OP64. OSTEOCLAST-MEDIATED BONE RESORPTION: REGULATION BY HYPOXIA-INDUCIBLE FACTOR (HIF) AND ANGIOPOIETIN-LIKE 4 (ANGPTL4)

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Background: Hypoxia is a feature of the hyperplastic synovium in rheumatoid arthritis (RA). Many cellular components of RA express the hypoxia-inducible transcription factor, HIF. We have recently demonstrated that osteoclast-mediated bone resorption is enhanced by hypoxia in a HIF-1α-dependent manner (1). We continue our investigation of the molecular mechanisms regulating hypoxia-induced osteoclast activation to further understanding of bone resorption in RA.

Methods: Osteoclasts were differentiated from CD14+ PBMC with M-CSF (25 ng/ml) and RANKL (50 ng/ml) for 16 days. Osteoclasts were then exposed to hypoxia (2% O2) for 24 h prior to fixation, analysis of resorption (toluidine blue staining of dentine slices) or collection of RNA, protein or cell supernatant. To identify potential genes of interest, performed using RNAiMAX (Invitrogen).

Results: Use of a panel of normoxic inducers of HIF (CoCl2, dimethyl sulfoxide, hydroxyurea, L-mimosine) revealed that HIF expression is sufficient to enhance osteoclast resorption in the absence of a hypoxic stimulus. Analysis of microarray data therefore focussed on known HIF target genes.

Conclusion: These data demonstrate that expression of HIF is sufficient to stimulate osteoclast-mediated bone resorption in the absence of a hypoxic stimulus. Analysis of microarray data therefore focussed on known HIF target genes.

Disclosures: All authors have declared no conflicts of interest.

References:

OP65. MOLECULAR AND CELLULAR EVOLUTION OF FUNCTIONAL TERTIARY LYMPHOID STRUCTURES IN SALIVARY GLANDS OF NOD MICE

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Background: Tertiary Lymphoid Structures (TLSs) are common features of chronic inflammatory diseases including Sjögren’s syndrome (SS). We recently showed that these ectopic structures acquire secondary lymphoid organs properties and are capable of supporting B cell activation and autoantibody production including expression of activation-induced cytokine deaminase (AID) and Ig class switching. Dissecting TLSs dynamics in humans is technically and ethically challenging. Thus, we used the NOD mouse, a spontaneous model of autoimmune sialoadenitis, to characterize the cellular and molecular basis of autoreactive B cell activation and evolution of functional Ectopic Lymphoid Structures (ELS) in the chronically inflamed NOD salivary glands.

Methods: Submandibular glands from 110 female NOD mice from 4 to 35 weeks of age were collected. Paired snap-frozen samples were analysed by immunohistochemistry (IHC) for T and B lymphocytes (CD3/CD20) to evaluate cell infiltration and the degree of B/T cell segregation, ELS were detected by staining for FDC-M1 (follicular dendritic cell networks), GL7 (germinal centre B cells) and AID (marker for ELS functionality). Characterization of B cell subsets within the infiltrates was carried out by immunostaining and by FACS analysis with CD19, CD21, CD23, IgD, IgM, CD1d and CXCR5 antibodies. Quantitative TaqMan real-time PCR was performed to identify the mRNA expression of ELS-related genes. Sex/age matched Balb/c and C57BL/6 mice were used as controls.

Results: NOD infiltrates in glands displayed progressive features of ELS from week 8, with 75% of mice developing B/T cell segregation, FDC networks and GL7+ ectopic germinal centers from week 20. Evolution of TLSs was closely associated with mRNA upregulation of genes regulating ELS organization and function such as lymphoid chemokines CXCL13/CCL19 and their receptors CXCR5/CXCR7, lymphotaxis and B cell survival factors BAFF and APRIL. In agreement with CXCL13/CXCR5 mRNA expression, B cells in infiltrates display strong CXCR5 expression and were mostly characterized by a follicular phenotype (B220+IgD+IgMlow/CD23+/CD21low) as demonstrated by both IHC and FACS analysis on isolated cells. Finally, functional analysis of ELS was demonstrated by expression of AID mRNA and protein within FDC networks, which paralleled the detection of circulating SS-related autoantibodies.

Conclusions: This work provided the first in-depth characterization of cellular and molecular mechanisms underlying the evolution of functional TLSs within submandibular infiltrates of NOD mice. These data strongly support the hypothesis that B-cells can be activated within TLSs in the target organ and promote in situ autoantibody production. Overall, these data support the critical importance of ELS formation in chronic autoimmune inflammation and identified NOD mice as a suitable model to test therapeutic strategies aimed at modulating B cell functionality.

Disclosure statement: All authors have declared no conflicts of interest.

References:
were estimated according to the human capital approach, which attempts to value an individual's contribution to the economy and measures indirect costs in terms of time lost from work due to illness, either due to work force or unpaid work at home and time spent by another individual (helper) assisting the person obtaining healthcare services. Time lost from work also has a number of components. At its simplest, is sickness absence from the existing work schedule. Individuals with ill-health, however, may also reduce the total number of hours they choose or are able to work during their working week and/or reduce the number of weeks they work in a year.

**Results:** In the most conservative approach (low estimate), we combined sickness absence with self-reported reduction in hours worked due to ill-health within the individual's existing work schedule, for those individuals who were employed. In the intermediate estimate, we added to this the estimated costs of time lost from work by those not employed who would have done so had it not been for their ill-health. Finally, in the high estimate, we also included the costs associated with those who were employed reducing the number of weeks worked in a year due to ill-health.

Using a conservative model the estimated total annual indirect costs (and 95% CIs) were £7,677 (£5,560, £9,794) for pSS, £10,444 (£8,206, £12,681) for RA and £982 (£307, £1,478) for controls. Using a model that maximizes the estimates, the equivalent figure was £5,502 (£9,542, £17,463), £7,070 (£13,112, £21,028) and £3,382 (£2,187, £4,578) respectively. These were all significantly greater at P < 0.001 for patient groups than for the control group.

**Conclusions:** PSS is associated with significantly increased indirect costs equivalent to 69–83% of that for RA patients. This needs to be taken into account when evaluating the overall economic consequences of PSS.

**Disclosure statement:** All authors have declared no conflicts of interest.

**OP67. THROMBOCYTOPSY AND HIGH SKIN SCORE ASSOCIATE WITH ELEVATED SERUM IL-6 LEVEL IN SYSTEMIC SCLEROSIS**

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**Background:** IL-6 is a pivotal cytokine that has been implicated in SSc pathogenesis with increased fibroblastic expression of IL-6 and upregulated collagen production. We hypothesise that thrombocytosis identifies a subset of SSc patients that is associated with high levels of IL-6. We analysed the relationship between serum IL-6 levels and the inflammatory response and modified Rodnan skin score (mRSS) in SSc.

**Methods:** This was a cross-sectional study of SSc patients and controls. Serum IL-6 levels were subdivided into three categories: High (>10 pg/ml), Low (>1 pg/ml, <3.12 pg/ml) and undetectable (<1 pg/ml). Categorical data were analysed by Chi-square test.

**Results:** A majority of the SSc cases were female: 77% and 96% for SSCc with elevated platelets and low platelets, respectively. IL-6 levels were significantly elevated in 55% of the entire cohort (r = 0.5, P < 0.001). There was no significant difference in IL-6 levels in entire cohort (P = 0.16) and in the SSc with elevated platelets cohort (P = 0.23). There was a strong association between serum IL-6 and CRP in the entire cohort (r = 0.74, P < 0.001) and this correlation remained significant in the entire cohort (r = 0.8, P < 0.001). There was a significant correlation between serum IL-6 levels and concurrent mRSS (r = 0.48, P = 0.02). There was moderate correlation between platelet count and concurrent mRSS (r = 0.33, P = 0.05).

**Conclusions:** This study suggests that IL-6 may be an important biomark for skin disease and that thrombocytosis in SSc patients may selectively identify those with high IL-6. These data support further exploration of the pathogenic role of IL-6 in SSc and suggest that IL-6 ligand-receptor axis may be a logical target for therapy in selected cases of dcSSc.

**Disclosure statement:** All authors have declared no conflicts of interest.

**OP68. A POTENTIAL ROLE FOR INTERLEUKIN-6 IN THE PATHOGENESIS OF SYSTEMIC SCLEROSIS**

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**Background:** Endothelial cell cytotoxicity and activation are thought to be pivotal to the pathogenesis of systemic sclerosis (SSc). We have previously demonstrated that neutrophils isolated from patients with SSc have a functionally distinct phenotype. The purpose of this study was to examine the potential for neutrophils to activate endothelial cells and cause endothelial cell apoptosis in co-cultures containing SSc serum.

**Methods:** Human dermal microvascular endothelial cells (HDMECs) in passage 4–6 were cultured with healthy control neutrophils in the presence of 25% control or SSc serum for 24 h. Patients included had limited or diffuse SSc. Apoptosis was measured using annexin V-FITC staining. Endothelial cell activation was measured using a fluorescently conjugated antibody against E-selectin. Fluorescence was quantified and localized using confocal microscopy. Statistical analysis was performed using the paired t-test.

**Results:** Co-culture of neutrophils and endothelial cells in the presence of SSc serum resulted in significantly more annexin V staining (P = 0.05) and E-selectin (P = 0.00004) expression than control serum. Both neutrophils and endothelial cells exhibited an increase in annexin V staining. The increase in E-selectin reflected an increase in endothelial expression. SSc serum alone, in the absence of neutrophils, did not elicit an increase in apoptosis or E-selectin expression.

Heat inactivated, AB serum spiked with 200 ng/ml rIL-6, reproduced the increase in apoptosis (P = 0.02) and E-selectin (P = 0.006) expression, but only when neutrophils were present in the cultures. The increase in E-selectin expression in response to SSc serum was abrogated by immunodepletion of IL-6 (P = 0.01) and IL-6 neutralization (P = 0.005). In addition, soluble gp130, an inhibitor of IL-6 transsignaling decreased the effect of SSc serum on E-selectin expression (P = 0.033) and apoptosis (P = 0.041) to control levels.

**Conclusions:** These data show that when stimulated with SSc serum, there is evidence of endothelial cell apoptosis and activation, which are thought to be relevant to the pathogenesis of SSc. IL-6 plays a significant role in this effect. Endothelial cell activation has previously been described in response to IL-6. However, endothelial cells do not express the IL-6 receptor (gp80) and are only able to respond to IL-6 when it is bound to soluble IL-6 receptor. This so called transsignalling occurs through the endothelial cell gp130 receptor. Neutrophils are the major source of soluble IL-6 receptors. This may explain why the effect of SSc serum is only seen when endothelial cells are cultured in the presence of neutrophils. IL-6 has also been implicated in fibroblast activation, autoreactivity and the maintenance of chronic inflammation, all of which may have pathological relevance in this disease. Anti-IL6 receptor antibodies (Tocilizumab) have been successfully used in rheumatoid arthritis and might be of therapeutic benefit in SSc.

**Disclosure statement:** All authors have declared no conflicts of interest.

**OP69. SUPERIOR MESENTERIC ARTERY VASCULOPATHY IN SYSTEMIC SCLEROSIS PATIENTS**

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**Background:** Gastrointestinal involvement is extremely frequent in both diffuse and limited systemic sclerosis (SSc), all gastrointestinal segments can be affected and symptoms are frequently absent until...
late stages. The aim of this study was to detect affection of Superior mesenteric artery (SMA) by coloured Doppler ultrasound (CDU) to assess subclinical gastrointestinal involvement to improve quality of life in SSC patients.

Methods: SMA blood flow in the fasting state was measured by CDU in 20 SSC patients and in 10 apparently healthy controls. The outcome variables emphasized in this study were whole vessel diameter and internal diameter with abnormalities, which were recorded as severity of stenosis that is normal, minimal disease (<20% lumen stenosis), moderate (20-49%), severe (50-69%) and critical disease (>70%). Mean velocity, pulsatility index (PI) and resistive index (RI) were all measured. Clinical manifestations and presence of small bowel affection were detected including nausea, vomiting, abdominal pain and early satiety.

Results: In SSC patients there was highly significant (P < 0.001) reduction in mean SMA diameter (0.66 ± 0.06 cm) vs (0.8 ± 0.036 cm) in controls, PI was significantly (P < 0.05) lower (1.53 ± 0.32) vs (1.82 ± 0.11) in controls, while RI was significantly (P < 0.05) higher (0.72 ± 0.13) vs (0.57 ± 0.037) in controls. Severity of stenosis was mild in 45% and moderate in 55% of the patients, on correlation of severity of stenosis with the disease duration, there was a positive significant correlation (r = 0.46, P < 0.05). Small bowel manifestations were detected in 8 (40%) of SSC patients, they showed a PI (1.24 ± 0.27) which was highly significant lower than those without small bowel manifestation (1.42 ± 0.18) (P = 0.001) whereas the RI was highly significant increased in SSC patients with small bowel manifestations (0.83 ± 0.07) when compared with patients with small bowel manifestations (0.64 ± 0.11), (t = 4.43, P < 0.001). On comparing diffuse 12 (60%) and limited 8 (40%) subtypes, there were no significant statistical difference (P > 0.05) regarding their CDU of SMA results.

Conclusions: CDU, which is a safe and non-invasive procedure, can be used in detection of SMA vasculopathy in SSC patients, even in those without small bowel manifestation reflecting its reliability in detecting subclinical affection of SMA in SSC and may be implicated on the further management. Also, CDU is informative technique in monitoring progressive SSC patients.

Disclosure statement: All authors have declared no conflicts of interest.

OP70. BODY MASS INDEX IS ASSOCIATED WITH MALABSORPTION BUT NO OTHER CLINICAL MANIFESTATIONS IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is a connective tissue disease that can affect multiple organ systems including the gastrointestinal (GI) tract. Despite there being several reasons why body mass index (BMI) might be either high or low in patients with SSc, there has been very little previous research into this. Our aim was to examine BMI in patients with SSc and to determine whether certain specific clinical features, including GI manifestations of the disease, are associated with low BMI. Specifically we set out to test the hypothesis that BMI is reduced in patients with upper GI involvement and with malabsorption.

Methods: Patients with SSc were identified from a clinical database at a single centre. Clinical data were entered approximately yearly on each patient, which included height and weight. 199 patients had recent height and weight data available and were included in the analysis. An unpaired t-test was used to compare BMI in patients with and without upper GI involvement, malabsorption, positive anti-centromere antibody and positive anti-topoisomerase antibody. BMI was also compared between patients with limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) disease.

Results: The mean age of the 199 patients was 59 years (s.d. 11.5). 163 (82%) were female. 164 (82%) had upper GI involvement, 23 (12%) were known to have malabsorption, 66 (33%) were anti-centromere antibody positive and 21 (11%) were anti-topoisomerase positive. 147 (74%) had lcSSc and 52 (26%) had dcSSc. Mean BMI was 24.9 (S.D. 5.1). BMI results in the different clinical subgroups are shown in the Table.

Conclusions: Patients with SSc are more likely to have a low BMI if they have malabsorption, emphasizing the need for careful nutritional assessment especially in this patient group. BMI did not differ between patients with and without upper GI involvement, anti-centromere or anti-topoisomerase positivity, nor between patients with lcSSc and dcSSc. The heterogeneity of patients with upper GI involvement is likely to have influenced results. In patients with complex multisystem disease, multiple factors can affect BMI such as cardiorespiratory involvement, musculoskeletal disease and corticosteroid treatment. These factors should be taken into account in future, larger studies.

OP71. NATURALLY OCCURRING FREE THIOLS WITHIN j2-GLYCOPROEIN I IN VIVO: FUNCTIONAL IMPLICATIONS IN THE REGULATION OF OXIDATIVE STRESS INDUCED ENDOTHELIAL CELL INJURY

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Background: j2-glycoprotein I (j2GP) is the major autoantigen of antiphospholipid antibodies. Structural biochemical study of the cysteine residues within j2GP reveals that some harbour an allosteric configurational suggesting that under specific conditions, disulfide bonds between such residues may break, exposing free cysteine thiols. We set out to investigate whether j2GP ex vivo harbour free thiols and aimed to assess the functional implications of this with regards to endothelial cell biology and oxidative stress.

Methods: A novel capture ELISA was developed to specifically detect j2GP with free thiols within serum or plasma. Serum or plasma was incubated with a thiol specific reagent with labelled proteins then captured on an ELIZA plate and probed with a specific murine monoclonal or rabbit polyclonal anti-j2GP antibody. Both EAhy926 cells of endothelial cell lineage and primary human umbilical vein endothelial cells (HUVEC) were exposed to oxidative stress with a high dose of hydrogen peroxide (H2O2) to induce cell death. The effect of reduced vs non-reduced j2GP on H2O2 induced cell death was then quantified.

Results: Using this novel ELIZA, it is shown for the first time that j2GP exists in vivo in a reduced state, upon screening 18 healthy human serum samples. Controls included non-labelled human serum probe with a monoclonal anti-j2GP and both labelled and non-labelled serum incubated with an isotype control antibody. All controls revealed a noise-like signal (OD (405 nm) < 0.1). However, as the ideal negative control a murine j2GP-/- mouse was employed. Performing the same ELIZA with j2GP with and without reduction demonstrated a strongly reduced signal with labelled mouse j2GP-/- serum (OD ± s.e.m. 3.27 ± 0.09) but a significantly reduced signal vs labelled j2GP-/- serum (0.08 ± 0.007, P < 0.0001, n = 2). The in-plate coefficient of variation (CV) was 5.08% ± 0.1. Both EAhy926 cells and HUVEC were shown to have a dose response loss of cell viability with incremental doses of H2O2. Incubating cells with H2O2 in the presence of j2GP reduced resulted in a significant abrogation of H2O2 induced cell death compared with cells incubated with H2O2 and non-reduced j2GP or buffer alone.

Conclusions: This novel finding, that j2GP exists in vivo in a reduced state represents a paradigm shift, as hitherto studies investigating the biology of this autoantigen have employed purified protein which spontaneously oxidizes when fractionated from blood. With respect to endothelial cells, reduction of j2GP protects against oxidative stress induced cell injury. It would be of interest to investigate how levels of reduced j2GP differ in disease states such as APS and SLE and also whether reduction of j2GP affects antigenicity, potentially revealing novel insights into the pathogenesis of such conditions.

Disclosure statement: All authors have declared no conflicts of interest.

OP72. HYPOVITAMINOSIS D IN PATIENTS WITH CONNECTIVE TISSUE DISEASE

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BMI in different clinical subgroups

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Yes</th>
<th>No</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper GI involvement</td>
<td>24.92±0.52</td>
<td>24.69±0.44</td>
<td>0.814</td>
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<tr>
<td>Malabsorption</td>
<td>22.06±0.50</td>
<td>25.24±0.50</td>
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<tr>
<td>Anti-centromere antibody positive</td>
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<td>24.75±0.57</td>
<td>0.294</td>
</tr>
<tr>
<td>Anti-topoisomerase antibody positive</td>
<td>24.75±0.58</td>
<td>24.75±0.58</td>
<td>0.294</td>
</tr>
<tr>
<td>LcSSc subtype</td>
<td>25.03±0.51</td>
<td>24.43±0.49</td>
<td>0.470</td>
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</tbody>
</table>

Results are mean (S.D.)

Disclosure statement: All authors have declared no conflicts of interest.
Background: There is increasing recognition of the many diverse and important roles that vitamin D plays in autoimmune and inflammatory processes and growing concern regarding the prevalence of hypovitaminosis D in rheumatology patients. Given the lack of data on hypovitaminosis D in connective tissue disease (CTD), we undertook a study of 25-hydroxyvitamin D [25(OH)D] levels to establish the prevalence and predictors of hypovitaminosis D in CTD patients.

Methods: The medical records and laboratory results of 72 outpatients (63 female, 67 white) aged 18–75 years (mean age 47.8 years) attending the Addenbrooke’s Hospital CTD Clinic for 12 months from September 2008 were retrospectively reviewed. The data were analysed separately for individual CTDs: systemic lupus erythematosus (SLE), Sjögren’s disease (s.d.), systemic sclerosis (SSc) and mixed connective tissue disease (MCTD) and was compared with a healthy adult population. In line with previous studies, vitamin D insufficiency, deficiency and severe deficiency were defined as 25(OH)D levels of <75 nmol/l, <40 nmol/l and <25 nmol/l respectively.

Results: In the 72 subjects with CTD the mean 25(OH)D level and s.d. was 42.6 nmol/l (20.2). Sixty-six (91.7%), 37 (51.4%) and 15 (20.8%) patients were found to have 25(OH)D levels of <75 nmol/l, <40 nmol/l and <25 nmol/l, respectively. SLE, SSc, MCTD and s.d. were associated with an increased risk of vitamin D insufficiency, with relative risks (RR) of 2.05, 2.04, 2.30 and 2.04, respectively, compared with healthy controls. SLE (RR, deficiency = 2.57; RR, severe deficiency = 1.96), SSc (Relative Risk (RR) = 4.11; 8.05) and MCTD (RR = 3.52; 4.83) were associated with both an increased risk of vitamin D deficiency and severe deficiency. Calcium/cholecalciferol supplementation was associated with a reduction in this risk (RR = 0.44). Age over 65, female sex, tobacco smoking, photosensitivity and treatment with oral prednisolone or hydroxychloroquine were not associated with significant alteration of the risk of hypovitaminosis D. No significant seasonal variation in 25(OH)D levels was detected in the patients studied.

Conclusions: This study suggests that patients with CTDs, particularly SLE, SSc and MCTD, are at an increased risk of hypovitaminosis D. 25(OH)D levels should be routinely measured and the use of calcium/cholecalciferol supplements considered in patients attending CTD outpatient clinic.

The prevalence of vitamin D insufficiency, deficiency and severe deficiency compared with a healthy, southern-English adult population.

<table>
<thead>
<tr>
<th>25(OH)D Status</th>
<th>Percentage</th>
<th>Relative Risk (95% CIs)</th>
<th>P-Value</th>
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<td>Insufficiency</td>
<td>91.7</td>
<td>71.4</td>
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<tr>
<td>Deficiency</td>
<td>51.4</td>
<td>28.0</td>
<td>1.83 (1.39-2.41)</td>
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<tr>
<td>Severe Deficiency</td>
<td>29.2</td>
<td>7.6</td>
<td>3.83 (2.57-5.71)</td>
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</tbody>
</table>

Disclosure statement: All authors have declared no conflicts of interest.