well as C4 (R = -0.280, p = 0.028). Clinical parameters presenting with significance between active LN and non-LN include serum TWEAK and urine beta2-MG (p = 0.026 and p = 0.039, respectively). Additionally, serum TWEAK level is significantly associated with renal SLEDAI (R = 0.299, p =
0.018). **Conclusions:** Serum TWEAK is closely correlated with lupus disease activity and also may be considered a useful biomarker to indicate disease activity in patients with SLE.

**POL.B.10**
Machine learning models using multiple low abundance protein biomarker levels is superior to clinical laboratories in diagnosing ISN/RPS class of lupus nephritis
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**Objectives:** Treatment and prognostication in lupus nephritis (LN) are often driven by renal biopsy findings, and traditional biomarkers are not predictive of renal pathology. We hypothesized that levels of multiple candidate low abundance urine proteins, when analyzed by multivariable machine learning techniques, would create more effective models of International Society of Nephrology/Renal Pathology Society class of nephritis (ISN/RPS Class) than biomarkers now available to clinicians. **Methods:** 95 subjects from the Charleston and Baltimore LN inception cohorts and the Genentech phase III trial of Rituxan in LN (LUNAR) study population were recruited. ISN/RPS Class was determined prior to induction therapy (as per the primary rheumatologist or the LUNAR study protocol). Urine samples were collected at entry for analysis. Urine levels of 52 candidate low abundance proteins (chemokines, growth factors, cytokines, and renal damage markers) were determined by the multiplex bead array or ELISA. Levels of individual markers were used to create receiver operating characteristics (ROC) curves, and those with ROC area under the curve (AUC) values > 0.65 were used to create multivariable models of LN Class at baseline using artificial neural network (ANN) and nearest related neighbor (NNR) machine learning modeling algorithms. Levels of proteins were either used alone to train models or were combined with baseline clinical variables as inputs. Clinical variables alone were also used to train a model for comparison. Input variables were Pr/Cr, DNA, C3, C4, serum Cr and the selected biomarker panel. The output variables were the individual biopsy classes. **Results:** The biomarker NNR models of class III, IV, V, and proliferative disease (ROC AUC 0.65, 0.83, 0.75, and 0.81 respectively) significantly outperformed the clinical models (ROC AUC 0.53, 0.76, 0.31, and 0.46 respectively). The most predictive markers for Class III and IV disease were IL6, IL1α, IL8, GM-CSF, MCP1, NGAL, IFNα2, IFN γ, and IL.12. ANN modeling improved ROC AUC values for class II and V more than III and IV (ROC AUC 0.97, 0.95, 0.95, and 0.96 respectively). 93% of the diagnostic model’s predictive power for Class IV disease derived from 11 variables: sIL2Ra, MCP1, IP10, IFNα2, IL1α, IL8, N-acetyl-beta-D-glucosaminidase (NAG), MIP1j, GMCSF, ecotaxin, and IFNy. **Conclusions:** This is the first study to systematically evaluate multiple biomarkers representing diverse pathogenic mechanisms by machine learning modeling techniques. It demonstrates that when markers of cell activation, migration, and damage are combined into a single model, diagnostic power is superior to models using traditional biomarkers.

**POL.B.11**
Oxidative stress during exercise in lupus patients and controls
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**Objectives:** Recently, oxidative stress as measured by plasma F2 isoprostane has been linked to fatigue in SLE. The aim of this study was to investigate the relationship of F2 isoprostane to aerobic capacity and exercise tolerance in SLE patients and healthy subjects. **Methods:** Plasma F2 isoprostane provides a reliable index of oxidative stress. We measured change in F2 isoprostane from baseline during a bicycle exercise test in Lupus patients and healthy controls. Phlebotomy was performed via forearm catheter at baseline, at peak aerobic capacity, and at 30 minutes post exercise. Plasma was processed and frozen prior to measurement of F2 isoprostane by mass spec liquid chromatography. Physiologic measurements included peak aerobic capacity (VO2peak), exercise duration, peak heart rate, ratings of perceived exertion (RPE). Fatigue was assessed with the Fatigue Severity Scale (FSS). We compared exercise parameters and change in plasma F2 from baseline in lupus patients and controls by t tests (significance threshold p<0.05). Spearman correlation coefficients were calculated to investigate the relationship between FSS, oxidative stress and exercise variables. **Results:** SLE patients (N=14) were similar to controls (N=8) in age, body mass index and baseline F2 isoprostane. Control subjects were able to reach a peak (p=0.0366) higher peak heart rates during exercise than the Lupus patients, even though there was no significant difference in RPE. There was a trend towards higher VO2peak and increased exercise duration in the controls. We observed a reduction in the exercise-induced oxidative stress response in lupus patients compared to healthy controls. In controls, peak exercise was associated with increased F2 isoprostane reflecting transient low grade oxidative stress. F2 isoprostane was increased over baseline at peak exercise in the controls. Both fatigued (FSS> or =4) and not-fatigued (FSS<4) lupus patients had reduced F2 isoprostane at peak exercise. In both lupus patients and healthy controls, F2 isoprostane returned to baseline during a 30 minute post exercise recovery period. **Conclusions:** We observed impaired generation of F2 isoprostane during peak exercise in lupus patients suggesting a defect in the protective adaptive response to exercise. In healthy persons, repeated bouts of oxidative stress during exercise provide a favorable stimulus resulting in adaptive up-regulation of anti-oxidant defenses. Failure of the adaptive response to oxidative stress generation during exercise could lead to a vicious cycle of oxidative stress, activation of redox sensitive signaling pathways and persistence of a pro-inflammatory milieu which could contribute to the pathogenesis of physical fatigue in SLE.

**POL.B.12**
Impaired control of the tissue factor pathway of blood coagulation in Tasmanian patients with systemic lupus erythematosus
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**Objectives:** Thrombosis is a frequent manifestation in patients with systemic lupus erythematosus (SLE), although the precise mechanisms remain uncertain. This study investigated whether the major physiological trigger of blood coagulation, the tissue factor (TF) pathway, was altered in SLE patients. Furthermore, we investigated potential associations between the TF pathway, the presence of antiphospholipid antibodies (APA), e.g., anticardiolipin (aCL) antibodies, lupus anticoagulants (LAC) and anti-beta2-glycoprotein-I (anti-beta2GP1) antibodies, and other abnormalities present in SLE. **Methods:** 101 subjects [40 SLE patients and 61 age- and sex-matched controls] were recruited from Tasmania, Australia. Markers of the TF pathway [TF, free and total tissue factor pathway inhibitor (TFPI) antigen, and TFPI activity], hypercoagulability [thrombin-antithrombin (TAT) complexes and prothrombin fragment 1+2 (F1+2)], inflammation [interleukin-6 (IL-6)], and endothelial cell damage [soluble E-Selectin (sE-Selectin)] were measured in the plasma of both patients and controls. Additionally, serum levels of APA (aCL IgG and IgM isotypes, LAC, anti-beta2GP1 and anti-prothrombin antibodies) were also determined. **Results:** SLE patients had higher levels of LAC (p=0.0102), anti-beta2GP1 (p=0.0139) and anti-prothrombin (p=0.0319) compared to normal controls. Furthermore, SLE Patients had almost twice the plasma levels of TFPI free antigen (patients vs controls; mean ± s.E.M) (11.64 ± 0.89 ng/mL vs 6.43 ± 0.42 ng/mL; p<0.0001), but approximately half the TFPI activity (0.66 ± 0.07 U/mL vs 1.22 ± 0.03 U/mL; p<0.0001), of normal controls. SLE patients had elevated TAT (18.18 ± 6.27 µg/L vs 4.79 ± 0.96 µg/L; p=0.01) and F1+2 (472.8 ± 51.6 nmol/L vs 355.9 ± 22.2 nmol/L; p=0.0218). Conclusions: Serum TWEAK is closely correlated with lupus disease activity and also may be considered a useful biomarker to indicate disease activity in patients with SLE.
controls and SLE patients (all \( p \leq 0.05 \)). No TF pathway marker correlated with APA, inflammation or endothelial cell damage in SLE patients. **Conclusions:** Although a significant increase in the ‘bioactive’ free form of TFPI was demonstrated in SLE patients, this was not matched by a corresponding increase in TFPI activity. Indeed, the reduction in TFPI activity reflects significantly impaired functional control of the TF pathway in these patients. Moreover, changes to the TF pathway were not associated with abnormalities in SLE, including the presence of APA, type of APA, inflammation or endothelial cell damage. The results from this study suggest hypercoagulability in SLE may (in part) be due to reduced TFPI activity, a mechanism that appears to be independent of other abnormalities of SLE.

**PO1.B.13**

Monocyte surface expression of Fcy receptor RI (CD64), a biomarker reflecting type-I interferon levels in SLE

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**Introduction:** More than half of SLE patients show evidence of excess Type I interferon (IFN-I) production, a phenotype associated with renal disease and certain autoantibodies. However, detection of IFN-I proteins in serum is unreliable and measurement of interferon stimulated gene (ISG) expression is expensive and time consuming. The aim of this study was to identify a surrogate marker for IFN-I activity in clinical samples for monitoring disease activity and response to therapy. **Methods:** Monocyte surface expression of Fcγ receptors, chemokine receptors and activation markers were analyzed by flow cytometry in whole blood from patients with SLE and healthy controls. FcγR expression also was measured in PBMCs from healthy controls cultured with Toll-like receptor (TLR) agonists, cytokines, or serum from SLE patients. Expression of ISGs was analyzed by real-time PCR. **Results:** Circulating CD14+ monocytes from SLE patients showed increased surface expression of FcγRI (CD64). The mean fluorescent intensity of CD64 staining correlated with ISG expression. Flow cytometry analysis of CD64 expression on monocytes from healthy controls. Exposure of monocytes from healthy controls to SLE sera also up-regulated the expression of CD64 in an IFN-I-dependent manner. Decreased CD64 expression was observed concomitantly with the reduction of ISG expression following high-dose corticosteroid therapy. **Conclusion:** Expression of CD64 on circulating monocytes is IFN-I inducible and highly correlated with ISG expression. Flow cytometry analysis of CD64 expression on circulating monocytes is a convenient and rapid approach for estimating IFN-I levels in SLE patients.

**PO1.B.14**

Hyperprolactinemia is correlated with anemia and proteinuria in SLE patients

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**Objective:** Patients with systemic lupus erythematosus (SLE) have excess cardiovascular morbidity and mortality due to accelerated atherosclerosis that cannot be attributed to traditional cardiovascular risk factors alone. YKL-40, a chitinase, is increased in serum of patients with acute myocardial infarction. Patients with stable coronary artery disease high serum levels of YKL-40 are associated with cardiovascular mortality. One prior study has shown that patients with SLE have increased serum levels of YKL-40. Flow-mediated vasodilation of the brachial artery (FMD) has become a broadly accepted indicator of endothelial dysfunction. Flow-independent vasodilation (FID) represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function.

**Methods:** YKL-40 was measured by radioimmunoassay in serum from all patients. In vitro, IFN-I as well as TLR7 and TLR9 agonists induced CD64 expression on monocytes from healthy controls. Exposure of monocytes from healthy controls to SLE sera also up-regulated the expression of CD64 in an IFN-I-dependent manner. Decreased CD64 expression was observed concomitantly with the reduction of ISG expression following high-dose corticosteroid therapy. **Conclusion:** Expression of CD64 on circulating monocytes is IFN-I inducible and highly correlated with ISG expression. Flow cytometry analysis of CD64 expression on circulating monocytes is a convenient and rapid approach for estimating IFN-I levels in SLE patients.

**PO1.B.15**

Elevated serum YKL-40: predictors of preatherosclerosis and subclinical atherosclerotic manifestations in systemic lupus erythematosus

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**Objective:** Patients with systemic lupus erythematosus (SLE) have excess cardiovascular morbidity and mortality due to accelerated atherosclerosis that cannot be attributed to traditional cardiovascular risk factors alone. YKL-40, a chitinase, is increased in serum of patients with acute myocardial infarction. Patients with stable coronary artery disease high serum levels of YKL-40 are associated with cardiovascular mortality. One prior study has shown that patients with SLE have increased serum levels of YKL-40. Flow-mediated vasodilation of the brachial artery (FMD) has become a broadly accepted indicator of endothelial dysfunction. Flow-independent vasodilation (FID) represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function. Intima-media thickness of the carotid bifurcation represents vascular smooth muscle function.

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traditional cardiovascular risk modifiers were assessed: male sex, age, blood pressure, smoking, body mass index, serum cholesterol, insulin resistance (HOMA model), C-reactive protein, SLEDAI, treatment ever with glucocorticoids, HCsQs and other immunosuppressive drugs. Results: Twenty-eight patients (30%) had increased serum levels of YKL-40. The median FMD was 5.9% (range: 0 – 15%), the median FID was 17% (range: 4.9 – 37%) and the median cIMT was 0.57 mm (range: 0.37 – 0.93 mm). Using non-parametric Mann-Whitney test we found high YKL-40 to be associated with low FID (P-value = 0.014) and high cIMT (P-value = 0.010). There was no significant difference in FMD between high and low serum YKL-40 (P-value = 0.614). The difference in cIMT remained significant in multivariate analysis including traditional and non-traditional cardiovascular risk modifiers (P-value < 0.001). The difference in FID between low and high serum YKL-40 was not significant in a multivariate analysis (P-value = 0.097). Conclusion: High serum YKL-40 was associated with cIMT independently of the effects of traditional and non-traditional cardiovascular risk modifiers but not to FMD and FID. Prospective studies are needed to further substantiate if YKL-40 could be a marker of preatherosclerosis and subclinical atherosclerotic manifestations in patients with SLE.

PO1.B.16

Neither anti-dsDNA-antibodies nor C3, but type I interferon-regulated SIGLEC1 correlates longitudinally with disease activity in systemic lupus erythematosus

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Introduction: Contradictory reports exist about the longitudinal association of type I interferon regulated genes or proteins with disease activity in SLE. We recently described SIGLEC1, an adhesion molecule exclusively expressed on blood monocytes and tissue macrophages, as surrogate marker for IFN responses detectable by flow cytometry. In the present report, autoantibody titres, level of complement component 3 and the expression of SIGLEC1 were longitudinally monitored in parallel and correlated to disease activity of SLE patients. Methods: 25 outpatient and inpatient SLE-patients were visited in free time intervals (4 until 12 weeks) - in total 82 patient-visits. SLEDAI2k, BILAG2004, medication, standard laboratory, autoantibodies, complement components and SIGLEC1 were assessed. Differences of SLEDAI2k and BILAG2004 were correlated with changes of biomarkers using linear regression. Results: Neither anti-dsDNA-antibodies (SLEDAI2k: P = 0.14; BILAG2004: P = 0.53) nor C3 (SLEDAI2k: P = 0.11; BILAG2004: P = 0.37) were significantly associated with lupus activity over time. Only changes in expression of SIGLEC1 were significantly correlated with changes of BILAG2004 (P = 0.005), but not with SLEDAI2k (P = 0.44). Conclusions: SIGLEC1 outperformed routine biomarkers of SLE with respect to detect changes in disease activity and therefore, is a convincing parameter that can be easily and quickly formed routine biomarkers of SLE with respect to detect changes in disease activity and therefore, is a convincing parameter that can be easily and quickly measured in standard diagnostic labs to adjust appropriate immunosuppressive therapies in a more personalized approach. Furthermore, Siglec1 is a promising biomarker to accompany currently discussed IFN-directed therapies.

PO1.B.17

Levels of low abundance proteins three months after start of induction therapy reflect treatment response at one year

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Objectives: Lupus nephritis (LN) results in renal failure in up to 42% of patients after five years. However, traditional biomarkers and clinical indicators of treatment response often cannot detect treatment failure until irreversible damage to the kidneys has occurred. Therefore, a more reliable methods of determining early response to induction therapy in LN is needed. We hypothesized that levels of multiple candidate low abundance urine proteins at the time of biopsy and three months after start of induction therapy would be different between treatment responders and nonresponders, offering the possibility of using the result panel to create predictive models of response to therapy. Methods: 62 subjects from the Charleston and Baltimore LN inception cohorts and the Genentech phase III trial of Rituxan in LN (LUNAR) study population were recruited. Urine samples were collected at entry and after three months of induction therapy (per the primary rheumatologist or per the LUNAR protocol for analysis). Urine levels of 17 candidate low abundance proteins (chemokines, growth factors, cytokines, and renal damage markers) were determined by the multiplex bead array or ELISA. American College of Rheumatology renal function response criteria were used to determine responders (n=46) and non-responders/partial responders (n=16). Entry and three month urine candidate protein levels were compared between responders and partial/non-responders by Mann-Whitney U test or Student t-test, and p values < 0.05 were considered significant. Results were reported as mean ± standard error. Results: Only one marker, IFNα2, was higher in non-responders than responders (26 ± 8 vs. 19 ± 5 pg/ml) at baseline. Levels of the following proteins were greater in non-responders at three months: IFNα2 (34 ± 16 vs. 6 ± 2) IL1α (77 ± 35 vs. 10 ± 3), soluble IL2 receptor antagonist (Ra, 1143 ± 491 vs. 360 ± 72), IL8 (266 ± 123 vs. 39 ± 12), IL12 (408 ± 204 vs. 31 ± 14), cystatin C (331 ± 1 vs. 242 ± 8), N-acetyl-beta-D-glucosaminidase (NAG, 20 ± 8 vs. 31), and neutrophil gelatinase associated lipocalin (NGAL, 105 ± 3 vs. 30 ± 7). Conclusions: This study demonstrates that several biomarkers represent multiple pathogenic mechanisms measured 3 months after initiation of therapy reflect response at one year, offering clinicians an early window into treatment response. Machine learning algorithms will be used to create predictive models of response to therapy when one-year followup is complete for the 95 subjects in this multicenter cohort.

PO1.B.18

Vitamin D deficiency in Korean patients with systemic lupus erythematosus

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Objective: Vitamin D is a pleiotropic hormone with immunoregulatory properties. Low levels of vitamin D were found in systemic lupus erythematosus (SLE) and other autoimmune diseases. We have investigated the prevalence of vitamin D insufficiency in SLE and the relationship between vitamin D and disease activity markers of SLE. Method: Blood samples were prospectively collected from SLE patients (n=104) and normal healthy controls (NC, n=49) from March 2008 to May 2008. The level of serum 25-hydroxyvitamin D (25(OH)D) was measured by radioimmunoassay and expressed as ng/mL. The levels of antichromatin antibodies were measured by enzyme linked immunosorbent assay (ELISA) and expressed as arbitrary unit (AU). The SLE patients were also evaluated for clinical, laboratory parameters, systemic lupus erythematosus disease activity index (SLEDAI) and systemic lupus international collaborative clinics/American College of Rheumatology damage index (SLICC/ACR DI). Results: The 25(OH)D levels of the SLE patients (42.49±15.08 ng/mL) were significantly lower than NC (52.72±15.19 ng/mL, P<0.001). Additionally, 17 SLE patients (16.3%) had vitamin D insufficiency, defined as 25(OH)D levels below 30 ng/mL and two in NC (4.1%). Three of SLE patients (2.9%) had vitamin D deficiency, defined as 25(OH)D below 20 ng/mL but none in NC. The risk of vitamin D insufficiency was 4.6 fold increased in SLE (P=0.032).

Conclusions: Vitamin D is a pleiotropic hormone with immunoregulatory properties. Low levels of vitamin D were found in systemic lupus erythematosus (SLE) and other autoimmune diseases. We have investigated the prevalence of vitamin D insufficiency in SLE and the relationship between vitamin D and disease activity markers of SLE. Method: Blood samples were prospectively collected from SLE patients (n=104) and normal healthy controls (NC, n=49) from March 2008 to May 2008. The level of serum 25-hydroxyvitamin D (25(OH)D) was measured by radioimmunoassay and expressed as ng/mL. The levels of antichromatin antibodies were measured by enzyme linked immunosorbent assay (ELISA) and expressed as arbitrary unit (AU). The SLE patients were also evaluated for clinical, laboratory parameters, systemic lupus erythematosus disease activity index (SLEDAI) and systemic lupus international collaborative clinics/American College of Rheumatology damage index (SLICC/ACR DI). Results: The 25(OH)D levels of the SLE patients (42.49±15.08 ng/mL) were significantly lower than NC (52.72±15.19 ng/mL, P<0.001). Additionally, 17 SLE patients (16.3%) had vitamin D insufficiency, defined as 25(OH)D levels below 30 ng/mL and two in NC (4.1%). Three of SLE patients (2.9%) had vitamin D deficiency, defined as 25(OH)D below 20 ng/mL but none in NC. The risk of vitamin D insufficiency was 4.6 fold increased in SLE (P=0.032).
The serum 25(OH)D levels, adjusted with BMI were positively correlated with hemoglobin (β=0.256, p=0.018) and serum complement 3 (β=0.365, p=0.002). Any significant correlation wasn’t found between the levels of serum 25(OH)D and disease activity markers like antichromatin antibody, anti-dsDNA antibody and SLEDAI. **Conclusion:** Serum vitamin D levels were lower and vitamin D insufficiency was more common in Korean SLE patients, however our study demonstrated that vitamin D levels might not be a good marker of disease activity.

**POI.B.19**

**Urine proteomics of active lupus nephritis**

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**Background:** Non invasive test of urine has been developed to diagnose active lupus nephritis. Urine proteomic tool can discover novel biomarkers. We evaluated whether 2-dimension gel electrophoresis (2-DE) of urine could distinguish active lupus nephritis. **Methods:** Pre-biopsy samples from 50 mL of spot urine were collected and its supernatant was kept in -80 °C. Protein precipitation with ethanol and further separated by 2-DE was performed. Protein spot identification was analyzed by Imagemaster 2D platinum software. Selected proteins were validated by ELISA method. **Results:** Thirty urine samples were collected from biopsy-proven lupus nephritis (active =15, inactive = 15). Ten and five samples were from healthy volunteers and other glomerular diseases. Thirty-seven protein spots were differentially expressed between active and inactive LN. Most proteins are transferrin, kininogen, immunoglobulin, alpha-1 beta glycoprotein, prosta-glandin D2 synthase, and zinc-alpha 2 glycoprotein (ZAG). Urine ZAG was significantly increased in active LN as compared to inactive LN and healthy volunteer (Table1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ZAG (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Volunteer</td>
<td>982.7±451.4</td>
</tr>
<tr>
<td>Inactive LN</td>
<td>2106±706.8</td>
</tr>
<tr>
<td>Active LN</td>
<td>927±1457</td>
</tr>
<tr>
<td>Glomerular disease</td>
<td>1444±3777</td>
</tr>
</tbody>
</table>

*p<0.01 compare with Healthy volunteer

**Conclusions:** Novel urine biomarker (ZAG) has been identified by proteomic approach. Urine ZAG should be further evaluated for its potential non invasive test in lupus nephritis.

**POI.B.20**

**Lipoprotein(a), oxidised LDL and carotid atherosclerosis in patients with systemic lupus erythematosus**

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**Objective:** The aetiology of atherosclerosis in systemic lupus erythematosus (SLE) appears to be multi-factorial. The inflammatory process may play a role in part by mediating alterations in lipids e.g. oxidation in addition, several studies have noted increased lipoprotein(a) [Lp(a)] in SLE patients. The aim of this study is to determine the association between lipid subtypes and subclinical atherosclerosis in a population with SLE. **Methods:** 168 women with SLE (≥4 1997 ACR criteria) and 56 healthy controls were studied. Sub-clinical atherosclerosis was assessed using B mode Doppler ultrasound of the carotid artery to measure the carotid intima-media thickness (cIMT) and identify carotid plaque. Oxidised-LDL and Lp(a) were measured by ELISA and immunonatex enhanced immunonassay methods, respectively. **Results:** The mean (SD) age of the patients and controls were 52(11) and 47(14) yrs respectively. There was no significant difference in the mean (SD) cIMT between the two groups: 0.06 (0.01) vs 0.07 (0.1) cm; P=0.1. Patients with SLE tended to have a higher prevalence of carotid plaque 26% vs 14%; P=0.07. Oxidised-LDL was significantly higher among SLE patients [median (IQR) 76 (57, 99) vs 56 (42, 88) U/l; P=0.004]. Lp(a) also tended to be higher in SLE patients (p=0.08). In SLE and controls there was a significant correlation between oxidised-LDL and mean cIMT (R=0.15, P=0.04 and R=0.29, p=0.02 respectively). There was a positive association between oxidised-LDL and carotid plaque in SLE only (R=0.16, P=0.04). Lp(a) was significantly associated with carotid plaque (R=0.19, p=0.01) and weakly associated with mean IMT (R=0.13, p=0.09) in SLE but not controls. **Conclusions:** Oxidised-LDL and Lp(a) are associated with subclinical atherosclerosis in patients with SLE. Control of the inflammatory process may reduce oxidative stress and reduce the development of atherosclerosis in this population.

**Abstracts of Poster Presentations**

**POI.B.21**

**Relationship between markers of inflammation and common carotid intima-media thickness in patients with systemic lupus erythematosus and antiphospholipid syndrome**

Seredavkina, Nataliya V.; Reshetnyak, Tatiana M.; Kondratieva, Lubov V.; Ostryakova, Ekaterina V.; Alexandrov, Elena N.; Novikov, Alexander A.; Much, Evelina S.; Nasonov, Evgeni L.

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**Background:** Low grade inflammation may present in patients (pts) with antiphospholipid syndrome (APS) in relation to specific risk factors, such as C-reactive protein (CRP) and cytokines. The only presence of antiphospholipid antibodies and traditional risk factors (TRF) of atherosclerosis cannot explain all spectrums of clinical features in APS pts. **Objectives:** to estimate levels of tumor necrosis factor α (TNFα), soluble TNFα-receptor 1 (sTNFα-R1) and high sensitive CRP (hs-CRP) in pts with systemic lupus erythematosus (SLE) and APS depending on common carotid intima-media thickness (IMT). **Methods:** A total of 156 pts (52 with primary APS (PAPS), 48 with SLE+APS and 56 with SLE without any APS manifestations) and 28 age– and sex–matched controls were assessed. They underwent electrocardiography, echocardiography, ultrasonography and laboratory testing including antiphospholipid antibodies and lipid profile of plasma. sTNFα-R1 and hs-CRP were measured in all pts and controls, TNFα – in 126 pts and 28 controls. TRF of atherosclerosis (according to the verified Framingham risk assessment formula) were assessed in all pts and controls. **Results:** Concentrations of TNFα and hs-CRP (3.59 [2.18; 6.48] pg/ml and 2.69 [0.95; 8.14] mg/l) were higher in groups of pts than in controls (0.34 [0.28; 0.89] pg/ml and 1.07 [0.43; 1.94] mg/l) respectively (p<0.05 in all cases), and did not differ between the pts’ groups. Serum levels of sTNFα-R1 in SLE+APS pts (3.86 [2.24; 6.25] ng/ml) and in SLE pts (2.71 [2.10; 5.08] ng/ml) were significantly higher than in PAPS pts (2.25 [1.90; 3.26] ng/ml) and in controls (2.19 [1.93; 2.56] ng/ml), p<0.001. Atherosclerotic plaques (ATP) and thick IMT were registered with the same frequency in pts and controls: 14% and 25% vs 3% and 11%, respectively (p<0.05 in all cases), and were more frequent in pts older 50 years (p=0.05). Concentrations of sTNFα-R1 were significantly higher in pts with ATP (4.00 [2.90; 4.12] ng/ml) than in pts with thick (2.94 [2.30; 3.50] ng/ml) and normal IMT (2.18 [1.81; 2.55] ng/ml). There weren’t any relationship between the TNFα, hs-CRP and IMT. Serum levels of sTNFα-R1 were correlated with IMT and Framingham risk score (R= 0.17 ± 0.20, respectively, p<0.05), levels of TNFα – with concentrations of hs-CRP (R= 0.28, p<0.05). There weren’t any relationship between the TNFα, hs-CRP and IMT. Serum levels of sTNFα-R1 were correlated with IMT and Framingham risk score (R= 0.17 ± 0.20, respectively, p<0.05), levels of TNFα – with concentrations of hs-CRP (R= 0.28, p<0.05). **Conclusions:** Increased levels of TNFα, sTNFα-R1 and hs-CRP were associated with thick IMT and the presence of ATP in pts with SLE with and without APS, but not with PAPS.
PO1.B.22

**Serum fetuin-A (alpha HS-glycoprotein): correlation with other markers of disease activity in systemic lupus erythematosus**

**About-Ray, Anna; About-Ray, Susan**

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**Objective:** To assess the serum level of fetuin-A, a negative acute phase reactant, in systemic lupus erythematosus (SLE) patients and to elucidate its correlation with clinical features, other laboratory parameters and overall disease activity. **Methods:** The study comprised 59 SLE patients (49 women and 10 men, mean age 44.7 years, mean disease duration of 6.2 years) diagnosed according to ACR criteria for SLE and 30 healthy age and sex matched controls. Disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Laboratory investigations included complete blood count, erythrocyte sedimentation rate (ESR), urine analysis, 24-hour urine protein measurement, serum creatinine, antinuclear antibody (ANA), anti-double stranded DNA (anti-dsDNA), complement component C3 and anti-C1q antibodies. Serum fetuin-A levels were measured by enzyme linked immunosorbent assay (ELISA). **Results:** Serum fetuin-A levels were found to be significantly lower in SLE patients compared to age and sex matched healthy controls, p < 0.01. Furthermore, the levels were significantly lower in active SLE patients compared to inactive SLE patients, p < 0.05. The results demonstrated a significant positive correlation between disease activity (SLEDAI) and serum fetuin-A levels, p=0.001; r = 0.551. There was a significant negative correlation between serum fetuin-A levels and ANA, anti-dsDNA, serum creatinine, 24-hour urine protein and C1qAb respectively (p = 0.03, r = -0.488; p< 0.01, r = -0.521; p< 0.08, r = -0.401; p< 0.06, r = -0.411; p< 0.001, r = -0.585 respectively). There was a significant positive correlation between fetuin-A levels and C3 levels, p< 0.05, r = 0.546. The findings showed that fetuin-A levels were significantly lower in patients with renal involvement than in those without renal involvement, p < 0.01. Renal involvement was present in 21/59 patients. No correlation was found between age, disease duration and fetuin-A levels. **Conclusion:** The findings of the present study suggest that measurement of serum fetuin-A levels in SLE appears to be a useful addition to the clinical and other laboratory parameters and may be a useful biomarker in the monitoring of disease activity and progression particularly in the presence of renal involvement.

PO1.B.23

**Evaluation of erythrocyte C4d to complement receptor 1 ratio in systemic lupus erythematosus by using CR1-2B11**

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Increased erythrocyte-bound complement 4d (E-C4d) and decreased erythrocyte-bound complement receptor 1 (E-CR1) ratios might be augmented by the reciprocal changes in lupus. This study focused on more accurately measuring E-CR1 independently of polymorphisms, determining the ratio by using CR1-2B11 and examining its cut-off value to differentiate SLE from other disease. We enrolled 80 lupus patients, 72 patients with other diseases, and 72 healthy controls. A newly identified CR1-2B11 antibody specifically recognized an epitope of CR1 and was used to detect E-CR1. The E-C4d and E-CR1 were detected with indirect immunofluorescence staining and analyzed by flow cytometry. The E-C4d/E-CR1 ratios were calculated and compared with patient diagnosis. SLE patients had higher E-C4d levels than healthy controls and patients with other diseases (5.47 ± 0.5 vs. 0.64 ± 0.07 and 91 ± 0.09 mean fluorescence intensity (MFI) respectively, P < 0.001 for both). Conversely, E-CR1 levels in the SLE group were lower than in these two groups (1.94 ± 0.15 vs. 5.58 ± 0.24 and 3.74 ± 0.21 MFI, P < 0.001 for both). Furthermore, the E-C4d/E-CR1 ratios in SLE patients were significantly increased compared with the two groups (3.76 ± 0.38 vs. 0.12 ± 0.01 and 0.3 ± 0.03, P < 0.001 for both). The range of E-C4d/E-CR1 ratio in all patients was between 0.017 and 13.9.

PO1.B.24

**Urine biomarkers of renal pathology in lupus nephritis**

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**Objectives:** Kidney pathology is important for choosing therapy and monitoring disease progress in LN. Because it is not practical to biopsy an SLE patient at every renal flare, a non-invasive test that accurately reflects renal pathology is highly desirable. The objective of this study was to identify urine biomarkers that can be used as surrogates for specific pathologic kidney lesions, such as necrosis, crescents, inflammation, or fibrosis/scarring. **Methods:** 47 urine samples were obtained at the time of diagnostic biopsy for LN, fractionated to remove proteins larger than 30 kDa, and spotted onto weak cation exchange protein chips (CM10 chips) for proteomic analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Urine samples were grouped by pathologic findings according to the type of glomerular injury (defined as no endocapillary proliferation, endocapillary proliferation, cellular crescents, necrosis, or the degree of interstitial inflammation, or the degree of interstitial fibrosis and atrophy (defined as none, mild, moderate-severe). Differential protein expression was tested by ANOVA followed by t-test and a p < 0.05 was considered significant. **Results:** SELDI-TOF MS identified 169 protein ions between 2-20 kDa with a signal-to-noise ratio ≥ 15. Protein ions present in ≥50% of samples were chosen for statistical analysis. Thirteen urine biomarker candidates were differentially expressed between specific pathologic findings in LN biopsies: 4 for glomerular injury, 8 for interstitial inflammation and 7 for interstitial fibrosis and atrophy. To date 6 of these candidates have been identified, including isoforms of hepcidin, fragments of α1-anti-trypsin, and albumin. To verify SELDI-TOF MS findings, urine hepcidin was quantitatively measured by ELISA and found to be increased specifically in moderate and severe interstitial inflammation. Urine hepcidin showed no correlation to liver fatty-acid binding protein or β2-microglobulin, both markers of proximal tubular injury. **Conclusions:** Urine protein profiling at the time of kidney biopsy for LN identified 13 potential biomarkers of specific renal pathologic lesions. One of these, hepcidin, may be a marker of renal interstitial inflammation. The lack of correlation with proximal tubular injury markers suggests that urine hepcidin is not increased because filtered hepcidin is not reabsorbed by the proximal tubule. This is consistent with our previous finding that hepcidin is expressed by interstitial leukocytes in LN.

PO1.B.25

**E-selectin and VCAM-1 as biomarkers of disease in patients with SLE**

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**Introduction:** Cellular adhesion molecules specifically E-selectin and Vascular Cell Adhesion Molecule-1 (VCAM-1) are produced in response to vascular activation and may reflect inflammatory or atherogenic stimuli. They are associated with increased cardiovascular risk in the general population. Some studies have found increased levels in lupus and correlation with disease activity, damage and specific disease manifestations such as nephritis and skin disease. We tested the hypothesis that circulating levels of VCAM-1 and E-selectin are increased in lupus, and that they correlate
with disease activity, overall damage and specific manifestations, in particular lupus nephritis and clinical cardiovascular disease. Methods: We conducted a cross sectional cohort-control study of patients with SLE and healthy controls. E-selectin and VCAM-1 were measured on fasting blood using standard ELISAs (R+D systems). We compared levels in patients and controls and also examined correlations with SLEDAI-2000, SLICC damage index and the presence of skin disease, nephritis and a history of cardiovascular events. Results: We studied 179 women with SLE and 69 healthy females. The mean (SD) age was 54 (11.17) and 49 (14.66) years respectively (P=0.019). There were significantly higher levels of E-selectin in SLE (P=0.009) however this increase was not found in any specific disease sub group and did not correlate with disease activity, damage or cardiovascular events. Levels of VCAM-1 were not significantly different in the two groups (P=0.192) however in patients with active nephritis levels, VCAM-1 levels were significantly increased (P=0.009). Levels of VCAM-1 were increased in all smokers (P<0.0023). Conclusions: E-selectin is significantly raised in SLE and while not correlated with a specific disease subtype may reflect ongoing low grade vascular inflammation in these patients. Although not significantly raised in lupus patients, VCAM-1 may be important in the pathogenesis of atherosclerosis as it is associated with smoking in both groups. The raised levels in active nephritis have been replicated in other studies and VCAM-1 may be a marker of disease activity and vascular risk in lupus nephritis.

PO1.B.26 Platelet C4d is associated with all-cause mortality in patients with systemic lupus erythematosus

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Objectives: Platelets bearing complement C4d (P-C4d) are reported to be specific for a diagnosis of systemic lupus erythematosus (SLE) and are associated with ischemic stroke. We investigated the association of P-C4d with all-cause mortality and prevalent cardiovascular disease (CVD) events in our longitudinal cohort of patients with SLE. Methods: We recruited 356 consecutive outpatients or inpatients with SLE since July 2001. Outcomes were all-cause death and cardiovascular events including myocardial infarction, coronary artery bypass graft, percutaneous coronary transluminal angioplasty, stroke, coronary artery bypass graft, percutaneous coronary transluminal angioplasty, stroke, paroxysmal embolism, deep vein thrombosis or other thrombosis. P-C4d status was determined by flow cytometry. Multivariable logistic regression was utilized to assess the independent association between P-C4d and all-cause mortality. Results: Mean age was 44.4 years (range: 18-81 years), 92% were female, and 81% were Caucasian. Mean SLE disease duration was 15 years at baseline. Mean duration of follow-up was 4.7±2 years. Seventy SLE patients (20%) had positive P-C4d at baseline. P-C4d-positive patients were more likely to have a history of renal disease, seizure disorder, hemolytic anemia, thrombocytopenia, anti-double stranded DNA (dsDNA) and/or antiphospholipid antibodies. Overall CVD event frequency was 21.6%. SLE patients with positive P-C4d had significantly more CVD events compared to those with negative P-C4d (35.7% vs. 18.2%, P=0.001). Positive P-C4d at baseline was associated with stroke, but not with other cardiovascular events (odds ratio 4.96, 95% confidence interval 1.75-14.06, P=0.003) after adjusting for age, race, smoking history, SLE disease duration, renal disease, dsDNA and antiphospholipid antibodies. The overall mortality was 3.9%. Causes of death were infection (n=4), cardiac arrest (n=2), congestive heart failure (n=1), cancer (n=2), hemorrhage (n=1), and unknown (n=4). Six of these 14 deceased patients had a history of cancer (ovarian carcinoma, lymphoma, lung cancer, anal squamous cell carcinoma). Positive P-C4d at baseline was associated with all-cause mortality (hazard ratio 7.92, 95% CI 2.13-29.48, p=0.002) after adjusting for age, race, sex, SLE disease duration, renal disease, cardiovascular event, cancer, dsDNA and antiphospholipid antibodies. Baseline SLE activity and smoking history were not associated with stroke or all-cause mortality and did not attenuate the significant association between P-C4d and all-cause mortality. Conclusions: Platelet C4d is associated with all-cause mortality and stroke. Platelet C4d may be a prognostic biomarker as well as a pathogenic clue that links systemic inflammation, complement activation, and thrombosis.

PO1.B.27 Interleukin-18 (IL-18) in systemic lupus erythematosus: a novel marker of disease activity and a potential target for therapy?

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Objectives: Systemic lupus erythematosus (SLE) is an autoimmune disease with a complex pathogenesis. Cytokines and the ensuing inflammatory process play a vital role in SLE leading ultimately to irreversible organ damage. Interleukin-18 (IL-18) is a proinflammatory cytokine that induces interferon-gamma and may thus produce much damage in SLE. Accordingly, the aim of the present study was to investigate serum IL-18 in SLE patients before and after 4 months of hydroxychloroquine phosphate only therapy and to assess its effect on disease activity. Methods: A total of 66 SLE patients diagnosed according to the ACR criteria for SLE and 34 age-sex-matched healthy controls were recruited in this study. The serum levels of IL-18 were determined by ELISA. Serum levels of IL-6 and TNF alpha were also measured using the ELISA method. Anti-dsDNA antibody, CH50, C3, C4 and circulating immune complex levels were analyzed. Disease activity was measured using the SLE Disease Activity Index (SLEDAI). All parameters were measured before and 4 months after therapy. Results: At baseline serum levels of IL-18, IL-6 and TNF alpha were significantly higher in SLE patients when compared to the controls, p<0.005 and significantly higher in those SLE patients with higher SLEDAI, p<0.05. After 4 months of hydroxychloroquine therapy, the mean serum levels of IL-18, IL-6 and TNF alpha decreased significantly in all patients, p<0.05. IL-18 levels correlated positively and significantly with disease activity (SLEDAI score), p<0.05; r = 0.466 and with anti-dsDNA antibody titer, p=0.05; r = 0.484. IL-18 correlated significantly with TNF alpha, p<0.05; r = 0.585. There was also a significant association between TNF alpha and SLEDAI score, p<0.001; 0.623. Conclusions: The proinflammatory cytokine IL-18 is increased in SLE patients, indicating that it may play a crucial role in the inflammatory processes in SLE. Hydroxychloroquine therapy lowers the proinflammatory cytokines and is thus a valuable drug in SLE. Furthermore, as IL-18 correlates significantly with TNF alpha, TNF blockade could also be considered for use in SLE, particularly in active SLE. However, the development of a specific therapeutic agent for blocking IL-18 would be a welcome addition to the therapeutic armamentarium of this autoimmune disease.

PO1.B.28 Potential biomarkers identified from systemic lupus erythematosus patient peripheral blood B cell, T cell and myeloid cell transcriptions

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Objectives: To characterize the transcriptiones of isolated leukocyte subsets obtained from SLE patients as compared to healthy controls and to determine whether proteins predicted to be differentially expressed by microarray an-
Lupus

**PO1.B.29**

**Lymphocyte-bound complement activation products (LB-CAP) as biomarkers for SLE**

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**Objectives:** Systemic lupus erythematosus (SLE) is immunologically characterized by polyclonal lymphocyte activation, autoantibody production, and complement activation. Complement activation products bound to circulating lymphocytes, erythrocytes, and platelets have recently been shown to be specific biomarkers for diagnosing SLE. In this study, we explored the possibility that lymphocyte-bound complement activation products (LB-CAP) may serve as biomarkers for specific clinical/serological features and disease activity of SLE.

**Methods:** A cross-sectional study was conducted which involved 224 patients with SLE, 179 patients with other diseases, and 114 healthy controls. LB-CAP on peripheral blood lymphocytes was measured by flow cytometry. Associations of LB-CAP with clinical and serological manifestations of SLE were investigated using logistic regression analysis.

**Results:** Significantly elevated levels of C4d were detected specifically on T and B lymphocytes (173 transcripts), CD3+CD4+ T cells (92 transcripts) and CD3+ myeloid cells (201 transcripts) as compared to controls. As predicted by the SLE transcriptomes, there was an increased frequency of Siglec-1 (CD169) positive SLE myeloid cells as compared to control myeloid cells. Likewise, the array transcripts for the endosomal molecules CD107a (LAMP1) and CD63 (LAMP2) were upregulated in SLE myeloid cells and correlated with active disease as did CD63 expression in SLE CD3+CD4+ T cells. High intracellular protein expression levels were observed for both CD63 and CD107a proteins in control and SLE leukocytes. Although activated cells can display these molecules on the cell surface, we rarely observed cell surface expression of CD63 or CD107a in SLE myeloid cells compared to express increased levels of intracellular CD63 compared to controls. No consistent difference was noted for intracellular CD63 expression levels in T cells or for intracellular CD107a expression levels in T cells or myeloid cells. Elevated expression levels of CD38 transcripts were noted in SLE B cells which correlated with an increased frequency of CD38 positive B cells in SLE patients with active disease. Finally, elevated transcripts and protein expression for IFN-inducible molecules, such as Stat-1, were observed in SLE leukocytes. Increased expression of other IFN-inducible molecules are being validated at the protein level. Elevated levels of both plasma thioredoxin and galectin-3 were observed in SLE leukocytes. Increased expression of other IFN-inducible molecules are being validated at the protein level. Elevated levels of both plasma thioredoxin and galectin-3 were observed in SLE leukocytes. Increased expression of other IFN-inducible molecules are being validated at the protein level.

**Conclusions:** These studies suggest that leukocyte subsets in SLE express unique transcriptional profiles and this might translate to hyper-expressed proteins with potential pathogenic relevance in SLE.

**PO1.B.30**

Anti-C1q IgG levels neither forecast nor mark a lupus nephritis flare

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**Objectives:** The recognition of biomarkers of lupus nephritis (LN) that reliably identify, or more importantly forecast, a LN flare would greatly improve LN management. Anti-C1q antibodies have been shown to associate with LN, and reports have suggested that anti-C1q antibody levels can be used to forecast or mark a LN flare. However, this hypothesis has never been directly tested using regular (unbiased) serial measurements leading up to a LN flare. The present study tested this hypothesis.

**Methods:** Anti-C1q IgG plasma levels were measured by ELISA in 38 normal individuals, and a cutoff of 3 standard deviations above the mean was used to identify anti-C1q positive samples. Single plasma samples were then tested from 101 SLE patients (66 with LN) enrolled in the Ohio SLE Study (OSS), a longitudinal study of flare pathogenesis in chronically active SLE patients who have been followed at regular bimonthly intervals for an average of over 4 years. The samples selected for this cross-sectional analysis were at the patients’ first LN flare, or at baseline if they never experienced a LN flare. For the longitudinal analysis, anti-C1q positive patients who experience LN flares with available plasma samples at 8, 6, 4, and 2 months before, and at the time of LN flare (together termed a flare cycle) were tested for anti-C1q IgG. All samples from a flare cycle were assessed together. The same positive and negative controls were used in all patient assays, and all optical densities were normalized to the positive control.

**Results:** For the cross-sectional analysis, 29% of the nonrenal SLE patients had positive anti-C1q IgG, compared to 64% of the LN patients (P < 0.001 by Fisher’s exact test). For the longitudinal analysis, 21 LN flare cycles were identified from the anti-C1q-positive LN patients. No significant change was found in median anti-C1q IgG levels at flare (0.847 normalized OD, P = 0.235) or 2 months before flare (0.743, P = 0.488), compared to the levels at 8 months (0.909), 6 months (0.700), and 4 months (0.740) before flare (analyzed by Friedman’s repeated measures ANOVA). **Conclusions:** The presence of plasma anti-C1q IgG is significantly associated with LN, confirming numerous previous reports. However, when comparing plasma levels at regular bimonthly intervals for 8 months leading up to and at the time of LN flare, anti-C1q IgG neither forecasted nor marked a LN flare.
PO1.B.31

Should therapy go beyond the control of immediate injury? Biomarkers of the vasculature and their association with longitudinal assessments in the induction phase of a randomized multicenter trial comparing mycophenolate mofetil and intravenous cyclophosphamide.

Robert, Clancy1 the MMF/IVC Lupus Nephritis Induction Trial., Investigators 1. Kim, Minni2 Ginzer, Ellen M.3

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Objective: A major barrier to understanding and treating lupus nephritis (LN) is the paucity of sensitive and validated biomarkers. Adiponectin is expressed on the endothelium of all vessels in biopsies from patients with LN but decreased at inflamed areas. Adiponectin knockout mice suggest that adiponectin may be a key regulator of proteinuria. Increased expression of membrane EPCR in LN biopsies predicts a poor response to therapy. Methods: This study leveraged the LN induction trial comparing intravenous cyclophosphamide (IVC) and mycophenolate mofetil (MMF) to evaluate the relationship between clinical response of LN and adiponectin and sEPCR as a proxy for vascular “protective” molecules. Methods: Adiponectin, sEPCR, e-selectin, and nitric oxide (NO) were measured in 109 plasma from 48 patients in the LN induction trial. Response was evaluated using a prospeced primary endpoints related to urinary protein/creatinine ratio and serum creatinine. Sample included visits 4 (4wks), 7 (15 wks) and 9 (24 wks-end of induction Rx). Results: A trend toward increased plasma adiponectin in responders vs nonresponders (19.2 ± 6.8 vs 16.4 ± 9.1 at visit 4, 13.0 ± 5.2 vs 11.7 ± 6.5 at visit 7, 13.7 ± 7.8 vs 10.9 ± 4.9 at visit 9) was observed. In patients with subnephrotic proteinuria (<3 g/day urine protein; 63% of the total), plasma adiponectin was similarly increased in responders vs nonresponders at all visits. Moreover, when combining data across all visits nonresponders had significantly lower adiponectin (p=0.0032). There was a tendency of sEPCR to decrease in responders vs nonresponders (243 ± 164 vs 284 ± 167 at visit 4; 338 ± 271 vs 341 ± 197 at visit 7; 260 ± 104 vs 368 ± 216 at visit 9). In comparing MMF vs IVC, sEPCR, levels were significantly higher in the IVC group when data was combined over all visits (p=0.005). Consistent with evidence that therapy in the responder arm mobilizes vascular protective molecules, NO changed in the predicted direction (62 ± 47 vs 68 ± 66 at visit 4; 39 ± 46 vs 52 ± 65 at visit 7; 27 ± 33 vs 92 ± 55 at visit 9; p=0.02). Combining data across all visits, nonresponders had significantly higher NO levels than responders (p=0.046). sEPCR did not track with response. Conclusion: These results demonstrate that although MMF and CYC represent therapies which control immediate injury, MMF but not CYC may protect the vasculature, thereby attenuating the overall burden of disease.

PO1.B.33

Clinical significance of CD40 ligand in systemic lupus erythematosus (SLE)

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Objective: CD40/CD40L interactions are important in SLE pathogenesis by inducing T-cell mediated humoral immune responses. The aim of this study is to evaluate the role of CD40L as a potential biomarker of SLE disease activity by correlating levels of this cytokine with clinical severity, cardiovascular risk factors and established markers of SLE activity. Methods: Twenty-five subjects with stable or disease exacerbation, ages 22-62 yrs, 2 males and 23 females, on various doses of steroids were evaluated in the Rheumatology clinic. The SLE Disease Activity Index (SLEDAI) was used to identify disease exacerbation that ranged from mild (2) to moderately severe (20), along with ANA, dsDNA, complement, urinalysis, urine protein-creatinine ratio and hematocrit levels. 13 of the 25 subjects were randomly selected and serum CD40L concentration via ELISA was measured and cardiovascular risk factors were assessed. Renal and hepatic profiles of these subjects were evaluated. All of the study subjects were on hydroxychloroquine. Results: sCD40L levels ranged from 0.26 to 3.9 ng/ml. Regression analyses using linear correlation were prepared. sCD40L level was lower in 4 patients with SLE flare (high SLEDAI score) and was higher in 6 patients with stable SLE (low SLEDAI score) (p = 0.014, r2 = 0.55). Mean steroid dosages in flare vs. stable groups were 27 mg and 6 mg, respectively. In the 13 selected patients, 3 of 8 in the stable group versus all 5 patients in the flare group had cardiovascular risks.

Disease category (1) Stable (2) Flare

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</tbody>
</table>

Conclusion: sCD40L plays a biologically active role in SLE with flares. Increased levels of sCD40L in serum and tissue are important in SLE pathogenesis. sCD40L concentrations correlated with subjects’ cardiovascular risk profile, however corticosteroids in these patients can mask the secretion of sCD40L. Levels of sCD40L correlated with established markers for SLE flare (dsDNA, complement levels) in an inverse manner.

PO1.B.34

Oxidative stress markers and their correlation with severity of systemic lupus erythematosus

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Objective: Oxidative stress has been implicated as a contributing factor in various autoimmune diseases (ADs) including systemic lupus erythematosus (SLE). However, potential of oxidative stress in eliciting an autoimmune response, and its role in disease prognosis and pathogenesis in humans remains largely unexplored. This study investigates the status and contribution of oxidative stress in SLE. Methods: Sera from 72 SLE patients with various SLE scores (SLE disease activity index; SLEDAI) and 36 age- and gender-matched healthy controls were evaluated for oxidative stress markers, such as malondialdehyde (MDA)- and 4-hydroxynonenal (HNE)-protein adducts and their corresponding antibodies (anti-MDA- and anti-HNE-protein adduct antibodies), superoxide dismutase (SOD), and various autoantibodies, including ANA, anti-dsDNA, anti-Sm, anti-RNP and scL. Results: Serum analysis showed significantly higher levels of MDA- and HNE-protein adducts and their corresponding antibodies, i.e., anti-MDA-and anti-HNE-protein adduct antibodies in SLE patients. Interestingly, our data showed not only increased number of subjects positive for anti-MDA- or anti-HNE antibodies and SLEDAI (r = 0.734 and 0.647 for anti-MDA and anti-HNE antibodies, respectively) suggesting a possible causal relationship.
between these antibodies and SLE. Serum SOD was significantly lower in SLE patients with higher SLEDAI group (≥ 6) showing much lower SOD levels, suggesting a compromised antioxidant balance. Sera from SLE patients also had higher levels of various autoantibodies, including ANA, anti-dsDNA, anti-Sm, anti-RNP and aCL. Conclusion: Our findings support an association between oxidative stress and SLE. Stronger response in samples with higher SLEDAI suggests that oxidative stress markers may be useful in evaluating the prognosis of SLE as well as in elucidating the mechanisms of disease pathogenesis.

PO1.C.1

Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus: prevalence, clinical manifestations and renal functional outcome

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Background: The presence of antiphospholipid antibodies (aPL) has been associated with small vessel renal injury and chronic renal ischemia. The renal histologic findings associated with antiphospholipid syndrome (APS) include thrombotic microangiopathy (TMA), fibrous intimal hyperplasia (FHI), fibrocellular and fibrous arteriolar and arterial occlusions (FAO), focal cortical atrophy (FCA) and tubular thyroidization (TT). The common clinical manifestations are hypertension, acute renal failure or chronic low-grade proteinuria. Although it has been suggested the term “associated nephropathy aPL”, renal involvement has not yet included within the classification criteria of APS. Objectives: To determine the prevalence, clinical manifestations and renal functional outcome of patients with lupus nephritis and associated APS nephropathy. Methods: Inclusions criteria: a SLE diagnosis of (ACR criteria) nephritis requiring renal biopsy. Patients with systemic sclerosis, hemolytic-uremic syndrome, systemic vasculitis, thrombotic thrombocytopenic purpura, diabetic nephropathy and preeclampsia were excluded. The histological samples were all analyzed by the same pathologist. Results: Seventy-nine biopsies were included (70 female [88.6%]). The mean age at time of biopsy was 33.3 ± 16.6 years. The follow-up period (time between the renal biopsy and the last medical visit) was 73 ± 51 months. Nine (11.4%) renal biopsies met diagnostic criteria for APS nephropathy. Three (33.3%) showed acute APS nephropathy. Histological lesions found: 3 (3.8%) cases of TMA, 4 (5%) of FHI, and 3 (3.8%) of FCA. The SLE patients with APS nephropathy showed higher prevalence of APS nephropathy (p<0.001). There was no association between APS nephropathy and the presence of hypertension, nephrotic syndrome, hematuria, proteinuria or elevated serum creatinine levels. No significant difference in complete renal response, partial renal response and no response between groups were found. Patients with TMA showed higher prevalence of APS (p<0.001), extrarenal arterial thrombosis (p=0.02) and venous thrombosis (p=0.02) along their evolution Conclusion: The prevalence of APS nephropathy was 11.4%. There was no association between APS nephropathy and clinical manifestations or laboratory features except for increased development risk of APS and arterial and venous thrombosis. The long-term renal outcome was similar in SLE patients with or without APS nephropathy.

PO1.C.2

Childhood systemic lupus erythematosus (SLE): analysis of clinical and immunological findings in 74 patients

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Tehran University, Tehran, Iran

Objective: The aim of this report is to describe the first instance of the clinical features of childhood lupus erythematosus. To define the pattern of disease expression in patients with childhood onset (SLE). Material And Methods: We studied prospectively 74 patients with childhood-SLE who were seen con-
secutorily either as inpatients or outpatients between 2000 and 2008. All the patients fulfilled the 1982 (ACR) revised criteria for SLE and had the disease at or before the age of 16 years. In 74 patients, defined as the initial manifestation clearly attributable to SLE, occurred before the age of 16, and they represent the childhood onset group described in this report. Results: A fifteen-year retrospective analysis of the clinical features and survival of 74 Iranian children with (SLE) was made. Sixty five (88%) patients from the childhood onset group were female and nine male (12%) (ratio female/male, 7/1). Range of age at onset was 3-16 years (Mean age 10 ± 2.2). During the evolution of the disease, the childhood onset patients had the mode of presentation was as follows: 74% had skin involvement, 77% had musculoskeletal involvement, 43% had renal disease, 33% had hematological abnormalities, 24% had pulmonary involvement, 17% had central nervous system involvement, and 16% had cardiovascular disease. Anemia in 59% of patients. Autoimmune thrombocytopenia purpura in 45% cases, Leukopenia with lymphopenia was the presenting feature in 16% cases. ESR >85 in 78% cases, and positive (C-reactive protein) in 59% patients. Hematurnia was the most frequent finding in these patients (47%). Proteimuria was the second finding in our patients (43%). Raised BUN and creatinine was seen in (21%). The Coombs’ test was positive in 21% children, false positive VDRL in 16% patients with childhood-SLE. ANA positivity was detected in 97% of cases at presentation; the mean titer was >1:160 in all patients except 2 cases. All 2 children who were ANA-negative had at least a malar rash, oral ulcer, and associated with several mild manifestations. Anti-d DNA was positive in 83% patients. Antiphospholipid antibody was in 13% patients. 10% of patients with SLE will be anti-Sm positive, low C3 (85%), low C4 (41%), and low CH50 complement (85%). Conclusions: Childhood-SLE is not a common illness in the pediatric population. Although Childhood-SLE has been reported in children in first the 10 to 20 years of life, it is rare in children under 5 years of age, childhood onset patients as presenting clinical manifestations, while malar rash, photosensitivity, musculoskeletal involvement, hematological abnormalities, and renal disease were more common during the evolution of the disease.

PO1.C.3
Factors predictive of thrombosis in a multiethnic cohort of SLE patients
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Purpose: Thrombosis is an important cause of morbidity and mortality in SLE and it occurs frequently at a younger age than in the general population. We have now explored the factors associated with time to the occurrence of thrombotic events in SLE patients to expand previous observations made on this cohort (larger number of patients and years of follow up). Method: SLE patients (ACR criteria), age ≥16 years, disease duration ≤5 years (T0), African-American, Hispanic (Texan or Puerto Rican) or Caucasian ethnicity, from a longitudinal cohort were studied. An event was defined as the presence of thrombosis in either an arterial or a venous territory. Time to the first thrombotic event was examined by multivariable (MV) Cox models (full and parsimonious) adjusting for pertinent baseline clinically and sociodemographic variables. Results: A total of 643 patients were studied, with a mean (SD) age of 36.4 (12.6) and time of SLE at enrollment of 1.4 (1.3) years, 90% were female and all ethnic groups were represented (Hispanic-Texas: 19%, Hispanic Puerto Rican: 16%, African American: 37%, Caucasian: 28%). At baseline, 80% of the patients had health insurance, 14% were smokers, and 33% were below the poverty line; 72% were hydroxychloroquine users, 3% had diabetes, 36% had hypertension, 3% antiphospholipid antibodies and 27% anti-DNA antibodies. Sixty five (13.4%) patients developed a thrombotic event [stroke: 7.5%, claudication: 0.6%, myocardium infarction: 2.3%, an-gina: 2.8%, and deep venous thrombosis: 1.7%]. The parsimonious Cox model is shown in Table 1. Conclusion: As expected, age, poverty, smoking, damage accrual, antiphospholipid antibodies and higher doses of glucocorticoids were independently associated with a shorter time to the first thrombotic event. Health insurance seems to have a protective effect. Modifiable risk factors at the personal (smoking, high doses glucocorticoids) or societal (poverty, health insurance) should be acted upon to prevent these events and improve the outcome of our lupus patients.

Table 1. Cox Model for Baseline Variables Predictive of a Thrombotic Event

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.03 - 1.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Health insurance</td>
<td>0.52</td>
<td>0.29 - 0.93</td>
<td>0.0271</td>
</tr>
<tr>
<td>Poverty*</td>
<td>1.60</td>
<td>0.95 - 2.70</td>
<td>0.0778</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.89</td>
<td>1.03 - 3.49</td>
<td>0.0409</td>
</tr>
<tr>
<td>SDI‡</td>
<td>1.65</td>
<td>1.41 - 1.93</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Antiphospholipid antibodies‡</td>
<td>2.06</td>
<td>1.16 - 3.69</td>
<td>0.0144</td>
</tr>
<tr>
<td>Anti-DNA antibodies</td>
<td>0.55</td>
<td>0.30 - 0.92</td>
<td>0.0589</td>
</tr>
<tr>
<td>Glucocorticoid, maximum dose</td>
<td>1.01</td>
<td>1.01 - 1.02</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*As per the US Federal government guidelines adjusted for the number of persons in the household; † SDI, SLICC (Systemic Lupus International Collaborating Clinics) damage index; ‡ IgG and/or IgM antiphospholipid and lupus anticoagulant.

PO1.C.4
Subclinical abnormalities in echocardiography and electrocardiography in patients with systemic lupus erythematosus
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Hospital civil de Guadalajara, Guadalajara, Mexico

Introduction: In patients with systemic lupus erythematosus (SLE), the cardiovascular system is frequently affected, and represents an important factor in determining their morbidity and prognosis. Objective: To describe the echocardiographic and electrocardiographic findings in patients with SLE without cardiovascular symptoms. Method: In a cross-sectional study of 30 patients with SLE and 10 controls a M-mode echocardiography, Doppler and electrocardiography (EKG) was performed. The statistical analysis was conducted using non-paired t Student, Chi-square, Fisher exact test for small samples and test of correlation with Spearman’s rho test. Results: Compared with the control group, patients with SLE had a higher prevalence of echocardiographic abnormalities and in the EKG. Percardial effusion in 37%, concentric left ventricular hypertrophy 10% (3 vs 1 p = 0.03), dilatation of right cavities 17%, dilated left cavities 3%, regurgitation: 20% mitral, tricuspid 35%, aortic 7% and pulmonary 10%. The ejection fraction was abnormal in 13% and diastolic dysfunction in 10% of the patients. 67% of cases had pulmonary hypertension (20 vs 1 p = 0.005). Sinus tachycardia was observed in 20% and 17% had left bundle branch hemiblock. Conclusion: We found a high prevalence of cardiac abnormalities, mainly of the pericardium, valvular and pulmonary vasculature in patients with SLE. Echocardiography is a sensitive method to detect these abnormalities and should be used routinely for evaluation of these patients.

Keywords: Echocardiography, systemic lupus erythematosus, cardiac abnormalities

PO1.C.5
Sensitivity and specificity of pleural fluid antinuclear antibodies in lupus pleuritis
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Chiang Mai University, Chiang Mai, Thailand

Introduction: serum antinuclear antibody (ANA) has long been serology test used for diagnosis of SLE. Pleural fluid ANA was considered to be used in diagnosis lupus pleuritis. Objectives: To determine sensitivity and specificity of pleural fluid ANA titer ≥1:160 and ratio of pleural fluid to serum ANA ≥1 in order to distinguish lupus pleuritis from other etiologies. Patients and

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Activity decreased to 62.5% but the specificities did not change. Using ratio of pleural fluid to serum ANA ≥1, the sensitivity was 90.91% when compared with exudates effusion group (para-pneumonic effusion group).

Interchangeably for follow-up proteinuria in patients with lupus nephritis. According to high correlation with the 24-hUP, clinicians should consider the quantitative 24-hUP collection for the diagnostic evaluation of lupus nephritis. Active inflammatory and/ or proliferative lesions were found in renal biopsies from 10 patients with class II LN, 2 with class IV LN, and 4 with class V LN. Activity and chronicity index scores were calculated using the scoring systems of the US National Institutes of Health.

Conclusion: The finding of a defective GRD obtained in these SLE clinical cases, focus on a cerebral functional abnormality whose relationship with the primary disease and with the cognitive disturbance is to be clarified. The impaired cerebral GABA-A receptor binding represents a previously unreported finding, which might be associated with CNS involvement in the course of SLE and induced by no obvious pathogenetic factors, as the disease-dependent vasculopathy, the presence of specific autoantibodies, a drug-induced receptor modulation, or, alternatively, an independent neuro-pathological status. Even though the series studied is small, the observation reported here represents a new finding open for further investigations in order to better assess the link between the imaging abnormality, NP manifestations, CNS involvement, and SLE.

PO1.C.7


Vacc, Alessandra1 Mathieu, Alessandro1 Serra, Alessandra2 Cauil, Alberto1 Piga, Matteo1 Porru, Giovanni1 Marrosu, Francesco1 Sanna, Giovanni1 Piga, Mario2

1. Rheumatology Unit, A.O.U of Cagliari, Monserrato, Italy; 2. Chair of Nuclear Medicine, Department of Medical Sciences, A.O.U of Cagliari, Monserrato, Italy; 3. Department of Neurological and Cardiovascular Sciences, A.O.U of Cagliari, Monserrato, Italy; 4. Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, London, UK

Objectives: Gamma-aminobutyric acid-A (GABA-A) receptors play a crucial role in regulating neuronal excitability and cognitive functions. SPECT analysis of GABA-A receptors binding by 123I-labeled Iomazenil (123-I-IMZ) has been applied in some neuropsychiatric (NP) disorders to investigate conditions where GABA-A receptor density (GRD) can be detected several pathophysiological conditions. In this study we investigate cerebral GRD in a small series of patients with systemic lupus erythematosus (SLE) and cognitive impairment characterized by recurrent, episodic memory loss.

Methods: Nine female patients with SLE and cognitive alterations underwent to a clinical neuropsychiatric evaluation including digital video-EEG, brain MRI, 99m-Tc-ECD brain SPECT and 123-I-IMZ brain SPECT.

Results: All the patients tested showed diffuse or focal reduced expression of rGABA-A by 123-I-IMZ brain SPECT, and most of them revealed neither EEG nor cerebral MRI abnormalities.

Conclusions: The finding of a defective GRD obtained in these SLE clinical cases, focus on a cerebral functional abnormality whose relationship with the primary disease and with the cognitive disturbance is to be clarified. The impaired cerebral GABA-A receptor binding represents a previously unreported finding, which might be associated with CNS involvement in the course of SLE and induced by no obvious pathogenetic factors, as the disease-dependent vasculopathy, the presence of specific autoantibodies, a drug-induced receptor modulation, or, alternatively, an independent neuro-pathological status. Even though the series studied is small, the observation reported here represents a new finding open for further investigations in order to better assess the link between the imaging abnormality, NP manifestations, CNS involvement, and SLE.

PO1.C.8

Low-level proteinuria does not preclude significant renal pathology in lupus nephritis.

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Objective: Diagnostic delay increases the risk of a poor renal outcome in lupus nephritis (LN). Patients with systemic lupus erythematosus (SLE) and suspected LN are typically referred to a diagnostic renal biopsy after detection of significant proteinuria. The aim of the present study was to describe renal histological findings in a group of LN patients with low-level proteinuria at time of first renal biopsy.

Methods: Patients with urinary protein excretion <1000 mg/day at time of first renal biopsy were selected from a cohort of 100 Danish LN patients. Renal biopsy findings were classified according to the WHO criteria for LN. Activity and chronicity index scores were calculated using the scoring systems of the US National Institutes of Health.

Results: In total, 13 patients (13%) displayed low-level proteinuria at time of first renal biopsy (median level of proteinuria: 600 mg/day; range: 0-900 mg/day). Three of these patients presented with hypertension. An elevated s-creatinine concentration was found in 4 patients, while 5 patients had haematuria and/or urinary cellular casts. One patient was diagnosed with WHO class I LN, 6 with class II LN, 2 with class IV LN, and 4 with class V LN. Active inflammatory and/or proliferative lesions were found in renal biopsies from 10 patients (median activity index score: 2.0; range: 0-9). Chronic renal damage.
was observed in biopsy specimens from 9 patients (median chronicity index score: 2.0; range: 0-6). Among patients with proteinuria ≤500 mg/day (n=5), 4 patients had class II LN, and 1 patient had class V LN. Four out of five patients with proteinuria ≤500 mg/day presented with chronic renal lesions (median chronicity index score: 3.0; range: 0-6). Conclusions: These findings confirm that low-level proteinuria can be associated with significant renal pathology in LN, including membranous and proliferative nephritis. Our observations underscore the need for sensitive and specific methods for early detection of renal inflammation in SLE patients.

POI.C.9
Depression is a risk factor for subclinical atherosclerosis in SLE
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Purpose: Women with SLE have increased rates of subclinical atherosclerosis compared to controls, as measured by coronary artery calcium (CAC) and carotid plaque. Women with SLE also exhibit higher prevalence of depression and depressive symptoms than controls. Although depression and other psychological factors have been linked to atherosclerosis in healthy women, their associations in women with SLE remain largely unexplored. The purpose of this study was to evaluate biological and psychological risk factors associated with subclinical atherosclerosis in women with SLE, as defined by presence of coronary artery calcium and/or carotid plaque.

Method: In this cross-sectional study, 161 women with SLE without prior history of cardiac events completed comprehensive cardiovascular risk factor assessments, SLE disease activity assessments, and the Center for Epidemiologic Studies Depression Scale (CES-D). Participants also completed an electron-beam computed tomography scan of the coronary arteries to determine the presence of CAC, and carotid artery ultrasound to detect presence of plaque. Subclinical atherosclerosis was defined as having either or both of these vascular indicators. In unadjusted logistic regression analyses, risk factors associated with subclinical atherosclerosis at p<0.15 were evaluated for inclusion in multivariable models. The final model was selected based on Akaike’s Information Criteria. Results: The mean age of the participants was 50 years, and 88% were Caucasian. Mean SLE duration was 16 years. Most (68%) had taken corticosteroids, with median duration of 10 years of use. The mean CES-D score was 11.6 (SD=9), with 27% of the participants scoring ≥16, a score consistent with minimal depression. Objectives: To evaluate potential risk factors for subclinical atherosclerosis in SLE patients. Methods: Clinical/ laboratory features of 1,200 SLE patients (ACR Criteria) followed at the Lupus Clinic of Rheumatology Division were obtained from the electronic register database from 2001 to 2009. Pulmonary TB was diagnosed in 20 patients (1.7%) [TB+ group]. As control group [TB- group], were arbitrarily selected 40 patients without TB matched for age, gender, age at SLE diagnosis and disease duration. Results: All 20 patients of TB+ group presented confirmed pulmonary TB from 1 to 23 years after SLE diagnosis (7.6 ± 8.1), and none of them had TB before SLE diagnosis. Radiologic evaluation revealed that nonmiliary infiltrates was the predominant form in 16 patients (80%), followed by miliary infiltrates in 3 (15%), and pleural effusion in one (5%). The apparent higher frequency of previous exposure to TB contact in TB+ group than TB- group did not reach statistical significance (20% vs. 5%; p=0.180). Frequencies of previous SLE involvements (cutaneous, articular, hematologic, renal, pericarditis and central nervous system) were alike in TB+ and TB- groups (p=0.05). In contrast, prior pleuritis was more frequently observed in TB+ group (40% vs. 5%, p=0.001), and the risk of pulmonary TB had an odds ratio (OR) of 12.74, 95% CI (Confidence Interval) 2.37-68.53. On the contrary, pneumonitis had similar frequency in both groups (p=0.107). No differences in the frequencies of anti-dsDNA, anti-Sm, anti-Ro, anti-La and antiphospholipid antibodies were observed in patients with or without TB (p>0.05). Immunosuppressive and corticosteroid (including daily dose of prednisone) therapies at the moment of TB diagnosis were also similar in both groups (p=0.05). Conclusions: Our study has identified pleuritis as the major risk factor for pulmonary TB in SLE, reinforcing the need of a careful surveillance of this subgroup of patients.

Table 1. Multivariable logistic regression analysis of risk factors for subclinical atherosclerosis in women with SLE (N=161).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.11</td>
<td>(1.00-1.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Years of Education</td>
<td>0.82</td>
<td>(0.68-0.99)</td>
<td>0.037</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.50</td>
<td>(1.50-4.66)</td>
<td>0.028</td>
</tr>
<tr>
<td>Waist-Hip Ratio*</td>
<td>1.11</td>
<td>(0.77-1.63)</td>
<td>0.867</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>1.11</td>
<td>(0.32-3.94)</td>
<td>0.867</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>2.00</td>
<td>(0.73-6.93)</td>
<td>0.422</td>
</tr>
<tr>
<td>4th quartile</td>
<td>1.11</td>
<td>(1.12-4.49)</td>
<td>0.032</td>
</tr>
<tr>
<td>CRP</td>
<td>1.12</td>
<td>(1.01-1.23)</td>
<td>0.029</td>
</tr>
<tr>
<td>Depression</td>
<td>3.85</td>
<td>(1.37-10.87)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*Reference Group = 1st quartile of waist-hip ratio.

POI.C.10
Pleuritis as the major risk factor for pulmonary tuberculosis in systemic lupus erythematosus (SLE)
Pasoto, Sandra G.; Shinjo, Samuel K.; Borba, EDUARDO F.; Bonfa, ELOISA
Reumatology Division, Faculdade de Medicina da USP, São Paulo, Brazil

Introduction: A high prevalence of tuberculosis (TB) is observed in SLE, but there are scarce studies in literature addressing the predisposing factors for pulmonary TB in this disease. Objective: To evaluate potential risk factors for pulmonary TB in SLE patients. Methods: Clinical/ laboratory features of 1,200 SLE patients (ACR Criteria) followed at the Lupus Clinic of Rheumatology Division were obtained from the electronic register database from 2001 to 2009. Pulmonary TB was diagnosed in 20 patients (1.7%) [TB+ group]. As control group [TB- group], were arbitrarily selected 40 patients without TB matched for age, gender, age at SLE diagnosis and disease duration. Results: All 20 patients of TB+ group presented confirmed pulmonary TB from 1 to 23 years after SLE diagnosis (7.6 ± 8.1), and none of them had TB before SLE diagnosis. Radiologic evaluation revealed that nonmiliary infiltrates was the predominant form in 16 patients (80%), followed by miliary infiltrates in 3 (15%), and pleural effusion in one (5%). The apparent higher frequency of previous exposure to TB contact in TB+ group than TB- group did not reach statistical significance (20% vs. 5%; p=0.180). Frequencies of previous SLE involvements (cutaneous, articular, hematologic, renal, pericarditis and central nervous system) were alike in TB+ and TB- groups (p=0.05). In contrast, prior pleuritis was more frequently observed in TB+ group (40% vs. 5%, p=0.001), and the risk of pulmonary TB had an odds ratio (OR) of 12.74, 95% CI (Confidence Interval) 2.37-68.53. On the contrary, pneumonitis had similar frequency in both groups (p=0.107). No differences in the frequencies of anti-dsDNA, anti-Sm, anti-Ro, anti-La and antiphospholipid antibodies were observed in patients with or without TB (p>0.05). Immunosuppressive and corticosteroid (including daily dose of prednisone) therapies at the moment of TB diagnosis were also similar in both groups (p>0.05). Conclusions: Our study has identified pleuritis as the major risk factor for pulmonary TB in SLE, reinforcing the need of a careful surveillance of this subgroup of patients.

POI.C.11
Multiple renal artery aneurysms in a patient with systemic lupus erythematosus
Fano, Patricia1 Rubio, Tomas2 Jimenez, Fernan1 Arteaga, Jesus3 Arnaez, Ruben1 Perez, Carlos1
1. Virgen del Camino Hospital, Pamplona, Spain; 2. Hospital de Navarra, Pamplona, Spain

Objectives: Vasculitis is a known complication of patients with systemic lupus erythematosus (SLE). Inflammation of the vessels can result in the development of arterial aneurysms. Renal artery vasculitis is rare in patients with SLE, and documented only in a few case reports. We report an SLE patient who presented with severe hypertension. Multiple aneurysms of the right renal artery were identified and treated with endovascular embolization.

Methods: A case was investigated retrospectively and literature was reviewed.

Results: A 29-year-old woman presented with a 4-week history of asthenia, anorexia, low grade fever, edema, weight loss, arthralgia, and hypertension. On physical examination we observed elevated blood pressure (200/120 mm Hg). Oral aphthous ulcerations, elevated central venous pressure, tachycardia, and leg oedema were noted. Laboratory tests revealed: impaired renal function (creatinine 1.3 mg/dl), normocytic anemia (haemoglobin 10.2 g/dl), lymphopenia, positive Coombs’ test, a high erythrocyte sedimentation rate, and C-reactive protein level, and low albumin (2.7 g/dl). Complement C3 and C4 were low. Urine analysis revealed many erythrocytes, and 1.5 g protein excretion/24 hours. Antinuclear antibodies were positive. Testing for double-stranded DNA antibody was positive. Positive cryoglobulinemia was detected. Anti-phospholipid tests were negative. Other serologic tests for autoimmune disorders, and common viral and bacterial infections were negative or normal. A transthoracic echocardiogram was normal. Histologic examination of...
POI.C.12

Prospective analysis of neuropsychiatric events in an international disease inception cohort of SLE patients

Handy, JG; Urowitz, MB; Su, L; Bae, S-C; Gordon, C; Wallace, D; Clarke, A; Bernatky, S; Isenberg, D; Rahman, A; Aralcis, GS; Gladman, D; Fortin, P; Sanchez-Guerrero, J; Romero-Diaz, J; Merrill, JT; Vasudevan, A; Bruce, I; Steission, K; Khamashta, M; Petrini, M; Munzi, S; Dooley, MA; Ramsey-Goldman, R; Van Vollenhoven, R; Nived, O; Sturfelt, G; Iavazzon, C; Kalunian, K; Ramos-Casals, M; Zoma, A; Douglas, J; Thompson, K; Farewell, V

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Objective: To determine the frequency, accrual, attribution and outcome of neuropsychiatric (NP) events and the impact on health-related quality of life over 3 years in a large inception cohort of SLE patients. Methods: The study was conducted by an international research network. Patients were enrolled within 15 months of SLE diagnosis. NP events were identified using the American College of Rheumatology (ACR) case definitions, and decision rules were derived to determine the proportion of NP events attributable to SLE and non-SLE causes. Physician assessment of outcome of NP events was recorded using a 7-point Likert scale (patient demise; much worse; worse; no change; improved; much improved; resolved), and patient perceived impact was determined by the mental component summary (MCS) score and physical component summary (PCS) score of the SF-36. Statistical analysis included Cox regression for examining the time to case resolution for NP events, multi-level ordinal regression for examining the association between explanatory variables and the probability of more favourable Likert outcome scores of NP events, as well as linear regression for SF-36 analyses. All analyses were adjusted for the correlation of multiple observations from the same patient.

Results: There were 1206 patients (89.6% female) with a mean (SD) age of 34.5 (13.2) years. The mean disease duration at enrollment was 5.4 (4.2) months. Over a mean follow-up of 1.9 (1.2) years 486/1206 (40.3%) patients had one or more NP events. Eighteen of the 19 ACR NP case definitions were identified and the frequency of individual NP events varied from 47.1% (headache) to 0% (myasthenia gravis). NP events were attributed to SLE in 13.0% of patients (17.7 – 30.6% of NP events) using two a priori decision rules. The most frequent NP events attributed to SLE were seizures, mood disorders, cerebrovascular disease and acute confusional states. The outcome was significantly better for those NP events attributed to SLE (p<0.001), especially if they occurred within 1.5 years of the diagnosis of SLE. Patients with NP events, regardless of attribution, had significantly lower SF-36 summary scores for both mental and physical health over the study compared to those without NP events (estimate for MCS scores -9.7, p<0.001; estimate for PCS scores -3.3, p<0.001). There were 18/1206 (1.5%) deaths and in 4/18 (22.2%) the primary cause was attributed to NP events (intracranial hemorrhage [2], stroke [1], seizures [1]). Conclusion: NP events in SLE patients are variable in frequency, commonly present early in the disease course and adversely impact patients’ quality of life over time. Events attributed to non-SLE causes are more common than those due to SLE, although the latter have a more favourable outcome.

POI.C.13

Lupus nephritis: a 33-year experience

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Objective: Patients with Lupus nephritis as a serious manifestation of Systemic Lupus Erythematosus (SLE), may express increased frequency of other sever lupus manifestations. The aim of this study was to compare the clinical and paraclinical manifestations of patients with and without lupus nephritis. Method: We used the electronic database of Rheumatology Research Center (RRC), which registered clinical and paraclinical manifestations of 2200 SLE patients during 1976–2009. Chi Square test was used to compare the two groups. Odds ratio and 95% Confidence Interval (CI) was used to present the strength of association (p-value<0.05). Results: Among 2200 lupus patients, 1468 patients (66.7%, 95% CI: 64.7-68.7) had lupus nephritis. Statistical analysis showed that constitutional manifestations, pulmonary involvement, cardiac involvement, neuropsychiatric manifestations, lymphopenia, thrombocytopenia, positive anti-dsDNA antibody, and low C3 were significantly higher, and discoid lesion was significantly lower in patients with renal involvement.

POI.C.14

Evans’ syndrome and systemic lupus erythematous. An analysis of clinical presentation and outcome of 20 cases

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Evans’ syndrome (ES) is a rare disease characterized by the simultaneous or sequential development of autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP). It is a condition with unknown aetiology that results from an alteration of the immune system that produces multiple autoantibodies targeting red blood cells and platelets. This disease can be associated with an...
underlying disorder such as lymphoproliferative disorders, common variable immunodeficiency and systemic lupus erythematosus (SLE). We describe the characteristics and outcome of ES in 20 patients with SLE. The data from these patients all of them women, 13 white and 7 non-white, fulfilling strict inclusion criteria for ES are reported. The mean age at time of Evans’ syndrome onset varied from 14 to 50 years. Four patients from 18 studies were under 16 years at onset of ES. All patients presented Evans’ syndrome at onset of disease, both cytopenias occurred simultaneously in all patients. Although corticosteroids and/or intravenous immunoglobulin (IVIG) are commonly used in its treatment, no standard strategy has been established. All patients were given corticosteroids (oral in all and pulse in 5), but 10 of them (50%) required at least one other drug, including azathioprine (n=8), intravenous cyclophosphamide (n=4) and rituximab (n=1). Some patients received more than one drug. Splenectomy and intravenous immunoglobulin were not used. At time of analysis, 19 patients (95%) were in remission of treatment; 1 patient with severe systemic lupus (5%) had died. These data suggests that Evans’ syndrome in SLE can have a good prognosis with a good response to treatment.

PO1.C.16

Diffuse alveolar hemorrhage in systemic lupus erythematosus (DAH) in patients with systemic lupus erythematosus (SLE) and to determine risk factors and clinical outcomes of DAH in SLE patients. Methods: Among the 1521 SLE patients admitted between January 1993 to June 2009 to affiliated hospitals of Catholic University of Korea, 21 SLE patients were admitted for DAH. The inclusion criteria for DAH was defined as new infiltrates on chest radiographs, an acute hemoglobin drop of at least 1.5 g/dL in the absence of obvious source of bleeding, and one or more of the following signs: hemoptysis, hypoxemia, bronchoscopic or biopsy evidence of DAH. 83 SLE patients matched for age and sex who were admitted for other manifestations, were included as disease controls. Data based on medical records were analyzed retrospectively. Results: There were no significantly differing demographic characteristics between SLE patients with DAH and those with other manifestations. Multivariate analysis demonstrated coexisting neuropsychiatric lupus (P=0.002) and high SLE disease activity index scores (SLEDAI > 10) as independent risk factors in the development of DAH (P=0.029). Among the 21 SLE patients with DAH, 13 died during the admission period (in-hospital mortality rate:61.9%). Mortality was associated with infection and requirements of mechanical ventilation. Conclusions: Collectively, SLE patients who have neuropsychiatric manifestations or are in the active stage of the disease have an increased risk for developing DAH. Due to the high mortality of SLE patients with DAH, early recognition of risk factors and appropriate intervention is essential.

PO1.C.17

The relationships between serum vitamin D levels, vitamin D receptor polymorphism, anti-vitamin D autoantibodies, IL-17 and IL-23 levels, and clinical parameters in SLE patients. Wozniacka, Anna1 McCauliffe, Daniel P2 Bogaczewicz, Jaroslaw1 Lukaszkiewicz, Jacek1 Kalota, Beata1 Sysa-Jedrzejowska, Anna1 1. Department of Dermatology and Venereology, Medical University of Lodz, Lodz, Poland; 2. Department of Dermatology, University of North Carolina, Chapel Hill, NC, USA; 3. Department of Pharmacology, Medical University, Warsaw, Poland

Objectives: To investigate relationships between vitamin D status, vitamin D receptor (VDR) gene start codon Fok I polymorphism, the presence of anti-vitamin D autoantibodies, interleukin-(IL)-17, IL-23 levels, and clinical parameters in patients with systemic lupus erythematosus (SLE). Methods: The study included 49 patients with SLE. Serum concentrations of 25(OH)D3 were measured with electrochemiluminescence immunoassay (ECLIA) in an automated analyzer (Elescsys 2010- under international control of Vitamin D External Quality Assessment Scheme -DEQAS). Fok I genotyping was performed based on real time polymerase chain reaction (RT-PCR), that identified FF, Ff and ff. In order to detect antibodies directed against 1,25(OH)2D3, and determine serum levels of IL-17 and IL-23 in SLE patients, enzyme-linked immunosorbent assays (ELISA) were employed. Results: The serum concentration of 25(OH)D3 in patients with SLE during summer time was 18.47±9.14 ng/mL, and was significantly decreased as compared with those of the control group (31.27±12.65 ng/mL) (p=0.005). During winter time a trend toward lower concentration of 25(OH)D3 in SLE patients was revealed, however it did not reach statistical significance in comparison to those of control (respectively, 11.71±7.21 ng/mL vs 16.01±8.46 ng/mL; p=0.054). 25(OH)D3 levels were significantly lower in SLE patients with renal disease or leucopenia as compared to SLE patients who did not have these manifestations (respectively, p=0.006 and p=0.047). Vitamin D deficiency in systemic lupus erythematosus was also associated with low interleukin-23 levels, but not lower interleukin-17 levels, anti-1,25(OH)2D3 autoantibodies or vitamin D receptor gene start codon Fok I polymorphism. Autoantibodies directed against 1,25(OH)2D3 were detected in 4 patients with SLE. No significant difference in 25(OH)D3 serum concentrations was found between SLE patients with and without these autoantibodies. Additionally, anti-1,25(OH)2D3 autoantibodies were not associated with clinical or laboratory findings including IL-17, and IL-23 levels. Serum concentrations of IL-23 were significantly lower in patients with vitamin D deficiency (p=0.037). The frequency of the VDR Fok I polymorphism in SLE patients was FF:9.52%; Ff:59.52%; ff:30.95%. No relationships were found between the Fok I polymorphism and the clinical and laboratory profiles of the SLE patients. Conclusions: Vitamin D deficiency in SLE patients, may have other causes besides that resulting from sun...
avoidance. From the presented findings, it is associated with renal disease, leucopenia, and low interleukin-23 levels, but not lower interleukin-17 levels, anti-I,-L(III)* antibodies or the vitamin D receptor gene start codon Fok I polymorphism. It is generally advisable to recommend supplemental vitamin D in SLE patients year round.

POL.C.18
Unusual presentations of antiphospholipid syndrome: report of three cases
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Objectives: Clinical presentation of antiphospholipid syndrome (APS) may vary widely. Therefore, its early detection requires a strong index of suspicion, especially when thrombosis occurs at unusual sites or non-specific symptoms predominate in early stages. We report three cases of APS with unusual clinical presentations. Methods: Three cases were investigated retrospectively and literature was reviewed. Results: Case 1: A 32-year-old woman presented during her 6th month of pregnancy with severe depression, anxiety, and hypertension. At 32 weeks of gestation foetal ultrasound disclosed signs of intratubal growth retardation. Therefore, the patient underwent caesarean section, which resulted in a live birth. Autopsy of placenta showed widespread infarctions. MR imaging of the brain revealed multiple small foci of increased T2 signal intensity in the periventricular and deep white matter, consistent with microvascular ischemic lesions. High titres of lupus anticoagulant and anticardiolipin (IgM and IgG) and antibeta-2-glycoprotein I (IgM and IgG) antibodies, were observed. Laboratory tests showed low titre of anti-nuclear antibody (ANA) positivity. Other serologic tests for autoimmune disorders were negative. The patient received therapeutic anticoagulation during puerperium, and a progressive recovery of the psychiatric disorder was achieved. Case 2: A 26-years-old man presented with recurrent episodes of transient visual loss and blurred vision in both eyes. He has a medical history of an episode of loss of vision in the right eye one year previously. Funduscopy disclosed a normal right eye and an ischemic area caused by an arterial obstruction in the superotemporal quadrant of the left eye, that was confirmed by fluorescein angiography. High titres of lupus anticoagulant and anticardiolipin (IgM and IgG) and antibeta-2-glycoprotein I (IgM and IgG) antibodies, were observed. A cardiac evaluation was performed with echocardiography and carotid artery ultrasonography, and findings of both were normal. The patient received anticoagulant therapy. Case 3: A 46-year-old male presented with a 1-week with history of worsening of chronic renal insufficiency and pain in the left leg. Venous ultrasonography disclosed deep venous thrombosis. MR imaging of the aorta and abdominal arteries revealed occlusion of the left renal artery. High titres of lupus anticoagulant and anticardiolipin (IgM and IgG) and antibeta-2-glycoprotein I (IgM and IgG) antibodies, were observed. The patient received anticoagulant treatment. Conclusions: Physicians should consider the diagnosis APS in patients with clinical presentations such as psychiatric disorders and leukoencephalopathy during pregnancy, retinal vascular occlusions, and renal failure associated to deep venous thrombosis.

POL.C.19
Primary cardiac disease (PCD) in systemic lupus erythematosus (SLE) of Latin American patients. Data from the multinational inception GLADEL cohort.
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Background and Purpose: Cardiac involvement is one of the major concerns in the management of SLE patients. The aim of this study was to investigate the prevalence of PCD, associated factors and mortality in SLE using data from GLADEL, a multi-ethnic, multinational Latin American cohort. Methods: SLE patients with a recent SLE diagnosis (<2 years) were recruited and followed longitudinally. PCD was defined as the presence of pericarditis, myocarditis, endocarditis, arrhythmias and/or valve abnormalities related to SLE pathogenic mechanism. Socioeconomic-demographic, clinical, serologic and therapeutic variables were compared between patient with/without PCD. Those significant variables at p<0.20 in these UV analyses were included into logistic regression models with stepwise selection with PCD endpoint. Results: Of the 1437 GLADEL cohort patients included in this study, 202 (14%) developed PCD (pericarditis 164, myocarditis 7, endocarditis 1, arrhythmia 23 and valvulopathy 35). Delay to SLE diagnosis was shorter in PCD patients (median 4.0 vs. 6.1 months, p<0.0002). In the UV analysis PCD was statistically more frequent in patients of African-Latin American origin (ALA) vs. Caucasian (OR:1.8, CI:1.8-2.8) and in lower/upper/middle vs. upper/upper-middle economic status (OR:1.8, CI:1.01-3.21). PCD patients have less chance to have had skin disease previous to SLE diagnosis (OR:0.7, CI:0.46-0.99), but more chance to have had lung disease (OR:4.7, CI:2.45-9.20), previous PCD manifestations (OR:7.1, CI:4.98-10.4), anti-DNAs (OR:1.68, CI:1.05-2.7), anti-SSB/LA (OR:2.36, CI:1.27-4.39) or low C3 (OR:2.38, CI:1.43-3.95). PCD patients had more chance to present infections (OR:1.9, CI:1.39-2.53), hypertrichydermidemia (OR:2.2, CI:1.31-3.57), hypercholerolemia (OR:1.8. CI:1.52-2.68) during follow-up and more chance to receive prednisone in a medium (<20-60mg/day) or high dose (>60mg) (OR:4.41, CI:1.58-12.34 and OR:4.47, CI:1.60-12.49, respectively), intravenous cyclophosphamide (OR:2.77, CI:2.05-3.75) and hemodialysis (OR:5.1.4, CI:3.05-8.68). Patients with PCD had higher activity (SLEDAI) (median 12.0 vs. 10.5, p<0.0057) and damage score (SLICC/ACR) (1 vs. 0, p=0.0002) at SLE diagnosis and higher damage score at follow-up (2 vs. 1, p<0.0001). Patients with PCD were significantly associated with mortality (16.8% vs. 4.1%, p<0.0001). In the MV analysis the presence of PCD during follow-up was associated to ALA ethnicity vs. Caucasian (OR:2.22, CI:1.01-4.87), PCD previous to SLE diagnosis (OR:4.49, CI:2.63-9.48), hypertrichydermidemia (OR:1.82, CI:1.02-3.23), intravenous cyclophosphamide (OR:3.27, CI:1.81-5.91) and hemodialysis (OR:5.68, CI:2.25-14.32). On the contrary, renal disease previous to diagnosis of SLE decrease the probability of having PCD during follow-up (OR:0.33, CI:0.17-0.61). Conclusion: ALA ethnicity, as well as some clinical, serological and therapeutics variables were associated with PCD in Latin-American SLE patients. A higher damage index and mortality associated with PCD should remind us of the importance of early diagnosis and appropriate treatment.

POL.C.20
Pleuropulmonary compromise in systemic lupus erythematosus (SLE) of Latin American prospective inception cohort (GLADEL)
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Pulmonary involvement in systemic lupus erythematosus (SLE) is common in SLE with clinical manifestations ranging from a benign course to potentially catastrophic manifestations. Objective: The aim of this study was to investigate the prevalence of pleuropulmonary compromise, to analyze the socioeconomic-demographic, clinical and serological features, and to assess

Lupus
their influence on the prognosis, in terms of mortality in patients with SLE. Patients and Methods: SLE patients from 34 centers of 9 Latin-American countries (Argentina, Brazil, Chile, Colombia, Cuba, Guatemala, Mexico, Peru and Venezuela) with a recent SLE diagnosis (≥2 years) had been recruited and followed longitudinally. Patients were subdivided into those without- out pleuropulmonary (WPPC), with pleuropulmonary compromise (PPC) and with pulmonary compromise (PC). Odds ratio with 95% CI was used to measure the strength of association between variables. Kaplan Meier survival curve was examined. Results were confirmed by univariate (UV) and multivariate (MV) logistic regression analysis Results: Of the 1,480 included in GLADEL cohort, 90% were female. Median age (years) at onset was 26 and at diagnosis 27. The median time of follow up was 55 months. Two hundred ninety-six patients had PPC (20%), 244 pleurisy (17%) and 90 PC (6%). Of these 90 patients with PC, 28 had pulmonary hypertension (31%), 25 pneumonia (28%), 16 pulmonary fibrosis (18%), 14 pulmonary hemorrhage (16%), 12 thrombosis (13%), 9 shrinking lung (10%) and 3 had pulmonary infarction (3%). The UV analysis showed that PC was significantly associated with SLE hematological (8.0% vs. 2.2%, p=0.0001), cardiovascular (9.9% vs. 3.2%, p=0.0001), neurological (8.4% vs. 5.1%, p=0.02) and renal disease (7.4% vs. 4.6%, p=0.03). The MV analysis confirmed the association of PC with hematological (OR=3.18, CI 1.65-6.13) and cardiovascular (OR=2.81, CI 1.75-4.51) manifestations. The MV analysis showed that the presence of PC increased the risk of death by more than 5 times (OR: 5.25, CI 2.92-9.42). When different types of PC were evaluated, pulmonary hypertension (OR: 4.93, CI 1.96-12.42) and pulmonary hemorrhage (OR: 12.60, CI 3.79-41.85) increased significantly the mortality. The overall cumulative estimated probability of survival at 6 years was significantly lower in patients with PC (73 vs. 93%, p=0.001). Conclusion: The prevalence of PPC was less frequent than in the other groups. The PC showed a significantly associated with hematological and cardiovascular involvement. SLE patients with PC had poorer survival, being pulmonary hypertension and pulmonary hemorrhage the main causes of death.

PO1.C.21
Renal transplantation in patients with systemic lupus erythematosus: 22 years experience from a single centre
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Objectives: To determine the clinical characteristics and outcome of renal transplantation in patients with SLE from our center. Methods: To analyze SLE patients with renal failure due to lupus nephritis, who were treated with renal transplantation in our Hospital from January 1986 to December 2008. Results: 40 renal transplantations were performed in 29 SLE patients (24 females (83%), mean age at transplantation 33±8 years (range 17 – 53). In 20 cases, only one transplantation was performed. The analysis included only the first transplantations performed in our centre. In 27 patients (93%), we obtained pre-transplantation histological diagnosis (according to the WHO classification): 22 (76%) type IV, 2 (7%) type III, 2 (7%) type II and 1 (3%) type VI. Six patients (20%) were positive for antiphospholipid antibodies (APL). Positive serologies for hepatitis virus were detected in 10 patients (9 HCV, 1 HBV). Twenty-six patients were on haemodialysis in the pre-transplantation period and 6 were on to peritoneal dialysis. Twenty (69%) transplantations were from deceased donors and 9 from living donors. The mean time elapsed between the diagnosis of lupus nephritis and the start of dialysis was 43±40 months, the mean time on dialysis was 62±52 months and the time on follow-up was 73±67 months. In 18 (62%) patients renal biopsy was performed for impair- ment in the renal function. Recurrence of lupus nephritis in renal allograft and flare-ups of lupus activity were not observed in this study. The graft survival rates were 76% at 5 years, 69% at 10 years and 62% at the end of the study. The patient survival rate was 93% at the end of the study. Graft rejection occurred in a total of 11 patients, 6 out of the 9 (66%) positive for HCV and S out of the 20 (25%) negative for HCV (exact Fisher test p = 0.047; OR = 6; CI = 1.08 – 33.4). In one case, graft rejection occurred twice, both produced by thrombotic microangiopathy, although studies were persistently negative for APL. Prior to her third transplantation, APL were detected as positive, which indicated anticoagulation immediately after transplantation, with a good evo- lution of this new graft. In the re-transplanted group (9 patients) a total of 20 transplantations were performed. There was a deterioration of renal function with re-entry into dialysis in 13 of them, 7 of whom had positive serology for HCV. There were 2 deaths in this group. Conclusion: Renal transplantation is a good alternative for renal replacement therapy in patients with SLE, but the existence of a thrombotic disease associated with the APL or the coexistence of HCV infection are related with the development of graft rejection.

PO1.C.22
Antiphospholipid syndrome in male lupus patients
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Background: Antiphospholipid syndrome (APS) is characterized by vascular thrombosis, and/or pregnancy morbidity associated with anticoagulipin anti- bodies (aCL) and lupus anticoagulant (LAC). Incidence of APS and antiphos- pholipid antibodies (aPL) in men is not known. We analysed 146 male patients with SLE to estimate frequency of aPL and aPL-associated complications. Materials and Methods: We studied 146 male patients (pts) fulfilling at least four of the American College Rheumatology criteria for the classification of SLE. Mean age was 30.5 years (range 15-64 years), mean disease duration was 84 month and ranged from 2 to 504 month. APL was measured by standard- ized ELISA. Presence of LAC was detected according to the guidelines of the International Society on Thrombosis and Haemostasis. Diagnosis of APS was based on the Sapporo criteria. Additionally we analyzed frequency of aPL-associated symptoms not included in the revised criteria (heart valve disease, livedo reticularis, thrombocytopenia, neurological manifestations).

Results: Vascular thrombosis developed in 39 from 146 male pts (26.7%), recurrent thrombosis occurred in 27 out of 39 pts (69.2%). The number of throm- bosis varied from 1 to 7 in one pts. 24 pts (61.5%) had only venous thrombo- sis, 8 pts (20.6%) – merely arterial thrombosis. Presence of arterial and venous localization was register in 7 (17.9%) pts. Positive titers of IgG-aCL (>25 GPL) was observe in 57/125 pts (39,3%), positive titers of IgM-aCL (> 25 MPL) – in 36/145 (24.8%) pts. LAC was positive in 22 from 60 pts (36,7%), 39 male pts (26,75) satisfied classification criteria of definite APS. Pts with APS had higher frequency livedo reticularis (33,3%), pulmonary hyperten- sion (20,5%), heart valve disease (46,2%) in comparison to pts without APS (10,3%, 5,6% and 16,8% respectively, p<0,05 in all cases). Statistically differ- ences were not observed in frequency of thrombocytopenia and neurological involvement between pts with and without APS. Clinical manifestations of APS added to SLE symptoms in 28 (72%) pts (average 2-199 month after SLE onset). In 9 pts (23%) aPL-associated signs preceded SLE-related manifesta- tions. Only 2 men (5%) developed APS and SLE simultaneously. Conclusions: We observed high incidence of definite APS in male patients with SLE. Main localization of thrombosis was vein vessels. Some aPL-associated manifestations not included in The Sapporo criteria (livedo reticularis, heart valve disease, pulmonary associated) were more frequently in patients with APS. Clinical manifestations of APS may precede SLE symptoms.

PO1.C.23
A concurrent case of systemic lupus erythematosus and ankylosing spondylitis
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Objectives: some co-existence case of systemic lupus erythematosus (SLE) and ankylosing spondylitis (AS) was described in literature but this co-existence is very rare. The case: our case is 26 years old female that referred for
1 year inflammatory low back pain. In physical examination she had bilateral positive FABER test and both sacroiliac tenderness and considerably limitation of motion in sagittal and coronal movement of spine. She had positive HLA B27 and bilateral symmetric grade 2 sacroiliitis in radiography. According to modified New York, 1984 criteria diagnosis of AS established. 6 month later she visited because of arthritis in PIP, MCP, wrists, elbows, knees and bilateral sacroiliacis. She had history of photosensitivity, oral ulcers, hair loss and urticarial rash. In further study she had positive FANA with homogenous pattern and positive Anti DNA (ds) but with normal complement. She met ACR criteria for SLE. Continuously she had spinal pain. Conclusion: this is a rare combination of SLE and AS disease. Sacroiliitis is seen 6% of SLE patients but symptomatic sacroiliitis and AS is rare. An unusual combination of genetically determined markers may cause increase rise for the development of both disorders.

POI.C.24
Cardiovascular risk factors predict rapid progression of atherosclerosis in lupus erythematosus
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Background: Patients (pts) with systemic lupus erythematosus (SLE) have a high cardiovascular (CV) risk, which may be due to a predisposition to atherosclerosis. Objectives: To estimate the rate of atherosclerosis in SLE cohort and to identify factors predictive of rapid progression. Methods: 227 SLE (156 women, 71 men, mean age was 35.6 ±0.7, disease duration – 132.9±7.7 months) pts fulfilling ACR criteria for SLE were included in the analysis. A high resolution ultrasound scan of the common carotid arteries was performed in pts with SLE. Digitized scans were used to measure the intima-media thickness (IMT). Traditional risk factors, SLE-related risk factors and biomarkers (hs-CRP, CD40L, sTNF-α, neopterin) of CV disease risk factors were recorded. We used linear regression model to identify baseline factors that were predictive of rapid atherosclerosis progression. Results: Ninety seven (43 %) pts had atherosclerosis (20 % - the IMT>0.9 mm, 24 % - carotid artery plaque). The mean (m) common carotid IMT was 0.77 (0.01) mm. Univariate linear regression model of increased IMT included traditional (age, gender, Framingham risk score, hypertension, dyslipidemia, high body mass index, smoking, family history of premature coronary disease, uric acid), SLE-related risk (disease duration, revised damage index (SLICC), SLEDAI-2K, duration of nephritis, duration prednisone, prednisone cumulative dosage) factors and levels of CD40L, sTNF-α. In multivariate prognostic model of increased IMT included age, Framingham risk score, hypertension, levels of systolic blood pressure (SBP), duration of nephritis, immunosuppressant (see table 1).

Conclusion: Subclinical atherosclerosis was associated with traditional risk and lupus-related factors (duration of nephritis and absence of immunosuppressive therapy) in pts with SLE.

POI.C.25
Autoantibodies and possible carbamazepine-induced lupus erythematosus: a clinical challenge
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Objectives: Drug-induced lupus erythematosus (DILE) has been associated with various medications including anti-convulsants like carbamazepine (CBZ). Immunologically in DILE unlike idiopathic systemic lupus erythematosus (SLE) less than 5% are associated with anti-dsDNA and ENA and less than 1% associated with hypocomplementemia. Anti-histone antibodies are positive in more than 95% of the individuals. Our case is unusual for the absence of anti-histone and dsDNA antibodies, and the presence of hypocomplementemia and leukenopia. Methods: A 34-year-old Caucasian lady developed epilepsy post surgery (January 2003) for a congenital arterio-venous malformation and was started on CBZ (October 2003). Five years later, she presented with an acute onset, photosensitive, scaly plaques on sun-exposed areas of her body which initially cleared with a topical steroid but recurred months later. When a skin biopsy revealed feature consistent with lupus erythematosus. Laboratory data revealed a positive IgG ANA (1:320) and anti Ro-SSA, reduced complement C4 and C3, leukenopia (WCC 2.6, neutrophils 1.03 and lymphocytes 0.88) and negative dsDNA, RNP, Sm and IgG and IgM cardiacolipin. Mepacrine 100mg was initiated for skin manifestations. She, subsequently developed myalgia, a generalized arthralgia and sicca symptoms in spite of continuing mepacrine. Serial serology was negative for anti-histone and anti-dsDNA antibodies repeatedly. The patient was reluctant to discontinue the CBZ as she had experienced numerous relapses and adverse effects whilst on Lamotrigin and Phenytinor prior to commencing CBZ and on switching from CBZ twice. She preferred to persist with the therapy despite the skin and musculoskeletal manifestations. However, should major organ involvement arise, management could prove challenging if she wishes not to withdraw CBZ. Results: Several cases of CBZ induced SLE have been reported in the literature. All but one was associated with anti-histone positivity. Despite the lack of standard diagnostic criteria for DILE, temporal association with a putative drug and resolution following withdrawal of therapy has been the best diagnostic tool. Conclusions: In this lady’s case, withdrawal of CBZ, in view of the fact that she is anti-histone and dsDNA negative, and a resulting resolution of symptoms would be diagnostic. Unfortunately her reluctance to stop CBZ leaves us with a diagnostic dilemma. The auto-antibody profile in DILE is not predictive of severity or extent of manifestation of symptoms. In our patient it is difficult to ascertain the course of the condition on continued challenge from a likely drug source.

POI.C.26
The medical outcomes study (MOS) sleep scale in lupus patients
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Objectives: To evaluate and to identify correlates of the psychometric properties of the Medical Outcomes Study (MOS) Sleep Scale in Systemic Lupus Erythematous (SLE) patients Methods: Sleep in 118 SLE patients was assessed using the self-administered MOS Sleep Scale. Bivariate correlations were determined using a Pearson’s correlation matrix between each of the six MOS sleep subscales and each potential predictor variable (age, educational level, SLEDAI, current prednisone dose, body mass index, visual analog pain scale, Beck Depression Inventory, State-Trait Anxiety Inventory). Serial hierarchical multiple regression analyses were computed to test the importance of demographic, clinical and psychosocial factors to the six sleep subscales. Results: The average age of this lupus population was 45.4 years; 92% were female and 57.6% were African-American. They had a mean SLEDAI score of 3.93 (range 0-22). SLE patients’ mean±SD MOS Sleep scores were generally poorer than the US general population in all six

Lupus
PO1.C.27

Myocardial ischemia in the absence of obstructive coronary artery disease in patients with systemic lupus erythematosus

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Objectives: Prior studies have demonstrated the presence of perfusion defects using adenosine stress cardiac magnetic resonance imaging (CMR) in the setting of microvascular disease with open epicardial coronary arteries. Our purpose was to use CMR to evaluate for microvascular coronary artery disease (CAD) in SLE women with anginal chest pain (CP). Methods: Twenty adult female SLE patients with anginal CP in the last 6 months and low to moderate SLE disease activity index (SLEDAI) were enrolled. Patients with known atherosclerosis or CAD were excluded. CMR was performed at 1.5 Tesla with 0.05 mmol/kg gadolinium first-pass perfusion three-slice stress followed by rest imaging, function and delayed enhancement imaging. CMR images were analyzed by visual semi-quantitative 5 point scoring in 16 segments for presence and extent of wall motion abnormality, hypoperfusion at rest and stress, or myocardial scar. Perfusion defects were categorized by the percentage of myocardium abnormal. SLE patients also underwent 64-slice coronary computed tomography angiography (CCTA). CCTA images were analyzed by visual semi-quantitative 5 point scoring in 16 segments for the following: 1) coronary calcium score (CAC) and 2) plaque type and location, and 3) degree of coronary luminal narrowing using a 5 point scale. Results: Eighteen subjects (mean age 41.3±11 years, mean SLE duration 13.2±12 years, 100% ANA positive, 56% dsDNA Antibody positive) had complete data available for analysis. Nine of 18 (50%) SLE patients displayed subendocardial perfusion defects on stress CMR. All had normal ventricular function and no scar. On CCTA, only 2 of these 9 subjects showed evidence of coronary artery plaque or calcification; one had CAC score of 5.9, (minimal calcification, 60th percentile for age and gender) and the other had an ulcerated plaque in the left anterior descending artery with a CAC score of 0. The Framingham risk score was <1% in all subjects except for 1 in subject where it was 2%. Conclusion: In our pilot sample of SLE patients with anginal CP, we observed the striking finding that 50% of our study group had abnormal stress myocardial perfusion by CMR in the absence of obstructive CAD on CCTA. The detection of subendocardial perfusion abnormalities suggests microvascular coronary involvement as a potential cause of the cardiac ischemic findings. Further validation testing of adenosine CMR in a larger SLE population is warranted.

PO1.C.28

CT findings of lung changes in systemic lupus erythematous: correlation with disease duration

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Objective: To evaluate lung changes in systemic lupus erythematous (SLE) and to analyze the relationship between the extent of lung abnormalities and duration of SLE. Materials and Methods: We studied 41 patients (1 man, 40 women; mean age, 42 years) with systemic lupus erythematous (SLE) who showed airway or interstitial lung disease on thin-section chest CT scans. Mean duration of disease was 9 years. Patients in whom pulmonary abnormalities were clinically thought to be due to infection or drug toxicity were excluded. Lung parenchymal abnormalities that included airspace consolidation, ground-glass opacity (GGO), reticulation, honeycombing, bronchovascular bundle thickening, nodules, bronchiectasis, traction bronchiectasis, and air trapping were assessed retrospectively by two chest radiologists. The relationship between the extent of each CT finding and duration of SLE were analyzed. One or 2 predominant HRCT findings were identified for each case, and patients were classified according to the predominant pattern. Results: The most frequent pulmonary abnormality was GGO (54%), followed by traction bronchiectasis (46%), reticulation (27%), honeycombing (27%), bronchiectasis (24%), consolidation (15%), air trapping (15%), bronchovascular bundle thickening (7%), and nodules (7%). None of these abnormalities correlated with the duration of SLE. We identified 15 patients with bronchiolitis pattern, 11 patients with nonspecific interstitial pneumonia (NSIP) pattern, 10 patients with usual interstitial pneumonia (UIP) pattern, 3 patients with organizing pneumonia pattern, and 2 patients with lymphocytic interstitial pneumonia pattern. Conclusion: Airway disease was more common than individual interstitial pneumonia patterns in patients with SLE. No correlation was found between duration of disease and the extent of CT abnormalities.

PO1.C.29

Analysis of clinical features of meningitis in Korean patients with systemic lupus erythematosus

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Objectives: Meningitis is a rare complication in systemic lupus erythematosus (SLE), which can lead to a fatal outcome. Meningitis is divided into septic and aseptic meningitis. The purpose of this study is to determine the demographic, clinical, laboratory features and outcomes of the meningitis in Korean patients with SLE. Methods: We conducted a retrospective medical record review of 1420 patients who were diagnosed as SLE in the rheumatology department of Seoul St. Mary’s and St. Mary’s Hospital in Korea between January 1997 and June 2009. We identified 20 patients (mean age 29.7±5.9 years) who developed septic or aseptic meningitis. The clinical characteristics, laboratory data, brain imaging findings and prognosis of these patients were analyzed. Results: In 11 cases, causative microorganisms were revealed (“septic meningitis”). Cryptococcus neoformans was identified in 5 patients, Listeria monocytogenes in 2 patients, Neisseria meningitidis, Streptococcus agalactiae, Haemophilus influenzae, and Mycobacterium tuberculosis in 1 patient, respectively. The other 9 patients were diagnosed as aseptic meningitis. Patients with septic meningitis were older than those with aseptic meningitis at the time of diagnosis of meningitis.
The most common manifestation was headache followed by fever and nausea in both types of meningitis. Mental changes were more frequently observed in patients with septic meningitis (P=0.005) although the presence of abnormal findings in brain imaging and prognosis did not differ significantly. Leukocyte counts in CSF were higher in patients with septic meningitis (P=0.044). The level of CSF protein tends to be higher in septic meningitis group (P=0.053) and the level of CSF glucose was lower in septic meningitis group (P=0.036). Plasma leukocyte count as well as neutrophil count was higher in patients with septic meningitis than in those with aseptic meningitis (P=0.037 and P=0.020, respectively). Conclusions: Meningitis was observed in 1.4% of the Korean patients with SLE. In 55% of the meningitis cases, microorganisms were isolated and Cryptococcus neoformans was identified most frequently. Alteration of mental status was more common in cases of septic meningitis. In addition, plasma leukocytosis and neutrophilia as well as CSF pleocytosis and hypoglycemia were more prominent in patients with septic meningitis.

PO1.C.30
Unusual presentation of antiphospholipid syndrome with cardiac failure and cutaneous thrombotic microangiopathy
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Objectives: Since the initial description of the antiphospholipid syndrome (APS) the spectrum of clinical manifestations has broadened, and it has become one of the most systemic conditions. Occasionally, the APS may present with predominant occlusive thrombosis of small vessels. We report a case of a patient who developed acute heart failure and cutaneous nodules, with histologic evidence of subcutaneous thrombotic microangiopathy. Methods: A case was investigated retrospectively and literature was reviewed. Results: A 38-year-old woman with a previous history of breast cancer and thrombocytopenia, presented with 2-week history of dyspnea and a skin rash. On physical examination she had a blood pressure of 150/100 mm Hg. Localized cutaneous areas with a mottled blue discoloration consistent with livedo reticularis, and nodular skin lesions were observed on the lower extremities. Laboratory tests revealed: lymphopenia, a high erythrocyte sedimentation rate, and C-reactive protein level. Complement C3 and C4 were low. High titres of anticardiolipin (IgM and IgG) and anti-beta-2 glycoprotein I (IgM and IgG) antibodies, were observed. Antinuclear antibodies, and anti-double-stranded DNA antibodies tests were positive. Other serologic tests for autoimmune disorders, and common viral and bacterial infections were negative or normal. High titres of anticyclophosphamide, diuretics, and ACE inhibitors. At 2-years follow-up, the patient was asymptomatic. In this patient, the close temporal relationship between the microvascular thrombotic lesions of the skin and the cardiomyopathy suggest that both disorders were produced by the APS. Conclusions: Clinicians should consider the diagnosis of APS in patients with acute cardiac failure and cutaneous lesions.

PO1.C.31
Comparison of the clinical manifestations and disease severity of systemic lupus erythematosus (SLE) among Vancouver residents of Asian and Caucasian origin
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Objectives: To compare Caucasian and Asian SLE patients followed by selected rheumatologists in the Greater Vancouver area for: 1) clinical manifestations and pattern of organ involvement; 2) Disease severity using the SLICC damage index. Methods: A retrospective medical chart review was conducted for the period 1999-2005 of rheumatology practices selected for large numbers of lupus patients of Asian origin. To be included patients had to meet 1982 ACR criteria for SLE and be Caucasian or Asian. Ethnicity was determined by chart review or by asking the treating rheumatologist if missing from chart. Asian ethnicity included Chinese, Japanese, Korean, Philippino, and Vietnamese. East Indians were not considered as Asians. Data were extracted using a standard form that included: demographics, SLE duration, 1982 ACR criteria, SLE treatment, SLICC, and involvement of the following systems, using predefined criteria derived from the SLAM: skin, myositis, arthritis, haematological, renal, cardiovascular, pulmonary, gastrointestinal, and neuropsychiatric manifestations. Results: Our sample included 165 Caucasians and 102 Asians (Mean age: 41.1 vs 44.9 years, resp. and 88.6% vs. 95% were female, resp.). Asian patients had more frequent renal involvement than Caucasians: proteinuria (41% vs. 17%, resp.), biopsy proven glomerulonephritis (39% vs. 13%), renal insufficiency (15% vs. 4%) and end-stage renal failure (8% vs. 2%); as well as haematological manifestations: lymphopenia (80% vs. 60%) and thrombocytopenia (37% vs. 22%) (all P < 0.05). Caucasian patients had more frequent skin disease compared to Asians: discoid rash (15% vs 7%) and photosensitivity (48% vs 30%), as well as neuropsychiatric manifestations, mainly cognitive impairment, anxiety, mood disorders and migraine headaches, but severe CNS involvement, such as vasculitis, CVA, seizures or psychosis, were not more frequent. The risk of SLICC score of 2 or more versus SLICC of 0 or 1, after controlling for age, gender, disease duration and smoking in a binary logistic and multinomial regression, was greater in Asian group (OR 1.868 95% CI 1.001-3.485 p = 0.0498).

Conclusion: In our sample of Vancouver residents, people of Asian origin had a different pattern of SLE manifestations, with more renal and haematological, but less CNS disease, compared to Caucasians. There was a significant difference in disease severity between the two groups using the SLICC damage index suggestive of a more severe disease in Asians.
PO1.C.32

Hidroxicloroquine and metabolic syndrome in patients with systemic lupus
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Background: The prevalence of the metabolic syndrome (MS) is increased in inflammatory and immune processes. In patients with systemic lupus erythematosus (SLE) this condition predisposes to cardiovascular disease concerning its morbidity and mortality. It has been reported a beneficial effect with antimalarial drugs such as hidroxicloroquine (HCQ) on lipid and glucose metabolism, but the potential protective effect on MS is controversial.

Objective: To describe the prevalence of MS, insulin resistance and secretion using homeostasis model assessment (HOMA IR) and disease activity (SLEDAl) in patients with SLE with and without HCQ therapy. Methods: A total of 51 patients with SLE (per the American College of Rheumatology classification criteria) were prospectively evaluated (October 2007-May 2009). MS was assessed using the World Health Organization (WHO) classification. Group 1: Patients without HCQ in the last 6 months. Group 2: Patients with HCQ ≥ 3 months. Dependent variable: treatment with HCQ. Independent variable: HOMA-IR (≥ 2.11), Waist; (≥ 88 cm), Hypertension (≥140/90), TG (≥150mg/dl), HDL (≥40mg/dl),fasting glucose (≥110 mg/dl). Statistical analysis: Significance level was set at probability value p ≥0.05. Chi-square and Fisher’s exact test were used for comparison of categorical variables or percentages.

Results: There were 51 patients, 49 of whom were women (96%). Group 1: 23/51 (45%); mean age: 38 years (DS: 13.72); mean duration of the disease: 8.9 years (DS: 7.33); mean GC dose: 6.7 mg (DS: 5.96). Group 2: 28/51 (55%); mean age: 34 years (DS: 13.47); mean duration of the disease: 7.3 years (DS: 7.15); mean GC dose: 5.1mg (DS: 6.14).

Conclusions: In this study we found that patients with SLE under treatment with HCQ presented lower disease activity and that the prevalence of MS was significantly lower. In this group there was no evidence of significant decrease in sensitivity to insulin (HOMA IR). Treatment with antimalarial drugs plays a role in the inflammatory process of SM and shows a protective effect in patients with SLE.

PO1.C.33

Regenerative nodular hyperplasia of the liver in systemic lupus erythematosus: case report and review of the literature
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Objectives: Regenerative nodular hyperplasia (RHN) of the liver represents a rare, but clinically important and likely under-recognized manifestation of systemic lupus erythematosus (SLE). To date, reported therapy in the available literature has focused on the treatment of associated portal hypertension and its sequelae, but not the underlying immunopathology. We describe a case in which systemic therapy for SLE was associated with evidence of improvement of RHN of the liver. Methods: Case report and review of the literature.

Results: A 27-year old female from Guyana was diagnosed with SLE on the basis of cutaneous, serosal, renal, and hematologic involvement and elevated antibodies to Smith and double-stranded DNA. Additional initial workup revealed elevations in serum alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT), with mild elevations inaminotransferase levels and low albumin, but normal bilirubin and coagulation profile. Abdominal ultrasonography and computed tomography revealed multifocal hepatic nodules, portal venous dilatation and splenomegaly, but no ascites. Guided transcatheter liver biopsy revealed only periportal inflammation, but no RHN, likely due to sampling error. Studies were negative for bacteria, fungi, parasites, mycobacteria, fibrosis and malignancy. Testing for hepatitis B and C, cytomegalovirus, antibodies to smooth muscle, mitochondrial and liver/kidney microsomal 1 antigens were negative, as was testing for antcardiopin antibodies and lupus anticoagulant. Most of the features, but not the hepatic biochemical abnormalities, improved with initial treatment including tapering prednisone and hydroxychloroquine. Subsequently, following a period of noncompliance, the patient presented with florid SLE including panniculitis and mononeuritis multiplex, necessitating more aggressive treatment including corticosteroids, intravenous pulsed cyclophosphamide and intravenous gammaglobulin. With this combination, the panniculitis and mononeuritis multiplex improved, as did the biochemical evidence of RHN. Conclusions: RHN of the liver is a rare (0.3% of cases by one estimate), but an important and likely under-recognized manifestation of SLE, with sequelae that can include portal hypertension with associated ascites and esophageal varices. The mechanisms are thought to include vasculitis, or, in some cases, primary thrombosis in the setting of antiphospholipid antibodies. Less than 25 cases of RHN of the liver in association with SLE have been reported, and therapies have focused on controlling portal hypertension (e.g. with non-specific beta blockade or porto-systemic shunting) and its sequelae (e.g. local treatment of esophageal varices). In the current case, systemic immunosuppressive treatment of severe SLE was associated with hepatic biochemical improvement, suggesting alteration of the suspected underlying vasculitic mechanism of RHN.

PO1.C.34

Abnormal regional cerebral blood flow in systemic lupus erythematosus patients with memory impairments
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Objective: Previous single-photon emission computed tomography (SPECT) studies have demonstrated multifocal hypoperfusion areas in systemic lupus erythematosus (SLE) patients. To our knowledge, there are no studies that explore the association between memory impairment and regional blood flow (rCBF) changes in SLE patients using statistical parametric mapping (SPM) analysis. This study investigated the association of rCBF changes with memory impairment in SLE patients using SPM.

Methods: 19 SLE patients (mean age 36.1±8.6, range 17-47) with subjective memory complaints underwent 99mTc ECD SPECT. Their SPECT images were analyzed by SPM2 software. The Korean-Wechsler Adult Intelligence Scale (K-WAIS) and Rey-Kim Memory Test (RKMT) were used to evaluate cognitive functions of the patient group objectively. On the basis of the Intelligent Quotient (IQ) - Memory Quotient (MQ) difference score, the patients were classified into 2 groups: a group with pronounced memory impairment (n=8) and a group without memory impairment (n=11). Results: There was no significant difference between 2 groups in clinical and demographic characteristics. However, we found decreased rCBF in the posterior cingulate cortex (PCC) in patients with pronounced memory impairment. Conclusion: This is the first study using SPM analysis of SPECT images in SLE patient complained of memory impairments. The PCC hypoperfusion may play a significant role in the memory function of SLE patients. Key words: SLE, memory impairment, SPECT, SPM
PO1.C.36

Clinical features, disease activity and damage accrual in systemic lupus erythematosus patients. Data from a Cuban cohort.

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Background and Purpose: systemic lupus erythematosus (SLE) is a chronic disease with flare-up and remissions episodes. An active disease may lead to a damage accrual. The aim of this study was to determine the prevalence of activity and damage accrual using data from SLE Cuban cohort. Methods: SLE patients from 2 centers were recruited and followed-up longitudinally. Demographic characteristics, cumulative clinical manifestations, laboratory data, disease activity using both SLEDAI and MEX-SLEDAI indexes (at diagnosis, 3, 6 and 9 years of follow-up) and Systemic Lupus International Collaborating Clinics damage index (SLICC/ACR) were compared between patients in an unvariable (UV) and multivariable (MV) logistic regression models. Results: of the 102 patients included in this study, 93.1% were female. The mean age at disease onset was 28 years (SD 10) and at diagnosis was 32 years (SD 10). The mean disease duration was 9 years. Eighty six percent of the patients fulfilled ACR criteria at the time of diagnosis and 94.1% during follow-up. Most frequently accumulated clinical manifestations were arthritides (86.7%), photosensitivity (67.6%), malar rash (58.8%), fever (53.9%), fatigue (35.2%) and renal disease (24.5%). Disease activity evaluation using SLEDAI and MEX-SLEDAI showed active disease at the time of diagnosis in 100% of the patients (by both indexes), at 3 years: 60.7% and 59.8%, at 6 years: 35.3% and 37.2% and at 9 years: 19.6% and 16.7% respectively. Median SLEDAI and MEX-SLEDAI scores: at diagnosis 10 and 5 and at 3 years 4 and 2 respectively. Evaluation at 6 and 9 years of follow-up the result was 0 by both indexes. The UV analysis performed to evaluate associated risk factors to lupus activity showed a significant association only with non-Caucasian ethnicity (OR: 2.54, CI: 1.06-6.18). Five hundred and six flare-up episodes were registered in a 732 cumulated years. An incidence of 0.69 flare-up/patient/year was observed. UV and MV logistic regression analysis showed significant association between damage accrual with non-Caucasian ethnicity (OR: 3.19, CI 1.23-8.38), a disease of more than 5 years (OR: 3.25, CI: 1.09-10.12) and with ≥2 flare-up during 1 year (OR: 3.70, CI: 1.26-10.96). Conclusion: in this cohort, active disease was present in 100% of the SLE patients at diagnosis but in less than 20% at the end of follow-up. Non-Caucasian ethnicity was the only risk factor associated with active disease. Damage accrual was significantly associated with non-Caucasian ethnicity, a disease of more than 5 years and with 3 or more flare-ups in 1 year.

PO1.C.37

Risk factors for arterial thrombosis in patients with lupus nephritis

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Objectives: To study the risk factors for arterial thrombosis in patients with lupus nephritis. Methods: Between 1996 and 2007, patients with SLE who fulfilled the ACR criteria for renal involvement were identified from our lupus cohort database. The cumulative incidence of arterial thromboembolic events since the diagnosis of lupus renal disease was analyzed by Kaplan-Meier’s plot. The effect of blood pressure control (mean systolic and diastolic blood pressure over time since the diagnosis of lupus nephritis), renal insufficiency (mean creatinine clearance [CrCl] over time as estimated by the MDRD formula) and other traditional and non-traditional risk factors on the risk of arterial thrombosis were studied by Kaplan-Meier and Cox regression analysis. Results: 232 patients with lupus nephritis were studied (88% women; mean age at diagnosis of lupus nephritis 35±3.15 years). Renal biopsies were performed in 211 (91%) patients - 100 (43.1%) patients had diffuse proliferative nephritis and 54 (23.3%) had membranous lupus nephritis. After a total follow-up of 1646 patient-years (mean 7.1 years) since the diagnosis of lupus renal disease, 20 arterial thromboembolic events occurred in 28 patients (incidence 12/1000 patient-year). The cumulative hazard rate for arterial events at 5 and 10 years after diagnosis of lupus nephritis was 3% and 18% respectively. In a Cox regression model, age, sex, SLE duration, chronic smoking (>3 years), diabetes mellitus, the presence of antiphospholipid antibodies, menopause, dyslipidemia (LDL ≥4.1 mmol/L or HDL ≤1.0 mmol/L), long-term use of prednisolone (>3 years), mean CrCl of <60ml/min and a mean systolic blood pressure of ≥130mmHg ever since the onset of lupus nephritis was not significantly associated with arterial thrombosis. However, a mean diastolic blood pressure of >85mmHg since the diagnosis of lupus nephritis was a strong independent risk factor for arterial thrombosis (HR 33.5 [95%CI 4.45-252]; p=0.001). Patients with histological membranous lupus nephropathy were not at higher risk of arterial thrombosis. Conclusions: In this large cohort of patients with lupus nephritis, histological classes, renal insufficiency and the mean systolic blood pressure were not significantly associated with arterial thrombosis. However, the mean diastolic blood pressure was a strong independent factor for the development of arterial thrombosis. Vigorous control of the diastolic blood pressure to a target of 85mmHg is recommended to reduce the risk of arterial thromboembolism.

PO1.C.38

Is osteoporosis a predisposing factor for subclinical coronary atherosclerosis in systemic lupus erythematosus (SLE)?

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Background and Objectives: Patients with SLE are prone to accelerated atherosclerosis, which is contributed by traditional and SLE-specific risk factors. Some of these factors such as chronic smoking, menopause, persistently elevated cytokines as a result of disease activity and long-term medications such as corticosteroids and cyclosporin are also unfavorable for bone mineral density (BMD). This cross-sectional study aims to evaluate the relationship between low BMD and subclinical coronary atherosclerosis in patients with SLE. Method: Consecutive patients who fulfilled ≥ 4 ACR criteria for SLE and had at least one vascular risk factor but without any ischemic symptoms were screened for osteoporosis and coronary atherosclerosis by DXA scan (Hologic, Bedford, USA) and multi-detector CT scan (Agatston calcium scores), respectively. Risk factors for atherosclerosis and osteoporosis were identified. Association between BMD values and the Agatston calcium scores was studied by correlation and regression analyses. Results: 144 SLE patients were studied (94% women). The mean age was 46.4±10.3 years and the mean SLE duration was 9.8±7.5 years. The frequency of vascular risk factors was as follows: smoking ≥2 years (10.4%), menopause (57%), diabetes mellitus (3%), hypertension (41%), body mass index ≥27kg/m2 (15%), LDL ≥2.6 or HDL ≤1.0 mmol/L (52%), antiphospholipid antibodies (36%), glucocorticoid treatment ≥3 years (78%). Thirty-one (22%) patients had osteoporosis at either the hip or lumbar spine (Z score < −2.5). Patients who had osteoporosis at the hip or spine had higher Agatston calcium scores than those without, but the difference was not statistically significant (37.7±80 vs 20.3±60; p=0.27). The proportion of patients who had abnormal coronary calcification (Agatston score ≥1) was also higher in osteoporotic than non-osteoporotic patients (42% vs 27%) but again statistical significance could not be achieved (p=0.13). The odds ratio of coronary atherosclerosis in patients with osteoporosis was 1.90 [0.82-4.40]. Age was an independent risk factor for both the occurrence of coronary atherosclerosis (relative risk 1.11 [1.04-1.19]; p=0.003) and osteoporosis at the hip / spine (relative risk 1.09 [1.01-1.18]; p=0.03). In a logistic regression model, osteoporosis had a positive relationship but was not significantly associated with coronary atherosclerosis after adjustment for age, sex and other risk factors such as SLE duration, smoking, hypertension, diabetes mellitus, hyperlipidemia, antiphospholipid antibodies, long-term glucocorticoid treatment, menopause and obesity. Conclusions: SLE patients with low BMD tend to have a higher risk of coronary atherosclerosis. A bigger sample size may be able to show a significant relationship.
Ultrasound in the assessment of Achilles tendon alteration in SLE
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Articular involvement, most frequently arthralgias or non erosive arthritis, is one of the most frequent clinical manifestation of Systemic Lupus Erythematosus (SLE). Few studies have evaluated joints and tendons involvement in SLE patients. In two studies, the ecographic analysis of tendons of a limited number of SLE subjects has showed the presence of finger flexor tenosynovitis and inflammation and thickness reduction of hand tendons. The purpose of this study was to evaluate inflammatory Achilles tendon involvement in SLE patients through three-dimensional ecography.

**Material And Methods:** We enrolled 10 SLE patients (M:F=9:1) referring to our Rheumatology Unit, with a medium age of 37.2 (±8.6) and disease duration of 10.8 years (±11.3). A three-dimensional ultrasound study (using a Logiq 9, General Electric Medical Systems, Milwaukee, WI) was performed by a rheumatologist who was not experienced in ultrasound, specifically trained for using the volumetric probe on Achilles tendon. The images volumes analysis was performed, at least 30 days after, by a specialist in muscle-skeletal ultrasound, unaware of the clinical picture of patients. Only two patients referred the presence of calcaneal pain. **Results:** The ultrasound showed tendinous alterations in all patients; 6 of 10 patients had calcific enthesopathy (bilateral in two cases). A mild swelling of the retrocalcaneal bursa (bilateral in three cases) was displayed in one-half of the patients, whereas only one patient (complaining of calcaneal pain) had tendinous power Doppler signal. Calcaneal bone profile was irregular in two cases (with bone erosions in one patient). Only in one case there was the increase of the thickness of the tendon. No Achilles tendon tears were observed.

**Conclusions:** The results of this study confirm the frequent tendinous and peri-tendinous involvement in SLE patients and show the presence of both inflammation and degeneration signs (usually not accompanied by clinical symptoms). Volumes, and not single images, acquisition could allow a ultrasound follow-up of patients.

Prolongation of the corrected QT interval in anti-Ro/SSA positive adults with SLE
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Electrocardiographic (ECG) abnormalities such as prolongation of the corrected QT interval (QTc) are known to occur in newborns who passively acquired anti-Ro/SSA antibodies through maternal transfer. In adults, studies of the association between QTc prolongation and anti-Ro/SSA are conflicting. Prolonged QTc can lead to life-threatening arrhythmias. Hence, the identification of risk factors for this syndrome is important. **Objective:** To examine whether anti-Ro/SSA antibodies are associated with an increased risk of QTc prolongation in a large SLE cohort. **Methods:** Patients fulfilling ACR criteria for SLE were enrolled at their first clinic visit. All patients were followed prospectively on an annual basis, at which time medication use, measures of disease activity (SLEDAI) and damage (SLICC/ACR damage index [SDI]), and laboratory data were collected. All registry participants were invited to undergo a 12-lead resting ECG at the time of their annual research visit between June 2002 and May 2007. Results of the last ECG obtained and corresponding clinical and laboratory data were analyzed. QTc greater than or equal to 440 msec was considered prolonged. Bayesian Information Criterion (BIC) was used to select factors more likely to be associated with the outcome of prolonged QTc and multivariate logistic regression models were performed to assess the association between anti-Ro/SSA antibodies and prolonged QTc. Other potentially associated factors examined included age, disease duration, SLEDAI, SDI, potassium and magnesium levels, antimarialials, and beta-blockers use. **Results:** For the 278 subjects with an ECG performed during the study, most (91%) were female, mean age was 44.8 years (SD=14.8), and mean disease duration was 12.9 years (SD=11.0). The prevalence of anti-Ro/SSA positivity was 41.9% and QTc prolongation was present in 6.5% of the entire cohort. Only 38.8% of patients with a normal QTc were anti-Ro/SSA positive compared to 72.2% of patients with a prolonged QTc. Of these, the 271 patients (97.5%) with complete data were used for the multivariate analysis. The only factors associated with prolongation of QTc were anti-Ro/SSA. **Conclusion:** Anti-Ro/SSA antibodies were associated with QTc prolongation in a large SLE cohort. Patients positive for anti-Ro/SSA antibodies may benefit from ECG testing. Those identified with QTc prolongation should receive counseling, including education about drugs that may put them at risk for life-threatening arrhythmias.
PO1.C.42

Findings on conventional MRI of the brain in active neuropsychiatric SLE
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Objective: The clinical manifestations of systemic lupus erythematosus with primary involvement of the nervous system (primary neuropsychiatric SLE or NPSLE) are highly diverse and their etiology is incompletely understood. The aim of this study was to provide an inventory of abnormalities on conventional brain MRI in patients with primary NPSLE and to interpret the findings in relation to possible underlying pathogenetic mechanisms. Methods: The first brain MRI exams of the first episode of active primary NPSLE of 74 patients were retrospectively reviewed. All patients fulfilled the revised 1982 ACR criteria for SLE and were classified according to the 1999 ACR case definitions for NPSLE syndromes. Patients with a history of brain disease or secondary NPSLE and patients in whom other mechanisms unrelated to SLE caused the neuropsychiatric symptoms were excluded. Results: The principal findings were 1) punctiform, focal or patchy hyperintensities in WM (40% of all patients) or both WM and GM (5%) suggestive of vascular inflammation (vasculitis) or multifactorial autoimmune mediated mechanisms of vascular occlusion or narrowing (vasculopathy with ischemia), 2) more widespread confluent hyperintensities in the WM, suggestive of chronic hypoperfusion due to the same mechanisms, 3) diffuse cortical GM lesions, most likely due to an immune response to neuronal components (12%) and 4) absence of MRI abnormalities, despite active signs and symptoms (42%). None of the MRI patterns, including normal MR images, appeared characteristic for a separate ACR NPSLE syndrome. A remarkable finding was the frequent occurrence (12%) of small cerebellar defects, which were probably old subclinical infarcts. Conclusion: Several distinct brain MRI patterns were observed in patients with active NPSLE, suggestive of different pathomechanisms. To advance our understanding of the various processes leading to NPSLE, the radiological manifestations may be a good starting point and useful for categorization of patients in further research.

PO1.C.43

Incidence and long-term prevalence of neurological manifestations in an inception cohort of 1480 SLE patients (NPSLE).
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Neurological manifestations of LES patients (NML) at diagnosis are partially known; however, the incidence of them during follow up is unknown. The object of the present was to show the NML at diagnosis and during the follow up. Up to the moment of the study, GLADEL cohort (Grupo Latino American Del Estudio de Lupus) had evaluated 1480 LES patients, with 5.5 years of median time follow up. We formed 3 groups for showing data: 1) patients with NML at diagnosis, 2) with NML during the follow up (no one of them had NML at diagnosis), and 3) patients without NML up to the moment. For group 1, 2 and 3 respectively: women 90.1%, 87%, 91% (p=0.18); Age in years at beginning of symptoms 29±12, 28±12 and 29±12 (p=0.81); Age in years at diagnosis 31±13, 30±12 and 30±12 (p=0.29); Urban residence 89%, 91%, 91% (p=0.70), and ethnicity: white 43%, 34%, and 43%, mestizo 42%, 53%, and 41%, African Latino American 11%, 10% and 14%, others 5%, 4% and 2% (p=0.0001).

PO1.C.44

Psychiatric syndromes in patients with systemic lupus erythematosus and rheumatoid arthritis
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Objective: To examine the frequency and reliability of depression and anxiety in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The cause of psychiatric syndromes in SLE patients with it multifactorial and includes primary immunopathogenic mechanisms, nonspecific sequelae of chronic disease, and concurrent illnesses. Depression and anxiety are more common in patients with SLE than in RA patients. Methods: Forty-three patients with SLE were matched by age and sex to 43 patients with RA attending ambulatory clinics in a single academic medical centre. All fulfilled the American College of Rheumatology (ACR) classification criteria for either SLE or RA. Anxiety and depression levels were assessed with the Beck Depression Inventory and Beck Anxiety Inventory. Health related quality of life (HRQOL) was evaluated by the SF-36. Results: The patients were well matched with regard to age, sex and disease duration. There were no significant differences in self-reported HRQOL, anxiety and depression symptoms between the 2 groups. Conclusion: Psychiatric syndromes such as depression and anxiety regardless of etiology, are common in both SLE and RA patients. Depression and anxiety in SLE patients may represent global changes in the central nervous system that require ongoing evaluation and treatment. Severe chronic pain accompanied by progressive joint destruction, disability, and disfigurement in RA patients increases the risk of depression and anxiety.