1

14-3-3η Induces Key Factors Associated with RA Pathogenesis and its Serum Expression in Early RA Predicts Higher Joint Damage

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Objective: Examine in vitro extracellular 14-3-3η"s stimulatory effects on key inflammatory and joint damage factors involved in RA pathogenesis; evaluate association of 14-3-3η serum levels in early RA with joint damage progression.

Methods: THP-1 cells were stimulated in vitro up to 18 hours with recombinant 14-3-3η using a dose range approximating the serum concentration seen in RA patients (0.1 to 100 ng/ml). mRNA levels of IL-1β, IL-6, IL-8, CCL2/MCP-1, MMP-1, MMP-9, TNFα, and RANKL were assessed by RT-PCR. Using the Augurex ELISA, serum 14-3-3η levels were measured at baseline in 40 patients with recent-onset polyarthritis (EPA) from the Sherbrooke EUPA cohort. Radiographic progression data was available for 33 of the patients and was defined as a change in Sharp/van der Heijde score (ΔSHS) ≥ 0.5. Differences between medians of 14-3-3η levels in the progression and non-progression group were analyzed by 2-tailed Mann-Whitney U test. The relationship of 14-3-3η positivity and titres with radiographic progression was investigated by contingency, univariate and multivariate logistic stepwise regression analyses. Variables entered into the multivariate model included titres of 14-3-3η, RF, CCP, CRP, ESR, age, gender and disease duration.

Results: 14-3-3η was found to have potent ligand-like activity, inducing inflammatory (IL-1 and IL-8) and joint degradative transcripts (MMP-1) by 2-fold with as little as 0.5 ng/ml. Twenty (61%) of the 33 early RA patients with 30 month follow-up data progressed. Median (IQR) baseline 14-3-3η levels were significantly higher in progressors [2.7 ng/ml (0.12-15.94 ng/ml) vs. 0.1 ng/ml (0.06 – 0.15 ng/ml), p=0.006]. Univariate analyses revealed that 14-3-3η and RF positivity were associated with radiographic progression with relative risks (RR) of 2.0 (95%CI, 1.1-3.7) and 2.8 (95%CI, 1.1-7.6), respectively. 14-3-3η titres were associated with joint damage progression, LR of 5.2, p=0.02. Stepwise multivariate analysis returned titres of 14-3-3η (LR=5.6, p=0.02), ESR (LR=6.4, p=0.01), CRP (LR=4.6, p=0.03) and gender (LR=4.4, p=0.04) as independent predictors of radiographic progression together informing 29.4% of the total variance (R^2) in radiographic progression. When 14-3-3η titres were excluded, the total variance for ESR, CRP and gender was 16.8%, indicating that 14-3-3η accounted for 12.6% of it.

Conclusion: Extracellular 14-3-3η is a potent inducer of key inflammatory and joint damage factors associated with RA pathogenesis. Serum 14-3-3η expression in early RA marks joint damage progression risk at 30 months follow-up. These data support an expanded evaluation to determine the extent to which 14-3-3η may aid in early RA patient prognosis and risk stratification.

2

IFN-alpha Induces Altered Transitional B Cell Signaling and Function in Systemic Lupus Erythematosus

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Objective: Previous experiments suggest that the B cells of lupus patients are hyper-responsive to B cell receptor engagement resulting in increased tyrosine phosphorylation and Ca2+ mobilization. However the precise B cell populations that are affected and the mechanisms leading to this hyper-responsiveness have yet to be determined.

Methods: PBMC were isolated from 27 healthy controls and 39 SLE patients with ≥ 4 ACR criteria. Phosflow was used to assess the levels of p-SYK, p-PLCgamma2, or p-ERK following crosslinking with goat anti-human IgM $F(ab')_2$ in distinct B cell subsets defined by anti-CD19,-CD27, -IgD, -IgM and -CD38. B cell proliferation and apoptosis following stimulation were assessed by flow cytometry, using CFSE and annexin V staining, respectively. For some experiments, healthy control B cells were incubated with IFN-alpha, or 50% plasma ± anti-IFN or irrelevant Ab. Lupus associated SNPs were determined by TaqMan genotyping.

Results: There were increased basal levels of p-SYK and p-ERK in naïve B cells ($CD19^+CD27^-IgD^+$) from lupus patients as compared to controls. The levels of basal p-SYK correlated with CD86 expression suggesting that these cells had been already activated in-vivo. Following crosslinking with anti-IgM, there was a significantly increased proportion of $p\text{-}SYK^+$ cells above basal levels in the naïve B cell population of lupus patients, with similar trends seen for the proportion of $p\text{-}PLCgamma2^+$ and $p\text{-}ERK^+$ cells. The increases seen in $p\text{-}SYK^+$ cells were most marked for the transitional B cell subset ($CD19^+CD27^-IgD^+CD38^{hi}IgM^{hi}$), where the levels of p-SYK correlated with enhanced proliferation and survival. There was no correlation between lupus associated SNPs in BLK, LYN, PTPN22, and CSK, and the proportion of $p\text{-}SYK^+$ cells following IgM crosslinking. The proportion of $p\text{-}SYK^+$ cells in the transitional B cell subset fluctuated between visits, suggesting a possible role for pro-inflammatory factors. Consistent with this, incubation of lupus plasma with control B cells enhanced SYK phosphorylation following IgM crosslinking, which was blocked by pre-incubation of plasma with anti-IFN but not irrelevant Ab. Incubation of healthy control cells with recombinant IFN-alpha enhanced SYK phosphorylation, proliferation, and survival following IgM crosslinking, particularly of the transitional B cell subset.

Conclusion: IFN-alpha alters transitional B cell function leading to enhanced survival and proliferation. As purging of transitional B cells plays an important role in preventing autoreactive B cells from entering the mature B cell pool, it is likely that elevated levels of IFN-alpha exacerbate the breach of B cell tolerance in lupus.

3

Investigating Access to Arthritis Health Services for Aboriginal People: A Framework for System Reform

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Objective: Aboriginal people (First Nations, Inuit, and Metis) in Canada have 1.3 to 1.6 higher prevalence rates of arthritis than non-Aboriginal peoples, and experience greater severity and earlier onset of the disease. Lower levels of healthcare utilization in this population suggest that

gaps in arthritis care access and provision exist. The objective of this study was to inform future health services reform by investigating health care access from the perspective of Aboriginal people with arthritis and health professionals.
Methods: This qualitative study employed a constructivist grounded theory methodology. Theoretical sampling techniques guided recruitment in clinics and community organizations across Alberta. Eligible participants with arthritis were 18 years of age or more, and self-identified their Aboriginal status and arthritis diagnosis. Eligible health professionals had experience providing arthritis care to Aboriginal patients. Participants included 15 health professionals and 16 people with arthritis. Semi structured interviews were conducted by trained research assistants and lasted from 24 minutes to 97 minutes in length. Each interview was recorded and transcribed verbatim and uploaded to NVivo 9© for analysis. Coding of the data followed standard procedures for grounded theory (i.e., open coding, axial coding to cluster codes into categories, and selective coding to develop themes and concepts). Analysis continued until saturation was achieved.
Results: Analysis of interviews revealed that patients and professionals often view arthritis health care access through different frames. Participants described living with arthritis as hard and often 'tough out' symptoms. Perceptions of arthritis as common in the community, combined with experiences of racism may contribute to the patients' frame. Interviews with health professionals revealed frustrations with poor patient outcomes. Professionals commonly spoke of lack of 'buy-in' among patients and framed failure to access services in terms of patient knowledge gaps. Health professionals discussed constraints imposed by complex healthcare systems which contribute to tensions between patients and providers. Examples of 'working around the system' to provide innovative models of services were revealed and show potential for improved access to care.
Conclusion: A theoretical framework was developed which models interactions between patients and professionals within the healthcare system and illustrates complex contextual factors that determine arthritis care for Aboriginal people. Following this framework, we conclude that equity in access will depend on the availability of broad culturally safe systems, rather than trying to change the characteristics of individual patients or professionals. Supported by a CIORA grant.

4

ERAP1 Variants Associated with Ankylosing Spondylitis Alter the Unfolded Protein Response in Cells Expressing HLA-B27

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Objective: Endoplasmic reticulum aminopeptidase 1 (ERAP1) has recently been identified to be strongly associated with HLA-B27 positive AS. We have shown that ERAP1 variants cause changes in free heavy chain (FHC) expression on peripheral blood mononuclear cells from HLA-B27 positive patients as well as on B27-expressing C1R cells by in vitro assays. Unfolding of HLA-B27 and the formation of FHC can cause the release of inflammatory cytokines by triggering the unfolded protein response (UPR). We tested if ERAP1 variants can affect the UPR.
Methods: Endogenous ERAP1 was silenced in C1R-HLA-B27 cells with ERAP1-shRNA ($C1R^{ERAP1sh}$). C1R cells with stable ERAP1-shRNA expression were identified by GFP expression and were selected with puromycin. Scrambled sequence shRNA was used as control.

Western blot (WB) for ERAP1 suppression was done using ERAP1 antibody. We then transfected either the common variant ERAP1 ($ERAP1^{WT}$) or one of the two AS-associated ERAP1 variants, K528R or Q730E into the $C1R^{ERAP1sh}$ cells. Lentivirus expression vector alone was used as control and exogenous ERAP1 expression was tracked with HA-tag. Stable cells expressing $ERAP1^{WT}$ or ERAP1-variants were selected by hygromycin. UPR was measured using PCR for spliced variants of XBP-1 and by qRT-PCR and western blot for BiP, CHOP and ATF-6.
Results: Almost all C1R cells that were selected by antibiotics were GFP positive indicating stable ERAP1-shRNA expression. Using WB we noted more than 90% suppression of ERAP1 and more than 75% suppression by qRT-PCR in $C1R^{ERAP1sh}$, compared to the cells with scrambled-sequence shRNA. Anti-HA WB showed uniform strong expression of $ERAP1^{WT}$ and variant forms of ERAP1 in the respective cell lines. Spliced XBP1, a marker of UPR, was upregulated in the $C1R^{ERAP1sh}$ cells. Re-introduction of ERAP1 (C1R-$ERAP1^{WT}$ cells) reduces the UPR response while C1R-$ERAP1^{K528R}$ and C1R-$ERAP1^{Q730E}$ cells expressing the ERAP1 variants had higher UPR activation compared to C1R-$ERAP1^{WT}$ cells. Other UPR markers including BiP, CHOP and ATF6 expression followed the same pattern with AS-associated variants leading to higher UPR.
Conclusion: ERAP1 suppression leads to increased UPR. AS-associated ERAP1-variants, which are known to have reduced function, leads to more UPR compared to the common variant of ERAP1.

5

Increased Risk of Autism Spectrum Disorders in Children Born to Women with SLE: Preliminary Data from the OSLER Cohort

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Objective: Experimental data suggest in utero exposure to maternal antibodies and cytokines as important risk factors for autism spectrum disorders (ASD). Women with SLE display autoantibodies and cytokines, which, in animal models, alter fetal brain development and induce behavioural anomalies in offspring. To date, no one has specifically assessed the risk of ASD in children of SLE mothers. Using the "Offspring of Systemic Lupus Erythematosus mothers Registry (OSLER)", we aimed to determine if children born to SLE mothers have an increased risk of ASD compared to children born to mothers without SLE.
Methods: OSLER is a large population-based cohort, which includes all women who had ≥1 hospitalization for delivery after SLE diagnosis, identified through Quebec's healthcare databases (1989-2009). OSLER also includes a randomly selected control group of women, matched at least 4:1 for age and year of delivery, who did not have a diagnosis of SLE prior to or at the time of delivery. We identified children born live to SLE mothers and their matched controls, and ascertained ASD based on ≥1 hospitalization or physician visit with a relevant diagnostic code, through to end of database follow-up. We performed multivariate analyses to adjust for maternal demographics, sex and birth order of child, and obstetrical complications. In a subsample analysis of children with maternal drug coverage throughout pregnancy, we further assessed relevant in utero medication exposures.
Results: 509 women with SLE had 719 children, while 5824 matched controls had 8493

children. Mean maternal age and follow-up were respectively 30.3 (SD 5.0) and 9.1 (SD 5.8) years. Children born to women with SLE had more records of ASD diagnoses compared to controls [1.4% (95%CI 0.8, 2.5) vs 0.6% (95%CI 0.5, 0.8)]. Mean age at ASD diagnosis was slightly younger in offspring of SLE mothers (3.8 years, 95%CI 1.8, 5.8) as opposed to controls (5.7 years, 95%CI 4.9, 6.5).In multivariate analyses, children born to women with SLE had substantially increased risk of ASD versus controls (HR 2.31, 95%CI 1.03, 5.16). In the subsample of children with drug coverage (n=1925), in utero medication exposures were rare in the 18 ASD cases: none were exposed to antimalarials, antidepressants, or immunosuppressants, while only one case born to a SLE mother and another born to a control mother were respectively exposed to corticosteroids and anticonvulsants.
Conclusion: Compared to children from the general population, children born to women with SLE have a substantially increased risk of ASD.

6

Predictive Validity of Low Disease Activity using Patient Reported Measures on Long-Term Outcomes in Early Rheumatoid Arthritis- Results from Study of New Onset Rheumatoid Arthritis and Ontario Best Practices Initiative

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Objective: Patient reported outcome measures (PROM) are used in routine practice for assessment of disease activity. They have been shown to correlate well with other composite measures. Current guidelines suggest remission or low disease activity (LDA) as the target of therapy in rheumatoid arthritis (RA). Our objective was to assess the predictive validity of early LDA defined by PROMs on future joint damage and disability in patients with early RA
Methods: We studied patients included in the Study of New Onset Rheumatoid Arthritis (SONORA), a multicenter early RA cohort and Ontario Best Practices Research Initiative (OBRI), a current clinical registry of RA patients followed in routine care. Patients with symptom duration ≤ 12 months at enrollment were included. In SONORA analysis, the main predictors were LDA (RADAI< 2.2) at 4mo and 12 mo. Multivariate linear regression analysis was used for assessment of LDA predicting HAQ at 3 years and mutivariate logistic regression models were used for assessment of the impact of LDA on x-ray progression over 2 years adjusting for potential confounders. In OBRI analysis, the predictive validity of LDA at6 months (RADAI< 2.2 and RAPID3< 2) on HAQ at 2 years was estimated using multiple linear regression analysis.
Results: There were 984 early RA patients in SONORA. Baseline (BSL) mean (sd) HAQ was 1.0 (0.7) that improved to 0.7 (0.7) at 3 years. At 2 years, 116(17%) patients developed radiographic progression. At 4 mo 25% achieved LDA and it increased to 37% at 1 year. LDA at both 4mo and 1 year was a significant predictor of lower future HAQ (p< 0.0001). LDA at 4 mo was associated with less radiographic progression (OR, 95% CI: 0.49, 0.25-0.95, p=0.03) in complete cases. Other significant factors associated with higher HAQ included higher BSL HAQ, older age and female sex and factors associated with future joint damage were BSL damage and positive RF and anti-CCP. There were 118 patients from the OBRI cohort who had at least 2-year follow-up with available outcome. At BSL 13(11%) were in LDA defined by RADAI that improved to 43(36%) at 6 mo. Mean (sd) HAQ 1.31 (0.8) at BSL improved to 0.78

(0.7) at 2 years. Based on RAPID3, 11% were determined to be in LDA at BSL which increased to 22 (22%) at 6 mo. LDA at 6 mo, defined by either PROM, was significantly associated with lower HAQ at 2 years (p=0.03 for RADAI, p=0.05 for RAPID3 criteria). Other significant factors associated with higher HAQ included older age, BSL HAQ and gender (female). **Conclusion:** Achieving LDA as early as 4-6 months is associated with improved long-term outcomes in early RA. Disease status using PROMs seems to have significant predictive validity for future outcomes.

7

C-Reactive Protein Gene Polymorphisms and C-Reactive Protein Levels in a North American Native Population that is Highly Predisposed to Rheumatoid Arthritis

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Objective: C-reactive protein (CRP) aids in host defense and CRP-deficient mice have accelerated arthritis, suggesting a role for CRP in immune tolerance. We examined associations between the rs3091244 and rs3093062 single nucleotide polymorphisms in the CRP gene, serum CRP levels, and rheumatoid arthritis (RA) susceptibility in a North American Native (NAN) population that has a high prevalence of RA. \
Methods: Two single nucleotide polymorphisms in the CRP gene promoter region were tested by sequencing: rs3091244 (C/T/A) and rs3093062 (G/A) in NAN patients with RA (n=545), their unaffected first degree relatives (FDRs) (n=338), and healthy NAN Controls (n=667) with no history of autoimmunity. Rheumatoid factor, anti-CCP, and high sensitivity CRP (hsCRP) were tested using commercially available ELISAs, and shared epitope (SE) alleles by specific primers. The genotyping data were analyzed using genotypic (CC vs CA vs TT vs TA vs TC), allelic (C vs T vs A), dominant (CC, CA, TC vs TT, TA; TT, TA, TC vs CC, CA; CA, TA vs CC, TT, TC) and recessive models (TT vs CC, CA, TA, TC; CC vs CA, TT, TA, TC). We report odds ratios (OR) with confidence intervals, and medians (interquartile range). Statistical significance was $p< 0.05$ using Chi Square, Mann Whitney U, and regression analyses.
Results: All subjects were homozygous (GG) for rs3093062. For rs3091244, significant differences between RA patients (58.9/3.1/6.2/0.4/31.4%) and NAN controls (61.3/2.7/3.2/1.8/31.0%) were found using the genotypic model (ChiSq 12.1, p=0.016) and the TT recessive model (RA=6.2 vs NAN controls=3.1%, ChiSq 6.6, p=0.012). In regression models including SE, anti-CCP, and smoking history, the C dominant genotypes predicted reduced risk of RA (OR 0.12, p=0.02, CI 0.02-0.76), whereas the T recessive genotype predicted increased risk of RA (OR 9.1, p=0.02, CI 1.4-59.6). Serum hsCRP levels differed between RA, FDRs, and Controls (9.5 (7.8) vs 3.6 (6.4) vs 1.2 (0.9) mg/L $p< 0.0001$). In analyses including RA, FDRs and Controls, the C dominant genotypes were associated with lower hsCRP levels (4.1 (7.5) vs other genotypes 4.5 (8) mg/L p=0.02), particularly for smokers (p=0.07). This association was less robust for asymptomatic FDRs and Controls (3.2 (21) vs 3.4 (6) mg/L p=0.08).
Conclusion: Although controversy remains as to whether CRP has a causative role in RA pathogenesis, the rs3091244 CRP promoter region polymorphism may modify the risk of

developing RA and influence circulating CRP levels in the NAN population.

8

Inter-Relationship of Sicca Symptoms, Autoimmunity and Systemic Sclerosis

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Objective: The relationship between ocular and oral sicca symptoms, autoimmunity and systemic sclerosis (SSc) is poorly understood. The objectives of this study are to determine the prevalence of glandular and extra-glandular manifestations of Sjögren's syndrome in SSc; and to evaluate the inter-relationships between serologic, ocular, oral, and extra-glandular manifestations of Sjögren's syndrome in SSc patients.

Methods: A cross-sectional study of consecutive SSc patients attending the Toronto Scleroderma Program were evaluated using patient-self reported and physician completed questionnaires, based on the Sjögren's International Collaborative Clinical Alliance questionnaires.

Results: One hundred ninety-four SSc patients (n=26 males, n=168 females) were included with a mean ± standard deviation age of 55.6±24.5 years and disease duration 9.3±8.7 years. Sicca symptoms included dry eyes (n=101, 52.1%), dry mouth (n=124, 63.9%), and vaginal dryness (66/168, 39.2%). Complications included dental caries (n=48, 24.7%), corneal ulcers (n=2, 1.0%), interstitial nephritis (n=1, 0.5%), parotid gland enlargement (n=1, 0.5%), and salivary gland enlargement (n=1, 0.5%). Twenty-two patients (11.3%) were told they had Sjögren's syndrome. SSc patients with the limited subtype and centromere antibodies were more likely to have ocular and oral sicca symptoms (odds ratio (OR) 1.89 (95% CI 1.02, 3.56). These patients were less likely to have interstitial lung disease (OR 0.15, 95% CI 0.03, 0.44). The presence of Ro and La antibodies (OR 0.70, 95% CI 0.29, 1.60), rheumatoid factor (OR 1.03, 95%CI 0.50, 2.10), and antinuclear antibody titre ≥1:320 (OR 1.15, 95%CI 0.64, 2.08) did not clearly differentiate SSc patients with ocular or oral sicca symptoms from those who did not.

Conclusion: Limited cutaneous SSc patients with centromere antibodies appear to be a distinct subset who have an increased burden of ocular and oral sicca symptoms, but less likely to have interstitial lung disease.

9

Subcutaneous Delivery of Methotrexate is Associated with Improved Treatment Survival Compared to Oral Administration for the Initial Treatment of Patients with Early Rheumatoid Arthritis

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Objective: To determine the comparative survival of initial treatment with subcutaneous (sc) methotrexate (MTX) versus oral MTX for patients with early rheumatoid arthritis (ERA) in routine care.
Methods: Patients with early rheumatoid arthritis (ERA) initiating methotrexate therapy were included from the Canadian Early Arthritis Cohort (CATCH), a multicenter, prospective cohort study of patients with ERA. In CATCH patients are treated at the discretion of the rheumatologist and followed every 3 months over the first year according to a standardized protocol. For this study, all patients had an age >16 years, a diagnosis of RA by 2010 criteria, symptom duration < 1 year, used MTX within 3 months of study entry and were MTX-naive or minimally exposed to MTX. We compared the survival between sc and oral administration over the first year. Treatment failure was defined as either a change in route of MTX or addition/switch of any DMARDs other than glucocorticoids. A Cox-Proportional Hazards model was used to adjust for important potential confounders: age, gender, comorbidities, smoking, education, symptom duration, serological status, erosions, baseline DAS28, functional status (HAQ-DI), mean starting dose of MTX (over first 3 months of treatment) and other concurrent DMARDs or corticosteroids.
Results: 674 patients were included (418 oral MTX, 256 sc MTX); mean age 53, 72% female, mean symptom duration 5.2 months, mean baseline DAS-28 5.5. Patients treated with sc MTX were less likely to receive other DMARDs (56% vs. 71%, $p< 0.01$), and had a higher mean starting dose of MTX (23 mg vs. 17 mg, $p< 0.01$). Other characteristics were similar between groups. Unadjusted Kaplan-Meier curves showed significantly improved survival with sc MTX (log-rank $p< 0.001$). After adjusting for confounders the association remained significant (Hazard ratio (HR) for treatment failure: 0.58 (95%CI: 0.37-0.92, $p=0.02$). Older age (HR: 0.98 (95%CI: 0.97-0.99) per year of age) and the use of other DMARDs in combination (HR: 0.53 (0.35-0.81)) were also associated with improved survival. The starting dose of MTX (HR: 0.98 (0.94-1.02)) and all other covariates demonstrated no significant association.
Conclusion: Subcutaneous MTX is associated with improved survival over oral MTX for initial treatment in patients with early rheumatoid arthritis. This is not a randomized trial so other confounding could have occurred.

10

Risk of Cerebrovascular Accidents in Patients with Systemic Lupus Erythematosus: A Population-Based Study

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Objective: Previous studies have shown that patients with systemic lupus erythematous (SLE) have an increased risk of cerebrovascular accidents (CVA). However, most of these studies have used clinic-based samples. Studies examining the risk of CVA at the population level are limited. To fill this knowledge gap, we estimated the risk of newly recorded CVA events among incident cases of SLE compared to controls from the general population using physician billing and

hospitalization databases from British Columbia (BC), Canada.
Methods: Our data includes all visits to health professionals and all hospital admissions covered by the comprehensive provincial medical services plan (1990-2010) and all dispensed medications (1996-2010) for all BC residents. We created an incident SLE cohort with cases diagnosed for the first time between January 1996 and December 2010 defined as follows: a) two ICD codes for SLE at least two months apart and within a two-year period on physician visits to a non-rheumatologist; or b) one ICD code for SLE on at least one visit to a rheumatologist or hospitalization data; and c) absence of prior SLE diagnosis between 1990 and 1995. For each case, ten controls matched by birth-year, sex and calendar-year of exposure were selected from the general population. The first CVA event during follow-up from hospital or death certificate was recorded as an outcome. We estimated relative risks (RRs) comparing the incidence of CVA in SLE cases and in controls before and after adjusting for confounders.
Results: Among 4,879 incident cases of SLE, 203 developed a first CVA. For the 49,555 non-SLE controls, there were 752 individuals with a first CVA. The age-, sex-, and entry-time-matched relative risk was significantly increased in the SLE cohort compared to the non-SLE cohort (RR=2.7; 95%CI 2.3–3.2). The risk was 6 times greater within the first year after the diagnosis (RR=6.1; 95%CI 4.6–8.1). After adjusting for angina, COPD, obesity, glucocorticoids, cardiovascular drugs, medications for diabetes, hormone replacement therapy, contraceptives, fibrates, statins, NSAIDs, Cox-2 inhibitors, mean number of hospitalizations, and Charlson's comorbidity index at baseline, the results remained statistically significant (adjusted-RR=2.8, 95% CI 2.4–3.4) The highest risk occurred in those younger than 45 years (adjusted-RR=8.8, 95% CI 5.7-13.5).
Conclusion: This large population-based study indicates a significantly increased risk of CVA in patients with SLE, especially in younger individuals and within the first year of disease onset. Our results support close CVA risk factor assessment in individuals with SLE and intervention when available.

11

Causes of Stillbirths in Women with Systemic Lupus Erythematosus (SLE): Preliminary Data from the OSLER Cohort

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Objective: It is believed that pregnant women with SLE face an increased risk of stillbirths, although there are few precise or recent estimates of the magnitude of the effect. As well, no one to date has investigated the causes of stillbirths in SLE pregnancy. Using the "Offspring of Systemic Lupus Erythematous Registry (OSLER)", we examined stillbirths and the cause of their death in SLE mothers versus those without SLE.
Methods: OSLER is a large population-based cohort, including all women with ≥1 hospitalization for delivery after SLE diagnosis, identified through Quebec's healthcare databases (1989-2009). OSLER also includes a randomly selected control group of women, matched ≥4:1 for age and year of delivery, without a diagnosis of SLE. We identified stillbirths (i.e. intrauterine deaths ≥20 weeks of gestational age) from SLE mothers and their matched controls, and ascertained the cause of death as indicated on the death certificates. We calculated odds ratios (OR) and their 95% confidence intervals (CI) using Fisher's exact test.
Results: 509 women with SLE had 729 births, among which 9 were stillbirths (1.4%), while

5824 matched controls had 8541 births including 47 stillbirths (0.6%). Versus controls, women with SLE had a substantially increased risk of stillbirths (OR 2.3, 95%CI 1.1,4.6). Among women having a stillbirth, the median maternal age was identical for both SLE and control mothers [respectively 31.0 years (IQR 29.0,32.0) and 31.0 years (IQR 26.5,30.5)]. There were more female offspring in stillbirths born to women with SLE (6/9) versus controls (22/47) (OR 2.3, 95%CI 0.5,10.2). Stillbirths in SLE mothers occurred at a younger median gestational age compared to controls [29 weeks (IQR 28,31) versus 35 weeks (IQR 27,38)]. Cause of death in SLE stillbirths were as follow: two (22%) occurred secondary to maternal hypertensive disorders, two (22%) following placental abruption, one due to a congenital anomaly, one secondary to chorioamnionitis, one caused by an umbilical cord abnormality, and one related to intrauterine growth restriction. Among control stillbirths, only two (4%) occurred secondary to maternal hypertensive disorders and two (4%) due to placenta abruption. We observed a trend for higher risk in SLE mothers versus controls for stillbirths due to maternal hypertensive disorders and placental abruption (identical OR 6.4, 95%CI 0.8,53.3).
Conclusion: Women with SLE have a substantially increased risk of stillbirths. Stillbirths in mothers with SLE may be more often caused by maternal hypertensive disorders and placental abruption, than stillbirths in mothers without SLE.

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High Mortality in North American Natives with Systemic Lupus Erythematosus (SLE): Looking for Solutions.

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Objective: Lupus outcomes including mortality have been found to be worse in most ethnic minorities, including African Americans, Asians, and Hispanics, but little is known about North American Natives (NAN). We compared mortality in NAN SLE patients to Caucasian SLE patients.
Methods: Patients from a single academic center were followed from 1990-2013 using a custom database. Variables included date of birth, diagnosis, year of disease onset, ethnicity, clinic visits dates, and vital status if known. Records of all patients with a diagnosis of SLE (≥ 4 American College of Rheumatology criteria) were abstracted. For patients who had not been seen in the last 2 years, updated vital status was obtained from the hospital medical records department. Ethnicity was by self-report, and categorized into NAN, Caucasian and other. The age at diagnosis, disease duration and age at last follow up or age at death was calculated and compared between ethnic groups. Survival time was compared between NAN and Caucasians using Kaplan Meier and Cox proportional hazard models.
Results: A total of 807 patients with SLE were identified: 201 (25%) patients were NAN, 501 (62%) were Caucasian, and the remaining 105 (13%) were of other ethnic backgrounds and were excluded from subsequent analyses. NAN patients were younger at diagnosis (NAN = 32±15 years vs. Caucasian = 37±15 years; p=0.001) and had a shorter disease duration compared to Caucasians: (NAN = 11±9 years vs. Caucasian = 15±11 years; p=0.001.) More NAN had died by the end of the follow-up period (NAN = 25% vs. Caucasian = 18% p< 0.001) and mean age at death was much younger in NAN (NAN = 50±16 years vs. Caucasian = 63±16 yrs p< 0.001). Survival rates were significantly worse in NAN compared to Caucasians: 5 year survival was 92% vs. 97%; 10 year survival 85% vs. 92%; 15 year survival 78% vs. 88% respectively (p< 0.001). In a cox proportional hazards model, the risk of death overall was higher for the NAN

(hazard ratio 3.3; 95%CI: 2.3-4.8) than for Caucasians, as was the risk of death following diagnosis (hazard ratio 2.1; 95%CI: 1.4-3.1).
Conclusion: While increased mortality in lupus patients compared to the general population is well described, our study demonstrates even greater excess mortality rate in NAN in comparison to Caucasians SLE patients. This study demonstrates the urgent need for improved care delivery for NAN with SLE to decrease the significant morbidity and mortality burden from this disease.

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T-Cell Receptor Excision Circles (TREC) Quantification in Inflammatory Polyarthritis of Recent Onset (EIA) and in Control Subjects

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Objective: Background: Patients with rheumatoid arthritis (RA) are reported to have an exhausted immune system. It is not known whether patients seen very early during early inflammatory arthritis (EIA) development already have evidence of immune exhaustion. Quantification of T-cell receptor excision circles (TREC) is a readily available measure of T cell thymic output. Objective: To compare TREC numbers in patients with very early EIA and in control healthy subjects (controls).
Methods: DNA from peripheral blood was collected at inclusion from a cohort of consecutive patients with EIA (1-12 month symptom duration) (EUPA cohort) and from normal controls with similar sex and age distribution. TREC numbers were quantified by quantitative polymerase chain reaction (qPCR) using the method described by Cheynier et al (1). The proportion of lymphocytes in nucleated blood cells was used to establish the denominator.. A Mann-Whitney U test was performed to compare TREC numbers in the two groups. The effect of age on the TREC numbers in each group was analyzed using a Spearman correlation.1- Dion, M-L, Sékaly RP, Cheynier R..Estimating thymic function through quantification of T-cell receptor excision circles. Methods in Molecular Biology. 2007;380:197-213.
Results: The cohort now comprises 666 patients with a median duration of follow-up of 4 years. At baseline, about 77% and 89% of patients fulfilled ACR 1987 and 2010 RA criteria, respectively. We report on 36 patients with a very short duration of symptoms (median (IQR) 1.8 (1.4-2.7) months) and on 35 controls. The median (IQR) number (per 10^5 lymphocytes) of TREC at inclusion for the EUPA patients was 16.2 (4.3-37.1) and 40.5 (16.9-98.3) for the controls ($p=0.006$). Among controls, increasing age was negatively correlated with TREC numbers ($r=-0.642$; $p< 0.001$) while among EUPA patients, age had no significant impact ($r=-0.228$; $p=0.196$).
Conclusion: In the literature, as well as in our controls, TREC numbers decrease with age. In very early EIA patients, irrespective of age, TREC numbers were low and similar to numbers found in older controls. This decrease in TREC numbers in EIA patients may be due to dilution of TREC-positive T cells (recent emigrents from the thymus) through active peripheral replication of T-cells or to a true decrease in the generation of new T cells by the thymus. Measuring both TREC numbers and the length of T cell telomeres sequentially in the same patients over various disease states may help to find out which mechanism is responsible for the lower TREC numbers in EIA.