3. Conditional deletion of GSK3β results in enhanced tissue repair and fibrogenesis in vivo via an endothelin-1-dependent mechanism

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Background: It has been previously hypothesized that Wntβ-catenin signaling pathway may play a key role in driving the fibrogenic responses in disease such as scleroderma. However, till date, no exact role of this pathway in driving fibrogenesis has been elucidated. To test this hypothesis, we targeted glycogen synthase kinase-3 (GSK-3), a key component of Wnt/β-catenin signaling pathway. GSK-3β normally phosphorylates β-catenin causing phosphorylation to be targeted for degradation. In the absence of GSK-3, β-catenin is translocated into the nucleus to activate transcription. To investigate the contribution of GSK-3β in fibrogenesis, we generated mice containing a fibroblast-specific deletion of GSK-3β and then subjected these mice to a model of wound repair.

Materials and Methods: To generate mice containing a fibroblast-specific deletion of GSK-3β, mice that carry a tamoxifen-inducible Cre-recombinase under the control of a fibroblast-specific regulatory sequence from the proα2(I) collagen gene were crossed with mice that carry homozygous conditional Lox-5/3 allele to generate Cre/ GSK-3β heterozygote mice. The second cross obtained Cre/GSK-3β mice. To delete GSK-3β, mice (age, 3 weeks) were given in injections of the tamoxifen and deletion of GSK-3β was tested by PCR. These mice were then subjected to the full thickness incisional model (skin punch biopsy) thermal wound healing the pre-inflammatory phase of wound was assessed on primary scleroderma fibroblasts. Results: Bleomycin treatment induced marked cutaneous thickening, inflammation and fibrosis in control mice. Deletion of Rac1 resulted in resistance to bleomycin-induced fibrosis and inflammation. Rac inhibition alleviated the persistent fibrotic phenotype of scleroderma fibroblasts.

Conclusion: Rac expression by fibroblasts is required for fibrogenesis. Inhibition of Rac1 may be a viable method to alleviate the development of cutaneous sclerosis.

4. The nuclear autoantigen centromere protein B (CENP-B) displays cytokine-like activities towards vascular smooth muscle cells

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Background: In vitro studies have demonstrated that some autoantigens have an additional biological function when they are released in the extracellular environment during injurious insults resulting in cell death. Indeed, it was previously suggested that the extracellular autoantigen releases during normal wound repair by cells like keratinocytes and/or chemoattractants and subsequently display pathogenic activities that contribute to the development of autoimmune diseases. Our present findings suggest that centromere protein B (CENP-B), a nuclear autoantigen specifically targeted in the limited cutaneous subset of systemic sclerosis (SSc), can be added to this list of bifunctional molecules.

Materials and methods: In SSc, autoantibodies to CENP-B are associated with the occurrence of prominent vascular manifestations such as pulmonary arterial hypertension (PAH), that in turn appears to be caused by intimal migration and proliferation of vascular smooth muscle cells (SMC). However the factors driving this vascular remodeling are unknown. Thus we examined the biological effects of extracellular CENP-B on human pulmonary artery smooth muscle cells (HPASM). Results: Purified CENP-B and CENP-B released from apoptotic endothelial cells bound specifically to the surface of HPASMC with a greater affinity for the contractile than for the synthetic type. CENP-B binding subsequently stimulated the migration of HPASMC in vitro, and stimulated the release of the pro-inflammatory cytokines and chemokines IL-6 and IL-8, respectively. The mechanism by which CENP-B mediates these effects involves the FAK, Src, ERK1/2, and p38 MAPK pathways. The induction induced by CENP-B was sensitive to peroxisome treatment, thus implicating one or several G protein-linked receptors in this process.

Conclusions: CENP-B has all the hallmarks of a bifunctional molecule that may participate in normal and pathogenic mechanisms where SMC are particularly involved. Our data support the concept that the primary role of autoantigens may be to alert the immune system to danger signals from invaded and damaged tissues to facilitate repair, thus promoting the occurrence of prominent vascular manifestations such as pulmonary arterial hypertension (PAH), that in turn appears to be caused by intimal migration and proliferation of vascular smooth muscle cells (SMC). However the factors driving this vascular remodeling are unknown. Thus we examined the biological effects of extracellular CENP-B on human pulmonary artery smooth muscle cells (HPASM). Results: Purified CENP-B and CENP-B released from apoptotic endothelial cells bound specifically to the surface of HPASMC with a greater affinity for the contractile than for the synthetic type. CENP-B binding subsequently stimulated the migration of HPASMC in vitro, and stimulated the release of the pro-inflammatory cytokines and chemokines IL-6 and IL-8, respectively. The mechanism by which CENP-B mediates these effects involves the FAK, Src, ERK1/2, and p38 MAPK pathways. The induction induced by CENP-B was sensitive to peroxisome treatment, thus implicating one or several G protein-linked receptors in this process.

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6. The prospective juvenile systemic sclerosis inceptions cohort

Ivan Foeldvari1, Jordi Anton2, Jeff Chaitow1, Eileen Baildam1, M. Hasegawa3, K. Takehara4, T. Matsushita1, T. Lehman5, Ivan Foeldvari1, Andreas Reiff6, Claudia T. L. Borg7, and Eloisa Bonfá7

Introduction: Juvenile systemic sclerosis (jSSc) is a rare disease. Currently just only retrospective data exist regarding organ involvement, and evolution of the disease, without standardized assessment of the patients. We developed a prospective assessment protocol for disease involvement, manifestations and progression of jSSc, which may become accepted as it presents the standard of a good clinical care.

Methods: Early jSSc patients, enrolled within 18 months after the first non-Raynaud symptom of the disease, will be followed over 36 months using a standardized assessment protocol. No specific therapy will be suggested. An Internet platform was created to make the project accessible. The demographic characteristics and pattern of organ involvement were evaluated to assess outcome.

From more than 1800 cases of SSc, 46 adults with jSSc were identified. The median age of onset was 13.06 years (range 5 to 16). 35 (76%) of the 46 patients were female. Median age at last visit was 32.67 years (range 16 to 71). The median disease duration was 21.15 years (range 3 to 58). 39% of the patients had a diffuse and 61% a limited subtype of SSc. 20 (43.5%) of the 46 patients showed overlap features of other connective tissue diseases, the most common overlap was with polymyositis in 10 of the patients. Three (6.5%) of the 46 patients had anticientromere antibodies. 12 (26%) of the 46 patients were anti-Scl 70 positive. The most common organ involvements were oesophageal in 33 patients (72%), pulmonary fibrosis in 22 patients (47%), bowel involvement in 9 patients (20%) and pulmonary hypertension in 7 patients (15%). Interestingly 7 patients (15%) did not have any major organ involvement beside skin and vascular involvement. The survival of the 46 patients after 15, 20 and 25 years was 97%, 93% and 83%. Seven of the 46 patients died during the observation period in the cohort. The disease remains active in these patients was 28.86 years (range 17 to 47).

This patient population has similar organ involvement and disease subtype characteristics as expected from an adult SSc cohort. It is interesting to see the high proportion of patients with overlap features. It is likely the study cohort of patients reflect a survival bias, representing the classical pauciarticular pattern of SSc but with under-representation the diffuse subtype due to higher early mortality of this subset. The antinuclear antibody pattern, with only 6.5% of all patients anticientromere positive, contrasts markedly with adults where this is a very common hallmark reactivity.

8. Mandibular function is severely impaired in systemic sclerosis patients

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Background: The temporomandibular (TMJ) has never been evaluated objectively and systematically in Systemic Sclerosis. Therefore, the objective of this study is to evaluate the TMJ function in SSc patients.

Methods: 35 SSc(ACR criteria) women and 30 age-sex-matched healthy-controls were selected. Helkimo’s index was performed and includes: anamnesis index(Ai) clinical dysfunction index(Di) and mandibular mobility index(Mi). Skin involvement was scored by the Modified Rodnan Skin Score(MRSS).

Results: Ai dysfunction was more frequent in SSc patients (80%) compared to controls (50%, p<0.001). The degree of Di was distinct in patients (8.6% normal, 48.6% mild, 22.8% moderate and 20% severe) and controls (50% normal, 33.3% mild 16.7% and moderate, p=0.001). Diffuse SSc patients (n=9) with moderate/severe Di had a trend of higher face MRSS score than those with mild (n=12) Di dysfunction (p=0.06). More than 80% of the SSc patients with severe Di dysfunction (86%) were on cyclophosphamide treatment (cutaneous fibrosis), contrasting with the remaining patients (p=0.001). Abnormal Mi index was universal in SSc patients and more frequent than controls (100% vs. 66.7%, p<0.001). The Mi dysfunction was severe in 77.1% and mild in 22.9% of the cases contrasting with controls (13.3% severe, 53.3% mild and 33.3% normal). p<0.001. Approximately half of SSc patients with severe Mi index (47%) were on cyclophosphamide treatment (cutaneous fibrosis), contrasting with the mild group (p=0.02).

Conclusion: Temporomandibular dysfunctions are very frequent in SSc and are possibly related to skin fibrosis. The concept that this clinical problem involves more than the TMJ and mastigatory muscles is certainly relevant for future therapeutic strategies.
9. Caveolin-1 scaffolding domain peptide inhibits the monocyte to fibrocyte differentiation of normal and scleroderma peripheral blood mononuclear cells

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Background: Peripheral blood mononuclear cells (PBMC) play an important role in inflammation and fibrosis. They also are involved in fibrosis by serving as progenitors for fibrocytes, a circulating population of collagen-expressing cells that enter tissues and differentiate into fibroblasts. We previously showed that caveolin-1 is a key signaling molecule in the regulation of collagen expression by scleroderma lung fibroblasts. In the current experiments we have determined that caveolin-1 is also a key signaling molecule in the differentiation and function of monocytes and fibrocytes in scleroderma lung disease patients.

Materials and Methods: PBMC were isolated from the blood of healthy volunteers and scleroderma patients, and treated with the CSD (caveolin-1 scaffolding domain) peptide or scrambled, control peptide. Caveolin-1 and signaling molecules activities/levels were determined by Western Blotting. MMP-9 secretion levels were determined by gelatin zymography. Monocyte to fibrocyte differentiation was evaluated by morphology and flow cytometry.

Results: 1) Less caveolin-1 is present in PBMC isolated from scleroderma patients than in these cells from healthy volunteers (caveolin-1 levels were only 41±5% as high in scleroderma PBMC as in normal cells), and the expression/activity of MAP kinases family members regulated by caveolin-1 is also altered. 2) Activation of normal monocytes with TNFα and TGFβ decreases the expression of caveolin-1 and increases the activation of ERK, JNK, and p38 kinases. 3) CSD peptide treatment inhibits TGFβ-induced MMP-9 secretion in normal monocytes and in normal and scleroderma fibrocytes. 4) Fibrocytes derived from the PBMC of scleroderma patients exhibit low caveolin-1 and high ASMA levels compared with fibrocytes isolated from the PBMC of healthy individuals. 5) The percentage of scleroderma monocytes that differentiate into fibrocytes in vitro is enhanced two-fold compared to normal monocytes. 6) CSD peptide treatment inhibits the transformation of normal and scleroderma monocytes to fibrocytes.

Conclusions: These observations suggest that the low level of caveolin-1 present in several cell types in scleroderma patients plays an important role in the progression of inflammation and fibrosis, and that the CSD peptide can provide remarkable protection against inflammation and fibrosis.

10. Cardiac conduction and morphological changes by electrocardiogram (ECG) in patients with systemic sclerosis (SSc) related interstitial lung disease (ILD)

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Methods and Patients: ECG findings of 163 patients with SSc and ILD recruited for a multi-center trial were reviewed. The mean subject age was 52.3 years ± 11.6, male: female ratio was 1.3:1, mean disease duration was 6.4 years ± 6.5. Of the 163 subjects, 95 had diffuse SSc (58%) and 68 (42%) were classified as limited SSc. Subjects had ILD by HRCT and were enriched for "active" ILD by using worsening FVC, Dlco and dyspnea criteria. Standard 12 lead ECG was recorded at rest with the patient in supine position for 5 minutes at randomization. Heart rate (HR), PR, QT, QT corrected for HR (QTC) and QRS intervals were recorded. ECG abnormalities related to rate, rhythm, morphology and repolarization were recorded. Screening echocardiography was utilized to exclude overt pulmonary arterial hypertension.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Sinus Bradycardia</th>
<th>First degree atrio-ventricular block</th>
<th>Intraventricular conduction delay</th>
<th>Right bundle-brach block</th>
<th>Left bundle-brach block</th>
<th>Left anterior hemiblock</th>
<th>Left ventricular hypertrophy</th>
<th>Left atrial hypertrophy</th>
<th>Right ventricular hypertrophy</th>
<th>Low voltage</th>
<th>Nonspecific ST/T wave abnormality</th>
<th>T wave inversion</th>
<th>Myocardial infarction sequela</th>
<th>Atrial ecotpy</th>
<th>Ventricular ecotpy</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.40</td>
<td>1.25</td>
<td>2.50</td>
<td>1.25</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>3.75</td>
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<td>5.75</td>
<td>5.00</td>
<td>2.50</td>
<td>1.25</td>
<td>4.50</td>
</tr>
</tbody>
</table>

N = 159

Background: Cardiac involvement in SSc is complex and includes pericardial disease, myocardial disease and disorders of rhythm and conduction. Electrocardiographic evaluation is a non-invasive and accessible measure. Its utility, sensitivity and specificity have not been established in well characterized SSc populations.

Results: 159 ECGs were available for analysis: mean HR was 76 bpm ± 12, mean PR interval was 157 msec ± 27, mean QT interval was 375 msec ± 31, mean QTc interval was 406 msec ± 50. The percentage of patients with ECG evidence of left axis deviation (LAD) was 75% of the abnormal ECGs related to conduction defects. Measures reflective of cardiac morphology (e.g. LVH, LAH, RVH) were noted in 22% of the abnormal ECGs. It is important to note that our protocol excluded pulmonary hypertension at all types and stage IILD (10% VAS-Breath). In this population felt to be representative of early, active SSc-ILD, ECG conduction abnormalities are common and may reflect concomitant myocardial involvement.

11. The utility of the SHAQ-DI and VAS-breathe as a subjective rating of respiratory function in systemic sclerosis (SSc) patients with interstitial lung disease (ILD)

Ann J. Impens, Elena Tushkowski, Kristine Phillips, Suparaporn Wangkawee, and James R Seibold for the BUILD-2 Investigators, Scleroderma Program
University of Michigan, Ann Arbor, Michigan, USA.

Background: Several physiological and subjective measures exist for the measure of pulmonary involvement in SSc. These outcome measures have not been fully validated. This study examined the utility of 6MWT, SHAQ-DI, and VAS Breathing.

Methods and Patients: 163 patients with SSc-ILD participated in a multi-center, randomized double-blind clinical trial. Baseline data is presented for the subgroup of 86 patients whom were randomized to the placebo group. Data gathered included: 6MWD, FVC, Dlco, Borg dyspnea, and SHAQ-DI. 39 (43.5%) had Limited and 47 (54.7%) diffuse SSc. 64 (74.6%) were female. Mean age was 54.5 ± 11 and disease duration 5.2 ± 6.6. Mean distance walked was 404.85m (SD 86.3m).

Results: Highest correlation with 6MWD was found with SHAQ-DI (-.48, p<.001). A regression analysis of the following variables: SHAQ-DI, 6MWD, BORG, weight, height, and age. When plotting the residuals 2 distinct subgroups were found: those with SHAQ-DI score above/below 1.50. Although only 16 patients fell into the category >1.5, t-test analyses showed that these groups were significantly different on the following variables: 6MWD (M= 418.2m, SE=19.05 vs M=350.4m, SE=19.16), Borg (2.46, SE=.24 vs M=3.94, SE=.62), SHAQ-Activity (M=.79, SE=.08 vs M=2.56, SE=.15), VAS Breathing (M=.53, SE=.34 vs M=.94, SE=.10), Dlco (M=4.2, SE=.13 vs M=3.61, SE=.19), FEV1(M=2.17, SE=.08 vs M=1.7, SE=.09), and FVC (M=2.65, SE=.10 vs M=2.14, SE=.14).

Correlations between 6MWD, SHAQ-DI, VAS Breathing, and some objective measures of lung function for the total group were:

<table>
<thead>
<tr>
<th>6MWD</th>
<th>FVC</th>
<th>Dlco</th>
<th>Borg</th>
<th>SHAQ- Activity</th>
<th>SHAQ- Breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>.26</td>
<td>-.20</td>
<td>-.29</td>
<td>-.33</td>
<td>-.53</td>
</tr>
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</table>

*P <.01 (2-tailed), **P <.05 level (2-tailed)

Conclusion: 6MWD had significant correlations with all other measures in this study except Dlco (selected results shown). The highest correlation for 6MWD with SHAQ-DI. This functional outcome measure correlated as high or higher with measures of lung functioning than the physiologic lung measures. The SHAQ-DI has the potential to be an inclusion criterion for clinical trials focused on cohort enrichment.
12. Therapeutic angiogenesis by local autologous progenitor cell implantation for ischemic digits in patients with systemic sclerosis

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Background: Microvascular abnormality is the most common finding in systemic sclerosis (SSc) that causes reduced blood flow and tissue ischemia, leading to digital ulcers. Insufficient vascular repair has been recently proposed to contribute to this process, thereby autologous progenitor cells implanted to promote angiogenesis could be a promising therapeutic strategy for SSc ischemic complications.

Objectives: This study was aimed to evaluate the short-term and long-term (3-6 months) effects of local bone marrow CD34-positive and mononuclear cell (MNC) implantation on clinical, functional and morphological characteristics of peripheral vascular disease in SSc patients.

Methods: Five dSSc patients with multiple intractable digital ulcers in both lower and upper extremities were treated with bilateral local injections of CD34-positive cells from peripheral blood (PB) after mobilization by G-CSF (case 1) and bone marrow (BM) (case 2,3,4,5) for ischemic skin ulcers in hands, while MNCs were implanted in lower extremities of the same patients. Ischemic status was evaluated by measuring ulcer healing, Raynaud’s Condition Score (RCS), visual analog pain, Raynaud’s phenomenon (RP) and ulcer scales. To evaluate vasculoprotective action of the implanted cells, we studied weekly during the first month and monthly later the changes in endothelial function, using measurement of flow-mediated brachial artery reactivity by high resolution ultrasonography and vascular endothelial injury markers, circulating endothelial precursors (CD34+VEGFR2, CD133+VEGFR2+ CEP) by FACS analysis, cutaneous blood flow (laser Doppler flowmetry), skin surface temperature (thermograph), peripheral arterial diameter and blood flow characteristics by Duplex ultrasonography, morphological signs of microangiopathy by nailfold videocapillaroscopy.

Results: Both CD34-positive cells (1.1±0.3 x 10⁶/patient, purity of selection using the M.A.C.S. technique – 91.3-96%) and MNCs showed rapid and evident beneficial effect on vascular symptoms in SSc patients: remarkable decrease in daily frequency and duration of RP attacks, RCS, VAS for RP, ulcers and pain. 15 out of 18 ulcers were completely healed and the mean surface area of the ulcers significantly decreased. Physical function and disability measured with HAQ and SHAQ improved in line with improved hand function (decreased finger-palm and increased interdigital indices).

Therapeutic efficacy of stem cell therapy was associated with restoration of altered endothelial function and significant (150 fold) increase in blood levels of early im

14. An objective method of measuring skin elasticity in systemic sclerosis: Results from a pilot study

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2University of Dundee, Department of Mechanical Engineering, Dundee, DD1 9SY, Scotland, UK.

Background: Systemic sclerosis (SSc) is an autoimmune disease with characteristic fibrosis of various organs, including the skin. The modified Rodnan score, used to assess skin involvement, is a subjective and user dependent method. As prognosis and response to therapy can be evaluated by assessing skin involvement, it is important to have an objective and reproducible technique available to measure this. The aim of this study was to test a newly designed skin torsion device in measuring skin elasticity in patients with systemic sclerosis as compared to healthy controls.

Material and Methods: 16 patients with systemic sclerosis and 71 healthy controls were recruited for the study. Skin elasticity was measured on the back of their hands and forearms with the newly designed hand held portable device. The device gently rotates the skin for 15 seconds to a maximum of 40 degrees. Total and localised (back of hands and forearms) modified Rodnan scores were also assessed.

Results: A statistically significant difference in the skin elasticity of the hands (1.9 degrees/second versus 2.3 degrees/second, Mann Whitney p<0.001) and forearms (1.9 degrees/second versus 2.5 degrees/second, Mann Whitney p<0.0001) was observed between patients and controls. On doing further linear regression analysis, the only significant predictor of the skin elasticity scores was having the disease.

Conclusion: In the present pilot study we found the portable skin torsion device to be a reliable non-invasive method that can be readily used in patients with systemic sclerosis to assess skin involvement. Further work is planned in this area.
15. Adenosine A2A receptor occupancy promotes dermal fibrosis by modulating IL-13 and Fli1 expression

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We have previously reported that adenosine enhances dermal matrix production. Adenosine A1 receptor (A1R)-deficient mice are resistant to dermal fibrosis stimuli such as bleomycin. To further clarify the mechanisms by which A1R receptor stimulation induces dermal matrix accumulation, we explored the effects of A1R receptor occupancy on key fibrogenic mediators in the dermis.

IL-13 is a potent fibrogenic cytokine. IL-13 levels were significantly increased in human and mouse dermal fibroblasts (DF) (real-time PCR), and IL-13Rα1 mRNA expression was increased by CGS21680 in membrane cell preparations from DF (Western blot, 24 hrs).

In vivo, treatment of ADA KO mice with ZM24385 significantly decreased IL-13Rα1 mRNA (p<0.01).

Fli1 is a known transcriptional regulator of fibrillar collagen genes and connective tissue growth factor (CTGF/CCN2) in DF. A1R stimulation with CGS21680 (10μM) suppressed Fli1 mRNA expression by 47±4.18% (vs. control) in DF nuclear extracts (4hrs, real-time PCR, n=4, p<0.05). Fli1 protein was also significantly reduced by CGS21680 (24hrs, 31.9±2.8% reduction, Western, n=5, p<0.05). Furthermore, IL-13 also stimulates a 38±7% (p<0.05, n=4) reduction in Fli1 protein in the nucleus.

Conclusion: A1R occupancy promotes dermal matrix production by (1) inducing the expression of the profibrogenic cytokine IL-13, (2) suppressing expression of the transcriptional repressor Fli1 in DF, and (3) augmenting CCN2 secretion by DF. These findings suggest that modulation of A1R function may be a novel therapeutic approach to limit dermal fibrosis as seen in conditions such as scleroderma.

16. Dermal recruitment following injury requires integrin-linked kinase

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The extracellular matrix is a key regulator of cell functions. Binding of matrix macro-molecules to integrins initiates the assembly of an intracellular multiprotein complex, the focal adhesion, of which integrin-linked kinase (ILK) is a central component. ILK binds to the intracellular tail of β1 integrins and recruits adaptor proteins, thus connecting the outside environment to the actin cytoskeleton. Focal adhesions are force-transmitted structures important for cell adhesion/migration and to counteract stress from the environment, such as during the contraction of wound granulation tissue. We showed previously that fibroblasts plated on collagen require integrin α2β1 for proper focal adhesion architecture and collagen lattice contraction in vitro. However, in mice, formation and contraction of granulation tissue proceed largely unaltered in the absence of collagen-binding integrin β1 (aSMA+/Cre;α2β1fl/fl) mice. By contrast, absence of ILK in fibroblasts profoundly not only disturbs focal adhesion interactions and in conveying environmental force cues.

17. Gravitational Stress in Linear Scleroderma of limbs

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Background and Rationale: Linear Scleroderma (LS) is the most common morphea subtype in children and young people. LS is a cutaneous disease of unknown etiology. The pathogenesis is not clear but the process of disease involves vascular damage, immunological mechanisms and fibroblast activation. It is a disorder that characteristically causes skin induration and pigmentary changes in a linear distribution, which runs down an arm or a leg. LS may involve the deep dermis, subcutaneous tissue, muscle and the underlying bone causing in some cases severe limb deformities, contractures and functional disabilities. In previous studies we have demonstrated that the endothelium critically situated at the blood-tissue interface, is an important target for gravitational stress (GS), which constitutes a mechanical load that the vessel wall that enhances Prostaglandin (PGI2) and nitric oxide (NO) synthesis. No therapy is universally accepted for the fibrotic stage of scleroderma. The evidence that EDRFs can regulate fibroblast properties led us to the present study.

Material and Methods: Seven cases of linear scleroderma involving limbs were reported (5F, 2M). The mean age at the entrance to GS protocol was 15±7 (range, 6 to 28 years). Disease duration at the entering ranged from 1 to 7 years. Clinical examination: The upper limb was involved in 3 patients, the lower limb in 4 pts and both upper and lower limbs ipsilateral in one patient. Linear scleroderma involving fat, fascia, muscle, and bone, was presented in two patients. Linear scleroderma and morphea coexisted in one patient. Joint contractures, arthralgias, flexion contractures, limb atrophy, leg length discrepancy, limb pain and impaired functional capacity were presented in four patients. Six patients had not received any treatment prior to entering the study. A pediatric patient, a 12 years old girl, with severe LS involving her left lower leg, confirmed by skin biopsy, underwent low-dose prednisone daily during 15 months prior the entrance to gravitational stress therapy. The main outcome measure was clinical evaluation before and after treatment using a skin severity score (SSS) to grade regional skin thickness and mobility (0 to 3 point scale). Laboratory and radiological studies were also done. The Ethics Committee of the Center approved the study and all patients or parents gave their informed consent.

Gravitational Therapy Procedure: All patients were placed on the couch of a human centrifuge in supine decubitus. After a week of training, all patients were exposed to different acceleration and deceleration profiles from 1 to 6.5 g’s, from head to feet (+GZ) as previously described, during one hour, two times per week, over three months.

Results: From the onset of GS treatment, the disease stopped progressing in all patients. Improvement in skin score from the patients was strongly associated with significant softening of sclerosis and functional recovery of limbs. A significant improvement regarding mobility and muscle strength, hand function, joint contractures and overall functional mobility of limb was also observed. Patients involved in gravitational therapy were followed-up over a period of 5±2.7 years (range 2 to 10 years).

Conclusions: Gravitational Stress as a therapy provides improvement in the management of linear scleroderma, reduces contractures and improves functional disabilities of lower or upper limbs related to pain, joint involvement, muscle force, growing and inducing the functional recovery of limbs. These data suggest that GS as a therapy is beneficial and safe in the treatment of patients with LS.

18. Cardiac involvement in systemic sclerosis

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Background: Systemic Sclerosis (ScS) is a multisystemic disorder characterized by the fibrosis of the skin and internal organs. Patients with ScS may have cardiac involvement, but no cardiac symptoms. Objective: to evaluate the frequency and type of cardiac involvement and other organs in a population of patients with ScS.

Materials and Methods: Two hundred patients (176 F, 24 M, mean age 49 ± 15 years) who fulfilled the criteria for systemic sclerosis according the American Rheumatism Association criteria were included in the study. According to skin involvement 59 patients had diffuse cutaneous ScS (involvement of the skin over 75% of the total body surface), 141 pts SSc (involvement of the extremities) and 141 pts had limited cutaneous involvement (the extremities distal to the elbows and knees + face) according the criteria of LeRoy et al. Screening of cardiac involvement included 12 lead ECG, 24-h ECG Holter monitoring and echocardiography with echo-Doppler. Lown classification for ventricular premature beats (VPB) was used.

Results: The female/male ratio was 78:1, the mean age at onset of symptoms was 44 (range 1-82), and the mean age at recruitment was 49 years (range 2-85). The duration of the disease was 8±7 years (range 9 months - 35yrs). In this population Raynaud’s phenomenon was present in 182 pts (91%), digestive tract disorders evidenced by endoscopic studies in 134 (67%), lung affection evidenced by CT chest and pulmonary function tests in 44 (22%), joint affection in 106 (53%), high blood pressure in 23 (11.5%), diabetes mellitus in 3 (1.5%). Only 6 pts (3%) had cardiac symptoms. Abnormal ECG was seen in 47 patients (23%) and abnormal echocardiograms in 24 patients (12%). ECG abnormalities included conduction system disturbances 21 (10.5%), low voltage in 3 (1.5%), signs of infarction 2 (1%), non-specific ST and T-wave changes in 11 (5.5%), supraventricular arrhythmia in 20 pts (10%), atrial premature beats (APB) were found in 19 pts (9.5%) and paroxysmal supraventricular tachycardia in 1 pt (0.5%), ventricular arrhythmia in 33 pts (16.5%), simple ventricular premature beats (VPB-Lown I) in 24 pts (12%), bigeminy of VPB Lown 2 pts (1%), multiformes of VPB Lown 4 pts (2%), complexed arrhythmias (> II ) 1 patient, coupled VPBs Lown IVa in 2 pts, ventricular tachycardia Lown IVb in 1 patient. In one patient of 27 years old with diffuse SS, syncopep palsies, episodes left posterior and right heart bundle block and a permanent pacemaker was implanted. Echocardiographic abnormalities included: LHV in 11 (4%); RVH in 5 pts (2.5%) secondary to pulmonary hypertension, or diastolic dysfunction in 10 pts (5%) and pericardial effusion in 11 pts (5.5%).

Conclusions: Patients with ScS may have cardiac involvement, but no cardiac symptoms. Our study suggests that ECG and ECHO examinations were valuable methods for detection of clinically silent cardiac involvement in patients with systemic sclerosis.

19. Increased prevalence of obstructive sleep apnea in patients with systemic sclerosis and pulmonary hypertension

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Background: Obstructive sleep apnea (OSA) can lead to pulmonary hypertension (PH) or increase its severity when PH is already present. While many PH centers screen for OSA when faced with a new PH diagnosis, suspicion for OSA in systemic sclerosis (SSc) and PH is lower. Our center's experience suggests that the prevalence of OSA in patients with SSc may be higher than expected compared to the general population.

Methods: A large academic PH center database (1/00-7/07) was reviewed. Patients fulfilling criteria for PH by right heart catheterization (RHC) or echocardiogram were included. OSA was defined as apnea hypopnea index (AHI) >5/hr. Data are presented as mean ± SD.

Results: We identified 43 patients (38 female, average age 62, range 40-79) with PH and OSA. 35 patients were examined by RHC in 3 patients and by echocardiogram in 10. As part of their initial PH workup, a sleep study was performed in 16 patients based on suspicion for OSA (snoring, witnessed apneas, obesity and/or daytime sleepiness). OSA was present in 13 of them with 30% prevalence for the entire cohort. Mean AHI was 11.6, SD 6.1 (range 5-22). Mean body mass index for the OSA group was 34.3, SD 6.0 (range 22-45) compared to 33.8, SD 9.4 (range 24-43) for those in whom sleep study was not included OSA.

Conclusions: Compared with a 2-4% OSA prevalence in the general population, the prevalence of OSA in this select group of SSc with PH appears to be higher than expected. Given that OSA carries an increased risk for cardiopulmonary complications—hypoxemia, pulmonary hypertension, and diastolic dysfunction—clinicians treating these patients should have a lower threshold to diagnose and treat this comorbid condition. Additional studies to define the incidence/prevalence of OSA in patients with SSc and PH, as well as factors which predict the presence of this disorder are warranted.

20. Associations with in-hospital mortality of scleroderma patients from 1990 to 2006

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Background: Studies examining clinical associations of in-hospital mortality in scleroderma patients have yielded varied results, although have examined periods of only one to two years. Literature from single-center scleroderma cohorts have suggested that causes of death in have changed over recent decades. We hypothesized that earlier studies may become more resistant following a prolonged low-level exposure to silica. The mechanisms by which certain chemicals may cause SSc are unknown. Dermal fibroblasts cultured from SSc patients show an altered phenotype that includes the over-expression of collagen and a reduced susceptibility to Fas-induced apoptosis, both of which may exacerbate fibrosis. Fas-resistant populations of fibroblasts can be selected using repeated cytokine treatment. Treatment of cells with silica has previously been reported to induce expression of transforming growth factor (TGF)β and other cytokines which may provide a pathway from exposure to resistance. The hypothesis of this study was that repeated treatments with silica may be able to induce/ activate this resistant fibroblast population.

Materials and Methods: MRC-5 human lung fibroblasts were treated repeatedly with medium alone (negative control), quartz silica, sonicated quartz silica (disrupted by ultrasound), or CH11 (an antibody stimulating Fas-induced apoptosis, positive control). Cells were treated at a concentration that was previously determined to cause 50% cell death. An MTT cytotoxicity assay was carried out following each treatment to determine the level of Fas-induced cell death in response to CH11.

Results: Following repeat treatments, average cell death induced by CH11 varied between cells treated with medium only and medium plus chemical. The cell death induced in medium treated cells was 34.5% ±4.0%, unsonicated silica 35.5% ±6.0%, sonicated silica 22.8% ±2.8% and CH11 23.6% ±1.8%. The results for sonicated silica and CH11 are statistically significant compared to the medium-treated control, with p<0.01. This effect was apparent following a minimum of two treatment cycles.

Conclusions: 1. An increased level of resistance to apoptosis was induced or selected for by treating repeated treatments with sonicated silica compared to unsonicated silica or medium only.
2. This suggests a possible mechanism by which cells in genetically susceptible individuals may become more resistant following a prolonged low-level exposure to silica.
3. Sonication exposes fresh surfaces of the silica, resulting in a greater production of reactive oxygen species, which is a possible cause of this effect.

21. Silica-induced resistance of cultured fibroblasts to Fas-induced apoptosis: implications for scleroderma

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Background: Systemic sclerosis (SSc) is a rare autoimmune disease that has been associated with exposure to specific chemicals, particularly silica and certain solvents. The mechanisms by which certain chemicals may cause SSc are unknown. Dermal fibroblasts cultured from SSc patients show an altered phenotype that includes the over-expression of collagen and a reduced susceptibility to Fas-induced apoptosis, both of which may exacerbate fibrosis. Fas-resistant populations of fibroblasts can be selected using repeated cytokine treatment. Treatment of cells with silica has previously been reported to induce expression of transforming growth factor (TGF)β and other cytokines which may provide a pathway from exposure to resistance. The hypothesis of this study was that repeated treatments with silica may be able to induce/activate this resistant fibroblast population.

Materials and Methods: MRC-5 human lung fibroblasts were treated repeatedly with medium alone (negative control), quartz silica, sonicated quartz silica (disrupted by ultrasound), or CH11 (an antibody stimulating Fas-induced apoptosis, positive control). Cells were treated at a concentration that was previously determined to cause 50% cell death. An MTT cytotoxicity assay was carried out following each treatment to determine the level of Fas-induced cell death in response to CH11.

Results: Following repeat treatments, average cell death induced by CH11 varied between cells treated with medium only and medium plus chemical. The cell death induced in medium treated cells was 34.5% ±4.0%, unsonicated silica 35.5% ±6.0%, sonicated silica 22.8% ±2.8% and CH11 23.6% ±1.8%. The results for sonicated silica and CH11 are statistically significant compared to the medium-treated control, with p<0.01. This effect was apparent following a minimum of two treatment cycles.

Conclusions: 1. An increased level of resistance to apoptosis was induced or selected for by treating repeated treatments with sonicated silica compared to unsonicated silica or medium only.
2. This suggests a possible mechanism by which cells in genetically susceptible individuals may become more resistant following a prolonged low-level exposure to silica.
3. Sonication exposes fresh surfaces of the silica, resulting in a greater production of reactive oxygen species, which is a possible cause of this effect.

22. First analysis of complex metabolic composition of synovial fluid in scleroderma by proton magnetic resonance spectroscopy

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Background: Magnetic resonance spectroscopy (MRS) is suitable for the simultaneous detection and measurement of the metabolic components of a biologic sample. Because of the difficulty of synovial fluid prevalece in scleroderma, previous studies have given little information and we tried to depict possible correlation between disease’s pathogenesis and complex composition of synovial fluid.

Materials and methods: We realized the first analysis of the complex metabolic environment of the joints affected by diffuse scleroderma in 5 patients with knee and elbow arthritis in comparison with rheumatoid arthritis (RA) analysis. Our previous studies used MRS method for the simultaneous detection and measurement of the metabolic composition of synovial fluid in different pathologies. NMR spectra have been recorded on a Bruker 400 MHz spectrometer.

Results: This method led to the possibility to attribute the signals for glutamine, threonine, lactate, hydroxybutyrate, glycine, dimethylamine and lipoprotein-associated fatty acids, ceramide and citrulline in synovial fluid. We have shown extremely weak signal intensity for citrulline at 3.15 ppm (highly specific for RA synovial fluid - sensitivity 80%, specificity 100% in our previous study, n=3/patients) and decreased concentration of glucose. Despite the difficulty of performing statistical analysis the overall aspect of synovial spectrum is completely different from RA.

Conclusions: MRS investigation of synovial fluid provides valuable information concerning in simultaneous detection and measurement of the metabolic composition of synovial fluid in different pathologies. NMR spectra have been recorded on a Bruker 400 MHz spectrometer.

Reference: This study excluded OSA.
23. Two dimensional tear protein electrophoresis for differential diagnosis of ocular manifestation in scleroderma

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Background: The eye is frequently involved in patients with scleroderma. Most often, this involvement consists in cutaneous abnormalities of the eyelids, resulting in tightness of the lids and blepharophimosis. Also, keratoconjunctivitis sicca (KCS) with a Sjogren’s-like picture has been described. Our study analyzed and compared electrophoretic tears patterns of normal subjects and patients with scleroderma with and without clinical manifestations of KCS. Thus, we try to determine the disease biomarkers that can be used for noninvasive diagnosis of KCS in patients with scleroderma.

Materials and methods: We analyzed three groups of subjects: patients with scleroderma without KCS (n=6), patients with scleroderma with KCS (n=6) and a control group comprising healthy volunteers (n=10). Tears were sampled using the Schirmer method. Tears were eluted in 40 microl, of elution solution containing sodium dodecyl sulphate, urea, EDTA, beta-mercaptoethanol and bromphenol blue.

Tear proteins were separated by two-dimensional electrophoresis (in the combination of isoelectric focusing with sodium dodecyl sulphate-polyacrylamide gel electrophoresis), and protein bands were stained with silver.

Results: We have depicted more than 20 bands, main components (tear-specific prealbumin, lactoferrin, lysozyme, secretory immunoglobulin A and immunoglobulin G) being identified using a marker of molecular weight. Isoelectric points of all proteins separated were determined by comparison with isoelectric point standards. The densitometric analysis of electrophoretic lanes was performed with ordinary flat scanner. The tear protein patterns of patients with scleroderma and KCS are different in number and intensity of spots from those of healthy subjects. Patients with KCS had decreased levels of lysozyme and lactoferrin in tears (p<0.03; p<0.05 at densitometric qualification).

Conclusion: Two-dimensional electrophoretic analysis of tear protein patterns of patients with scleroderma is a fast, reproducible and simple method that provided information for a rapid diagnosis of KCS in these patients.

24. Predictive value of microvascular imaging techniques for SSC-spectrum disorders

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Background: Systemic sclerosis (SSc) affects both microvascular structure and function. Laser Doppler and thermal imaging can be used to measure cutaneous blood vessel function. Nailfold capillaroscopy (NC) measures capillary morphology. The aim of this study was to investigate the relationship between capillary morphology and blood flow, and to determine which combination of techniques allows best discrimination between patients with SSc, primary Raynaud’s phenomenon (PRP) and healthy controls (HC).

Methods: Following acclimatisation, NC was performed in 16 patients with SSc, 14 with PRP and 16 healthy controls. In addition, participants underwent cold stimuli with cold water (15 °C, one minute). Hands were imaged for 15 minutes to monitor re-warming and reperfusion. Nailfold morphological features (width, density and tortuosity) were measured and baseline images and re-warming curves analysed (area under the curve, maximum temperature/blood flow after re-warming, initial gradient).

Results: Significant differences were found between groups (ANOVA) for capillary morphological features and re-warming curve characteristics. Correlation (p<0.001) was found between laser Doppler and thermal imaging for baseline (r=0.667) and maximum (r=0.729) blood flow and skin temperature and for areas under the re-warming curves (r=0.684).

ROC curves generated using logistic regression indicated that nailfold microscopy, thermal imaging and laser Doppler imaging allowed 89%, 74% and 72% respectively of SSc patient data to be correctly classified versus PRP patients and controls. A combination of all three techniques gave positive and negative predictor values of 93% and 94%.

Conclusions: Nailfold microscopy, thermal and laser Doppler imaging each independently provide good discrimination between patients with SSc and those with PRP and healthy controls. Laser Doppler and thermal imaging give equivalent information on dynamic changes in the cutaneous microcirculation, however these only weakly correspond to capillary morphology. Nailfold microscopy is the most suitable technique for classifying patient groups; however classification is further improved if all three techniques are combined.

25. A 3 month prospective study of nailfold capillary architecture changes with time


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Background: Structural microvascular disease is a hallmark of systemic sclerosis (SSc)-spectrum disorders and is well demonstrated using the technique of nailfold microscopy. We have developed software to allow automated measurements of nailfold microvasculature architecture (width, density, tortuosity and derangement). It is not known over what time period SSc related microvascular derangement evolves nor whether changes also occur in healthy controls. The aim of this sub-study was to assess changes in nailfold capillary architecture over a three month period, as part of a larger 2 year prospective study.

Methods: 19 patients with primary Raynaud’s phenomenon (3 male, median [range] age 41 [21 to 74] years), 12 patients with undifferentiated connective tissue disease (UCTD), all female, 51 [33 to 68] years), 38 patients with SSc (32 with limited cutaneous and 6 with diffuse cutaneous subtypes, 8 male, 55 [31 to 74] years) and 31 healthy controls (11 male, 42 [26 to 70] years) were recruited into the study. All patients were asked to refrain from smoking and caffeine for 4 hours prior to examination. Following acclimatisation for 20 minutes at room temperature (23°C), the ring finger nailfold of the non-dominant hand was imaged with video microscopy at 300x magnification. Features were extracted from images by automated software.

Results: As previously shown, capillary density and dimensions differed between diseased and healthy control groups. There were no significant changes within any of the 4 subject groups over the 3 month period.

Conclusions: 1) No within group changes were observed over 3 months, however, some individuals did demonstrate change over 3 months; 2) The computerized nailfold system was able to track changes in capillaries over time, lending further support to its potential as an outcome measure of microvascular change; 3) Longer term studies, in progress, will clarify the nature of microvascular disease progression in patients with SSc-spectrum disorders.

26. Mechanism of action of agonistic PDGFR auto-antibodies isolated from scleroderma patients

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Background: Systemic sclerosis (SSc) is characterized by fibrosis of the skin and visceral organs. We have identified stimulatory IgG auto-antibodies to the PDGFR receptor that are capable of converting normal fibroblasts into SSc-like cells inducing excess oxygen species (ROS) production by activating membrane NADPH oxidase complex. In order to elucidate the earliest mechanism involved in the molecular pathogenesis of systemic sclerosis we characterize the interactions between PDGFR and other components of the active NADPH system in the presence of PDGFR or SSc IgGs and the functional role of the lipid raft in the formation of the complex.

Material and Method: The receptor activation by PDGF or SSc IgGs was monitored immunoprecipitation of total cellular extract of stimulated fibroblasts with anti PDGFra antibodies and Western blotting with anti-phospho-tyrosine antibody. The interaction between PDGFR and NADPH oxidase complex subunits was evaluated by immunoprecipitation with anti PDGFR, anti gp91phox and anti Ha-Ras antibodies. For the membrane localization of the complex we pre-treated the cells with b-cyclodextrin for the cholesterol depletion of membrane and assayed the sample for tyrosine phosphorylation.

Results: We have found that SSc IgGs from several different patients selectively immunoprecipitate gp91phox in addition to PDGFR/PDGFR autoantibodies stabilized the interaction between PDGFR and the NADPH oxidase subunit, which is otherwise assembled transiently in response to PDGF, and lead to permanent activation of the signalling cascade. The receptor gets stabilized by these antibodies and form a stable membrane complex protected from degradation. This interaction is localized in the lipid compartment of the plasma membrane (lipid raft) and contains also Ha-Ras protein. Prolonged ROS production can be explained by the persistence of the receptor in the membrane bound to the NADPH subunit, gp91phox.

Conclusions: These data illustrate how stimulatory auto-antibodies from SSc patients are able to induce prolonged ROS production. SSc IgGs prevent down regulation of PDGFR receptor and lock the receptor in the active configuration. This results in persistent ROS production and appearance of the disease pathological signatures.
27. Reproducible and stable subsets in serial skin biopsies taken from patients treated in an open-label trial of rituximab

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Background: The purpose of this study was to assess the response of patients with diffuse systemic sclerosis (dSSc) to rituximab therapy, using genome wide gene expression profiling of skin biopsies. The other purpose of this study was to further characterize the global gene expression of dSSc skin to extend and validate previous results.

Methods: Using whole-genome DNA microarrays, gene expression was measured in skin biopsies from 13 patients with early diffuse cutaneous SSc (dcSSc), and 4 healthy controls. Both lesional and non-lesional back biopsies were analyzed for each patient; for 6 patients, samples were taken at pre-treatment and at 6-months post-rituximab therapy. A single patient underwent biopsies at base, 6 and 12 months.

Results: Three patients treated for 6 months showed significant changes in the gene expression profiles before and after rituximab treatment. This is consistent with the observation that the mean change in mRSS for treated patients was not significantly different between baseline (20.6) and 6-months (20.2). Using an intrinsic gene identifier algorithm to select the intrinsic genes in Ssc, we recapitulated three ‘intrinsic’ subsets (proliferation, inflammatory and normal-like) identified in a previous study of 24 patients with scleroderma. Analysis of skin biopsies from 6 early dSSc patients over the course of 12 months, and one patient over 18 months showed consistent and stable patterns of gene expression.

Conclusions: Rituximab treatment had little effect on the gene expression of skin biopsies from dSSc patients. Lesional and non-lesional skin from patients with SSc showed nearly identical patterns of gene expression, recapitulating the findings of two prior gene expression studies. The ‘intrinsic’ subgroups recapitulated here provide valuable information of gene groups that are an inherent feature of the disease. Serial biopsies taken from SSc patients at six-month intervals show nearly identical patterns of gene expression demonstrating that, over the time scale analyzed, the intrinsic subsets are a stable component of SSc gene expression.

28. Developing a Canadian Scleroderma Damage Index

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Background: Disease severity or activity scales for systemic sclerosis have been developed in Europe, Sweden, and the United States. A global damage index is under development in Canada.

Materials and methods: Data consist of over 1,000 variables collected on nearly 700 patients in the National Patient Registry of the Canadian Scleroderma Research Group. Statistical methods include multiple linear regression and multivariate procedures including but not limited to factor analysis and canonical correlation analysis.

Results: Challenges include data completeness and differences in diagnostic variables measured by Canadian rheumatologists compared to rheumatologists in other nations.

Conclusion: The development of the Canadian Scleroderma Damage Index is in progress. Variables in the National Registry are being assessed and variables not yet in this Registry are being considered.

29. Fibroblast-directed CTGF over-expression (in vivo) promotes connective systemic tissue fibrosis reminiscent of fibroproliferative diseases

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Background: Connective tissue growth factor (CTGF/CCN2) is a cysteine-rich secreted protein involved in wound healing and tissue repair. Its expression has been associated with many different forms of fibrosis.

Materials and Methods: We have developed transgenic mice with a high level of fibroblast-specific over-expression of CTGF/CCN2 using the Col1a2 enhancer/promoter sequence.

Results: These mice exhibit pronounced fibrosis of the skin, lung, kidney and small arteries and recapitulate clinical, histological and biochemical symptoms of many fibrotic diseases. Elevated collagen deposition in the dermis of these mice was mirrored by elevated collagen production and secretion by transgenic dermal fibroblasts in vitro. Examination of skin biopsies revealed enhanced fibroblast proliferation and a significant expansion of the myofibroblast cell population. Transgenic dermal fibroblasts also showed increased proliferation, enhanced migration in scratch wounds and increased remodeling of 3-dimensional collagen gels compared to wild-type controls. Gene expression analysis by Northern and Western blotting revealed elevated expression of endogenous CTGF, Coll1a1, Timp1 and 3, osteopontin, Fibronectine and α-SMA, and a series of other biochemical markers and gene clusters consistent with a fibrogenic response. Both phospho-p38 and phospho-Erk1/2 were increased in transgenic mouse fibroblasts whereas no change occurred in phospho-Smad3 compared to wild-type fibroblasts. Transgenic SRE, CTGF and CAGA promoters showed significantly increased basal activity compared to wild-type control fibroblasts, and enhanced induction in the presence of TGF-beta for the CTGF and CAGA promoters.

Conclusions: These data suggest that high levels of fibroblast-specific CTGF/CCN2 expression in vivo promote connective tissue fibrosis. Enhanced CTGF/CCN2 levels appear to synergize with non-canonical TGF-b signaling, modulating fibroblast responses to resemble a fibrogenic phenotype characteristic of many fibrotic diseases.

30. Systemic sclerosis – particularities related to capillaroscopy findings, Raynaud’s phenomenon characteristics and disease-specific autoantibodies

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Background: Among all connective tissue diseases, systemic sclerosis (SSc) has the most widespread microvascular abnormalities and also the greatest prevalence of Raynaud’s phenomenon (RP).

Objectives: The aim of this study was to assess the relationship between SSc, microangiopathy using nailfold capillaroscopy (NCS) and 1) the characteristics of RP, 2) the presence of digital ulcers (DU) and 3) the presence of SSc-specific autoantibodies.

Methods: 25 consecutive patients [M/F 3/22], median age 50.6 years (range 30-72) with median disease duration 7.6 years (range 2-12 years). Using an intrinsic gene identifier algorithm to select the intrinsic genes in Ssc, were enrolled in the study. Patients were assessed for DU. NCS was performed in all patients at the time of each exposure of RP and nailfold capillaroscopy. We classified NCS findings in "early stage" (ES)-capillary enlargement and minimal reduction of their density, "late stage" (LS)-multiple avascular areas with significant reduction of capillaries and few or none enlarged capillaries, "florid active stage" (FAS)-polymorphic capillaries with tortuosities, megacapillaries, bushy/rami fied capillaries, hemorrhages and moderate avascular areas, and "soft active stage" (SAS) - presence of 3 items from PAS. Patients fulfilled a RP questionnaire regarding 3 issues: changes in hands color - white (W), blue (Bl)and red (R), duration of crisis (minutes) and intensity of crisis - VAS scale [0-10]. Antitopoisomerase I Antibodies (Scl70) and anticientromere antibodies (ACA) were determined in all patients.

Results: A. Capillaroscopy findings. NCS stage frequency and SSc autoantibodies were as follows: ES -13 patients, all women: 5 ACA+, 4 Scil70+, SAS - 6 patients, all women: 3 ACA+, 3 Scil70+, 5 PAS - 5 patients: 2 men, both Scil70+, and 3 women - two Scil70+ and one ACA+. Late stage: 1 patient, male, Scil70+. Interestingly, the left hand presented more NCS abnormality than the right. 12 vs. 2 patients, equal NCS changes in 11 patients. Finger IV was most affected (13 patients), followed by fingers II (3 patients), III (5 patients) and V (4 patients).

B. Digital ulcers (DU). Eight patients had at least one hand DU (excluding those from the articular surface): 5 were ACA+ and 3 were Scil70+. Five out of 18 fingers with DU had FAS or SAS particularities. None of them had LS particularities. Four patients had toe ulcers, all being Scil70+, all with FAS finger capillaroscopy changes. Most frequent pathologic NCS finding were megacapillaries, followed by moderate avascular areas, bushy capillaries, hemorrhages, and multiple avascular areas.

C. RP findings: Score > 4 on VAS scale: 10 patients - 6 Scil70+, 3 ACA+. Three of them had severe NCS stages. On 19 subjects finger first involved in - and finger most exposed to RP had less severe or equal NCS findings than at least one of the others 7 times examined. Fifteen patients with DU had RP duration of < 8 years; 8 of them had minimal SAS. Ten patients had duration of RP > 8 years; 4 of them had minimal SAS. Of 9 patients who described color in crisis as B or B+R, 8 had minimal SAS. In another 9 patients who described color in crisis as W+R and/or R, only 2 had minimal SAS and 7 had ES. In 8 patients developing W+R color changes, 3 had minimal SAS. Eight patients had puffy fingers; 7 of these had minimal SAS.

Conclusions: A. Left hand and finger IV were the most frequently affected. Early NCS changes were met in half of the patients. More than 80% of patients with FAS and LS were Scil70+.

B. Fingers with DU did not display severe NCS findings. Toe ulcers correlated with severe NCS findings.

C. Higher intensity RP crisis scores were met more frequently in Scil70+ patients. Higher scores did not correlate with severe stages. Finger first involved in- and finger most exposed to RP did not correlate with the most severe NCS findings. None of the stages were strongly related to the duration of RP.

Patients with blue color alone or blue and red color of the hands in RP crisis most frequently had SAS. On the contrary, white plus red color associated with ES, while a severe RP had all kind of stages. Positive correlation was found in SAS particularities. Our results suggest there are 2 different microvascular involvement patterns in SSc patients: one associating digital ulcers and ACA, the other associating cyanosis, puffy fingers and severe NCS findings.
31. Cross-sectional study in systemic sclerosis

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Objectives: This study aims to overview the current clinical assessment of hospitalized systemic sclerosis (SSc) patients in three rheumatology units from Bucharest between 01-2007-01-2008.

Methods: Fifty-three SSc patients were included (8 men and 46 women); Mean age was 49,97±11,33 years; Fifty patients fulfilled ACR criteria for SSc. The others fulfilled LeRoy-Medsger (2001) criteria for early limited SSc. Our tools were: EUSTAR-Minimal Essential Data Set (Version August 2004) and supplementary 7 parameters from the extensive disease-specific clinical data-base.

Results: A: Quantitative: Forty-six parameters were evaluated to each subject; Another five were evaluated: DLCO to 34 (64,1%) patients, pulmonary hypertension (ECHO/PHT) to 38 (71,6%) patients, ARA to 48 (90,5%) subjects, ACA to 33 (62%) patients, SCL-70 to 44 (83%) patients. Mean disease evolution since first non-Raynaud’s symptom was 5,48±5,72 years. Mean age to onset of Raynaud’s was 39,49±13,69 years. Twenty seven patients (50,9%) had diffuse SSC (dc-SSc); 16 patients (30,2%) had limited SSC (lc-SSc) and 10 patients (18,9%) had other subsets. B. Qualitative: Most frequent parameters were: Raynaud’s syndrome (100%), ARA (97,9%), sclerodactyly (94,3%), esophageal symptoms (88,7%), DLCO <80% (88,2%), higher (79,2%) or lower (79,2%) telangiectasia (75,5%), pulmonary fibrosis plain x-Ray (PFx): 77,4%. Less frequent parameters were: friction rubs (11,3%), proteinuria (11,3%), reduced venular ejection fraction (9,4%), conduction blocks (9,4%), interstitial lung disease (9,2%), elevation of creatinine (5,7%), elevation of urates (4,7%), elevation of liver enzymes (3,7%), microvascular rarefaction (0,6%). SSc was active in 37,5% of cases; Overlap syndrom had 9,4% (one with myositis, one Systemic lupus erythematosus). ACA were present in 33%; Distribution of ACA: 82,6% SSc; 9% dc-SSC; 8,9% other subsets. From all ACA patients -11% had PHT. PHT-70 were present in 61,4%; Distribution of SCL-70: 74% dc-SSc, 15% other, 11% lc-SSc. From all SCL-70 patients -47,6% had PHT. PHT was met in 34,2% cases; Fifty procents of men had PHT and 30,33% of women had PHT. From patients with TLOCO<80%, 30,76% had PHT and 93,3% had PHT. Patients who had PHT, PFx, respective Rodnan score >14 have the probability (OR>1) of 5,10; 1,407 respectively to expose to chemical agents, while those who had PHT have equal chance to be smokers (OR=1) as those who did not have PFx in our schlerodema group. Next parameters: friction rubs, elevated phase reactants and men were met frequent in patients with both PHT and PFx. From those with active disease 60% were SCL-70 positive.

Conclusion: The overall disease activity was rather low in our group. Patients with active disease were mostly SCL-70 positive. Patients SCL-70 positive were predominantly in dc-SSc. PHT was more frequent in patients group with SCL-70 positive Men were more affected of PHT. As a test DLOCO <80% had good sensitivity for pulmonary fibrosis on X-Ray in the schlerodema group.The risk of exposure to chemical agents was higher in PHT, skin fibrosis and PFx patients from our schlerodema group. Our results confirm the higher prevalence of pulmonary involvement in SCL-70 positive patients.

32. Oxidative DNA damage in systemic sclerosis

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Background: Oxidative stress is implicated in the causation and perpetuation of tissue damage in systemic sclerosis (SSc). Several studies have shown increased lipid peroxidation in SSc1. There is some evidence to suggest that oxidative DNA damage is increased in systemic lupus erythematosus2, but there is no published data in SSc.

Objective: To investigate whether oxidative DNA damage is increased in SSc.

Subjects and Methods: Ten patients, fulfilling the ACR criteria for SSc and attending the Connective Tissue Diseases Clinic, Chris Hadza Baragwanath Hospital, Soweto, and 15 age- and ethnically-matched healthy controls were studied. In vitro baseline oxidative DNA damage and repair following hydrogen peroxide (H2O2)-induced DNA damage were measured in peripheral mononuclear cells using the single cell gel electrophoresis or ‘Comet’ assay as described by Singh et al3. Oxidative DNA damage was also assessed by measuring urinary 8-hydroxydeoxyguanosine (8-OHdG)-adducts using an ELISA assay (Assay Designs).

Results: No significant differences in baseline percentage (%) tail DNA in mononuclear cells was observed between the SSc patients (11,6%) and controls (10,8%). Mononuclear cells from both groups were able to repair H2O2-induced DNA damage after removal of the toxicant and culturing in fresh media at 37°C for 3 hours. However, DNA damage repair expressed as a decrease in % tail DNA to baseline levels was significantly faster in the control group compared to the SSc group (p=0,007). Urinary excretion of 8-OHdG was significantly higher in SSc group than in the control group (0,5±0,5±4µg/mg creatinine vs. 0,2±0,1±0,9±4mg/mg creatinine, respectively, p=0,0003).

Conclusions: Our preliminary results suggest comparable baseline DNA damage levels in SSc patients and healthy controls, but patients exhibited delayed repair of free radical induced strand breaks in vitro suggesting a defect in the DNA damage repair mechanisms in SSc. The increased urinary excretion of 8-OHdG in the SSc patients is further supportive evidence of oxidative stress in SSc.


33. Defects in matrix increase oxidative stress and endothelial mesenchymal transition

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Background: Systemic scleroderma (SSc) increases oxidative stress, fibrosis and microvascular rarefaction. Tight skin (Tsk-/-) mice have a defect in fibrillin-1, causing the mice to duplicate many of the cardiovascular features in humans with SSc. Previously we showed that D-4F, an apoA-I mimetic, reduces fibrosis and oxidative stress in Tsk-/- mice. Here we reasoned that defects in matrix might increase oxidative stress and inhibit EC function.

Materials and Methods: Microbfrils were isolated from ~6 month old C57BL/6 mice. Tsk-/- and TSK+/- mice were treated with DAPT (10^5 mg/kg 6-8 weeks) and Tsk-/- mice for 4-hydroxynonenal (4-HNE) content. Human umbilical vein endothelial cells (EC) were cultured on microbril preparations and examined for effects on proliferation and expression of fibroblast specific protein-1 (FSP-1) and expression of twist and slug, transcription factors involved in mesenchymal transition. Finally, microvascular EC and apoptotic cells in hearts of mice were immunostained and quantified.

Results: Defects in matrix increase oxidative modification of microbfrils in vivo that in turn impairs EC proliferation and increases endothelial mesenchymal transition. Thus, oxidative stress represents a second hit in SSc that promotes the transition of vascular EC into mesenchymal cells. Such changes in vascular EC function begin to explain why SSc increases microvascular rarefaceion.

34. Cloning of agonistic autoantibodies specific for the PDGF receptor from the B-cell repertoire of SSc patients

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Background: Systemic sclerosis (SSc) is a disorder characterized by fibrosi of skin and visceral organs. We have provided evidence that serum of SSc patients contains stimulatory autoantibodies directed to the PDGF receptor (PDGFR) that elicit Ha-Ras/ERK 1/2 signaling and collagen production in normal fibroblasts.

Materials and methods: IgG-positive B lymphocytes from peripheral blood of SSc patients were immortalized by EBV infection. Supernatants of single lymphocyte clones were screened for their ability to react selectively with F alpha cells (murine fibroblasts expressing the human PDGFR alpha) but not with F(-) cells (mock-transfected fibroblasts) by immunofluorescence and flow cytometry. Positive clones were further screened for the production of antibodies stimulating reactive oxygen species (ROS) and collagen production in normal human fibroblasts. Positive clones were expanded in serum-free medium. IgGs were purified from supernatants by A/G protein and tested to confirm both binding and biological activity on fibroblasts. mIgA was obtained from such positive single lymphocyte clones for sequencing and cloning of antibody variable regions.

Results: We isolated clones producing IgGs that i) reacted with F alpha cells, but not with F(-) cells; ii) stimulated ROS production, iii) induced Ha-Ras/ERK 1/2 cascade and type I collagen gene, iv) converted normal human primary fibroblasts into myofibroblasts. Several variable heavy and light chain IgG sequences were obtained by cloning selected cDNA fragments from total IgH.

Conclusions: Stimulatory PDGFR monocolonal autoantibodies were isolated from the immunoglobulin repertoire of scleroderma patients. These antibodies bind to PDGFR, induce ROS and collagen I production in normal fibroblasts and display the biological features identified in the total immunoglobulin pools purified from serum of SSc patients.
35. Association of interferon regulatory factor 5 (IRF5) polymorphisms with systemic sclerosis (SSc)

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Background: Type-1 interferon (IFN) signature has been shown to be the hallmark peripheral blood gene expression pattern in systemic lupus erythematosus (SLE). More recent studies have also noted a similar type-I IFN signature in systemic sclerosis (SSc). The transcription factor interferon regulatory factor 5 (IRF5) is a component of this IFN-gene expression signature and regulates the expression of other genes involved in cell-cycle regulation, cell adhesion, apoptosis and immune responses. IRF5 gene polymorphisms have been reported to be associated with SLE, rheumatoid arthritis, Sjögren’s syndrome, psoriasis, multiple sclerosis and inflammatory bowel diseases. The purpose of this work was to investigate the possible association between IRF5 polymorphisms with SSc.

Methods: We performed SNP genotyping for 3 SNPs on IRF5 gene using the Taqman Assay in 1,391 Caucasian, African-American, and Hispanic SSc patients along with 1,027 race matched controls. All SSc patients fulfilled ACR criteria or had at least 3 of the 5 CREST features. Chi-square, Fishers exact and logistic regression analyses were used for statistical comparisons. Illumina Human-REgF arrays were used for peripheral blood gene expression analysis.

Results: After HWE verification and correcting for multiple testing, two SNPs (rs2004640 & rs752637) showed significant association with White SSc patients. The TT genotype for the SNP rs2004640 had a frequency of 34.5% in White SSc patients as compared to 27.1% in White controls which was statistically significant. The bestfit model for the rs2004640 SNP was an additive model and was used for all comparisons. Logistic regression analysis controlling for gender and race showed that the TT genotype was an independent risk factor for SSc, including anti-topoisomerase-I antibody positive SSc and SSc with fibrosing alveolitis (Table 1). IRF5 was the topmost differentially expressed gene based on the rs200460 SNP genotypes in peripheral blood arrays of SSc patients (p-value 1.39x10^-14) (Fig. 1).

Table I. Estimated risk of TBX21 SNP rs17099436 in SSc patients versus controls, by logistic regression analysis.*

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT vs GG</td>
<td>1.56 (1.3-2.0)</td>
</tr>
<tr>
<td>TG vs GG</td>
<td>1.24 (0.99-1.6)</td>
</tr>
</tbody>
</table>

*The analysis was controlled for the confounding effects of sex and race. SSc: systemic sclerosis; OR: odds ratio; CI: 95% confidence interval; ACA: antinuclear antibody; ATA: antitopoisomerase-I antibody; RNA POL III: anti-RNA polymerase III antibody.

Control subjects are used as reference for all comparisons.

Conclusion: These data suggest an important role of this IRF5 polymorphism in its susceptibility to SSc. The TT genotype causes a splice variant containing exon IB whereas the GG genotype contains exon IA and exon IC. This IRF5 polymorphism leading to this isoform, upon stimulation, may facilitate expression of genes involved in maintaining fibrosis has not been extensively explored. The epidermal keratinocyte is a major source of pro-inflammatory and pro-fibrotic mediators. Antibodies directed against these cells have been reported in a number of other autoimmune diseases, and appear to lead to keratinocyte activation and secretion of potent soluble inflammatory/ pro-inflammatory mediators. We have previously shown that in early SSc the epidermis exhibits a phenotype resembling that observed in wound healing, and expresses a number of markers characteristic of epidermal differentiation.

36. IgG autoantibodies from scleroderma patients induces interleukin-1 alpha secretion

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Introduction: Tissue fibrosis caused by the excessive deposition of extracellular matrix is a common feature of many connective tissue diseases, notably scleroderma (cScl; SSC); SSc). Evidence suggests that complex intercellular interactions involving immune cells, endothelial cells and fibroblasts are important pathogenic events. However, the role of cellular events involving epithelial cells in initiating and maintaining fibrosis has not been extensively explored. The epidermal keratinocyte is a major source of pro-inflammatory and pro-fibrotic mediators. Antibodies directed against these cells have been reported in a number of other autoimmune diseases, and appear to lead to keratinocyte activation and secretion of potent soluble inflammatory/proinflammatory mediators. We have previously shown that in early SSc the epidermis exhibits a phenotype resembling that observed in wound healing, and expresses a number of markers characteristic of epidermal differentiation.

Objective: We studied the presence of anti-keratinocyte antibodies in SSc and examined the influence of the keratinocyte autoantibodies on the secretion of interleukin-1α (II-1α).

Method: Sera were obtained from 30 diffuse cutaneous SSc (dcSSc), 30 limited cutaneous SSc (lcSSc) patients and 30 healthy controls and evaluated for antibody binding to keratinocyte cells by cell-based ELISA. Immunoglobulin-G (IgG) was purified from 3 SSc patient and 3 controls selected from cell-based enzyme-linked immunosorbent assay (ELISA). Pre-purified IgG from SSc and from controls with keratinocytes was assessed for intracellular and extracellular expression of IL-1α. Interaction of IgG with keratinocyte binding and internalization was assessed using immunofluorescence. Biopsy sections were stained for human IgG. IL-1α expression in scleroderma epidermis was assessed by ELISA.

Results: We found that IgG purified from SSc patients bound to nucleolar antigens in keratinocytes compared to that of control IgG. In addition, pretreatment of human keratinocyte cells with purified IgG from scleroderma sera led to the induction and the secretion of IL-1α from normal human keratinocytes. Staining of human IgG in biopsy section from scleroderma patients showed cell membrane staining in the epidermis compared to control biopsies. Furthermore, IL-1α was overexpressed in dcSSc epidermis compared to control epidermis.

Conclusion: We have demonstrated the over-expression of IL-1α in dcSSc epidermis compared to that of control epidermis. Pre-treatment of keratinocytes with IgG purified from the sera of patient with SSc resulted in time dependent increase in the secretion of IL-1α. This data suggest over-expression of IL-1α by epidermal cells due to anti-body-mediated activation plays a key role in the abnormal function of both dermal and epidermal cells in dcSSC. The possibility exists that the epidermis in SSc is being activated by an autoimmune disease-driven mechanism.

37. Antibody profile of patients enrolled in the scleroderma lung study

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Background: The scleroderma lung study (SLS) was a double-blind randomized placebo-controlled trial that demonstrated modest but significant beneficial effects on pulmonary function, dyspnea, skin thickening, and health-related quality of life in scleroderma patients treated with one year of oral cyclophosphamide. The current study involved antibody analysis of patients enrolled in SLS to evaluate whether autoantibody status was associated with differences in baseline characteristics.

Materials and Methods: Of the 158 patients enrolled in SLS, 107 had serum available for autoantibody testing. Assays for anti-Scl-70, anti-centromere, and anti-RNA polymerase III (Pol3) were performed using enzyme immunoassay. Statistical analysis was performed using chi-square test to compare the mean and standard deviation of sex, race, and presence of positive bronchoalveolar lavage. Disease duration, skin score, high-resolution CT scan scores for fibrosis, ground glass and honeycombing, Mahler dyspnea index, and disability index of the scleroderma health assessment questionnaire score (HAQ-DI) were compared using Wilcoxon rank-sum test. Finally, age and comorbidities of the pulmonary function tests were compared using the two sample t-test.

Results: Of the 107 patients with antibody results available, one patient had both Scl-70 antibody and Pol3 and was excluded from further analysis. Although 41.5% of patients were classified as having limited cutaneous scleroderma, only 3 patients in this study had positive anti-centromere antibodies. Anti-Scl-70 antibody was positive in 29 patients, 66.67% of whom had diffuse cutaneous scleroderma. These patients were significantly younger than anti-Scl-70 negative patients (p=0.0051). Disease duration was similar in patients with and without anti-Scl-70 antibody. Anti-Scl-70 antibody was associated with higher baseline ground glass score (p=0.0133) and a trend toward a higher frequency of alveolitis by bronchoalveolar lavage (p =0.0690). In contrast, 93.75% of anti-Pol3 antibody positive patients had diffuse cutaneous scleroderma. Anti-Pol3 positivity was associated with higher baseline skin score (p=0.0019) and higher HAQ-DI (p=0.03495) but lower baseline fibrosis score (p=0.0576).

Conclusions: Scl-70 and Pol3 antibody status may account for some of the differences in baseline characteristics of patients enrolled in the SLS. Outcome analysis is ongoing, but it is hoped that antibody status might help identify patients more likely to respond to therapy in scleroderma-associated lung disease.
38. The German Network for Systemic Scleroderma: new data on disease subsets and organ involvement from more than 2000 patients


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Background: Systemic scleroderma (SSc) is a rare, heterogeneous disease, which affects different organs and therefore requires interdisciplinary diagnostic and therapeutic management. The German Network for Systemic Scleroderma (DNSS) was founded three years ago for basic and clinical research. Presently, it comprises dermatologists, rheumatologists, pulmonologists and nephrologists from 40 medical centers. To improve detection and follow up of patients presenting with early stages of the disease or overlap-syndromes, patients were classified in 5 subsets, i.e. limited, diffuse systemic sclerosis, overlap-syndrome, undifferentiated scleroderma and scleroderma sine scleroderma.

Results: Recent analyses revealed that 48% of patients suffer from limited SSc (lSSc), 31% from diffuse SSc (dSSc) and 11% of patients were diagnosed with an overlap-syndrome. 8% had an undifferentiated form while scleroderma sine scleroderma was present in 1% of patients. Pulmonary fibrosis was significantly more frequent in dSSc than in lSSc (61% vs. 24%). Accordingly, pulmonary arterial hypertension was more common in dSSc (20%) compared to lSSc (14%). Muscular involvement was typical for overlap-syndromes (69%). The onset of initial skin changes, following first attacks of RP, occurred earlier in dSSc than in lSSc. A family history of rheumatic diseases was associated with early disease onset. Follow up data revealed that the prevalence of joint contractures, hypertension and diastolic dysfunction increases significantly within one year.

In this register a classification of patients with disease manifestations characteristic of systemic sclerosis in 5 groups allows to include a broader spectrum of patients with features of systemic sclerosis and to gather new insights into disease evolution.

39. TGFβ-induced a-smooth muscle actin expression and extracellular matrix contraction in fibroblasts requires TAK1


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Background: The pro-fibrotic protein transforming growth factor-beta (TGFβ) is found in elevated amounts in scleroderma (SSc) patients. TGFβ-activated kinase 1 (TAK1) is thought to be a key signaling pathway of TGFβ. The effect of loss of TAK1 on transcriptional responses in fibroblasts is unclear. In this report, we use wild-type fibroblasts and those deficient in TAK1 to probe the contribution of TAK1 to mediate transcriptional responses in fibroblasts in response to TGFβ.

Materials and methods: Dermal fibroblasts (n=6) were obtained from control and SSc tissue. Fibroblasts derived from wild-type (WT), TAK1-/- (KO) embryos were used. The effect of TGFβ on the phenotype of TAK1-deficient cells was assessed by MOE430 Affymetrix gene arrays analyzed by Genespring software, real-time polymerase chain reaction (RT-PCR) and Western blot analysis. In addition, the ability of TGFβ-1 to influence matrix remodelling in collagen contraction models was also examined.

Results: TGFβ induced 265 transcripts greater than 2-fold in WT fibroblasts. Of these 194 were not induced greater than 2-fold in TAK1-deficient cells. TGFβ significantly enhanced the ability of WT to contract collagen lattices (p<0.05), whilst only marginally modulated TAK1-/- contraction. Western blot analysis shows that the ability of TGFβ to phosphorylate TAK1 is reduced by the FAK/sec inhibitor PP2. The ability of TGFβ to induce JNK phosphorylation is reduced downstream of FAK/sec.

Conclusions: Alterations in vascular and perivascular architecture were seen in the transgenic vessels. Some of the changes are indistinguishable from those seen in human pulmonary arterial hypertension. Our results support a role for TGFβ-overactivity in the vasculopathy of systemic sclerosis.

40. Pulmonary and systemic vasculopathy in a TGFβ-1 dependent mouse model of systemic sclerosis

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Purpose: Vascular complications of systemic sclerosis (SSc) are a major cause of mortality and morbidity. A robust mouse model of SSc-related vasculopathy is yet to be described. We have systematically examined vascular structure in a genetically determined model of SSc characterised by ligand-dependent activation of TGFβ signalling in fibroblasts.

Methods: The transgenic mouse strain TgRIIΔk-fib expresses a kinase-deficient type II TGFβ-receptor linked to a fibroblast-specific promoter leading to balanced ligand-dependent upregulation of TGFβ-signaling. Comparisons between transgenic and wildtype heart, lung and kidney were performed. We used immunohistochemistry to confirm excess TGFβ-1 and TgRII and evaluated vascular and perivascular architecture by H&E and special stains. Activation of TGFβ-dependent signaling pathways in cultured fibroblasts was confirmed by qPCR measurement of CTGF, PAI-1 and Collα1.

Conclusions: Aortic smooth muscle cell proliferation and phenotype assays, including signaling responses to exogenous TGFβ-1 and endothelin-1, were performed. Results: TGFβ-1 expression was increased in transgenic lung vessels and endothelin-1 and its specific receptors ET-RA and ET-RB were also upregulated. Figure 1 shows that transgenic intimal diameter, measured in 1000 vessels from 10 littermate pairs, was significantly increased, and particularly noted in the smaller 30-60μm pulmonary arterial vessels with an increase in intimal smooth muscle being the major contributing factor. α-SMA was also visible in very small (<20 μm) pulmonary arterioles in transgenic but rarely in wildtype samples (mean 3.0 compared to 1.8, p=0.02). Elastin deposition was disordered in the pulmonary vascular bed and downstream TGFβ-mediated signaling pathways were increased. Cardiac collagen content, measured by Sircol assay, was increased in transgenic mice.

Conclusions: Alterations in vascular and perivascular architecture were seen in the transgenic vessels. Some of the changes are indistinguishable from those seen in human pulmonary arterial hypertension. Our results support a role for TGFβ-overactivity in the vasculopathy of systemic sclerosis.
41. Thrombospondin 1 is a key mediator of TGFβ-mediated cell contrac-
tility in systemic sclerosis via a MEK/ERK-dependent mechanism
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Background: Sclerodermatous lesions result in excessive deposition and contraction of extracel-
lar matrix. The mechanism underlying the ability of fibrobasts to contract a collagen gel matrix is largely unknown. Fibrobasts from scarred (lesional) areas of patients with the fibrotic disease scleroderma show enhanced ability to con-
tract collagen relative to healthy fibrobasts. Thrombospondin-1 (TSP-1), an activ-
tor of latent TGFβ, is over-expressed by scleroderma fibrobasts. In this study we in-
vestigated whether activation of latent TGFβ by TSP-1 played a key role in matrix con-
traction by normal and scleroderma fibrobasts.

Materials and Methods: The matrix contraction of normal and SSC fibrobasts to re-
sponse altered TSP-1 activity were assayed by the fibroblast populated collagen lattices
(FPCL) model via the multi station tensioning Culture Force Monitor. Results: We have demonstrated that interfering with TSP1/TGFβ binding and knock-
down of TSP-1 expression suppressed the contractile ability of normal and sclero-
derma fibrobasts, basally and in response to TGFβ. During mechanical stimulation in the FPCL system using the mst- CFM we observed that TSP-1 expression and p-ERK activation in fibrobasts was enhanced. Inhibiting TSP1 activity reduced the elevated activation of MEK/ERK and expression of key fibrogenic proteins. TSP-1 may poten-
tially mediate fibroblasts responses to PDGF in the pathogenesis of SSC via MEK/ERK pathway.

Conclusion: TSP-1 is a key mediator of matrix contraction of normal and SSC fibrob-
lasts via a MEK/ERK dependant mechanism.

42. Serial anti-RNA-polymerase antibody levels in patients with systemic sclerosis
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Background: Anti-RNA-polymerase antibodies (ARA) occur in up to 20% of patients with systemic sclerosis (SSc), are strongly associated with the diffuse cutaneous (dc) subset of the condition and particularly with scleroderma renal crisis (SRC). We ana-
lysed ARA levels in SSc patients over time looking for relationship with clinical pre-
sentation and disease outcome.

Materials and methods: Subjects had definite SSc. A commercially available ELISA method was used to measure ARA levels.

Results: We included 33 patients who had ARA levels measured between 3 and 19
times over a follow-up period of between 21 and 142 months. Of them 88% had dcSSc; 48% had SRC, 30% had pulmonary fibrosis (PF), 9% developed pulmonary arterial hypertension (PAH) and 6% had clinically significant cardiac involvement. Over the follow-up period there were 4 deaths and survival at 3 and 5 years was 94% and 91%
respectively.

We observed considerable inter- and intra-patient variability in ARA levels (11-188U/
ml, mean±SD - 85±42U/ml). We divided subjects into subgroups according to degree of change in ARA levels over the first 36 months of disease and according to cumula-
tive antibody levels. We could not demonstrate any difference in survival or number of internal organ complications among the patients in the different subgroups. There was no significant correlation between absolute or peak ARA levels and onset of SRC.

Twenty-six patients had received immunosuppressive treatment during the assessment period. Although there was no significant difference in ARA levels when on and off treatment, subgroup analysis demonstrated a trend towards lower ARA levels with Mycophenolate mofetil (MMF) compared to no treatment or therapy with other im-
unosuppressants (p=0.058).

Conclusions: Despite the strong association of ARA with SRC, there is no clinically significant correlation between ARA levels and development of internal organ compli-
cations in patients with SSc. Change in ARA levels over the first 3 years of the disease occurrence do not appear to have major clinical significance. Antibody levels may be affected by treatment with MMF.

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son*, N. Manolios
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Background: Scleroderma finger clawing (Sfc) is associated with variable – some-
times profound – morbidity. We have devised new measures to semiquantitatively and quantitatively measure Sfc, describe these values in individuals with normal hands and in those with scleroderma.

Materials and Methods: Sydney Harbour Bridge (SHB) = arc/chorde at dorsal finger length/veolar linear finger length; ‘Perth’=hand height x 10/open span; Finger clawing grade: Hands opposed: Grade 0=mpc’s and p’ip’s contact; Grade 1= mcp’s contact; Grade 2= no mcp contact angle between plane of distal phalanx and palm=90°; Grade 3 = no mcp contact angle between plane of distal phalanx and palm=90°.

Results:

1. Normal values
Perth median = 1.44 [5.95 percentile 1.07–1.92].
SHB median = 1.00 [5.95 percentile 0.96–1.07]

2. Correlation
R vs L SHB (Pearson r=0.77; p<0.0001); R/L SHB vs Perth (Pearson r=0.05–0.005; p=0.63-0.97)

3. SHB/Perth–effect of age and gender: SHB age-dependent in males [p=0.0001] SHB/Perth possibly age dependent (role of Heberden’s nodes?). ‘Perth’ greater sensitiv-
ity than SHB in scleroderma hand. Both quantitative measures simple, quick, cheap. SHB widely adaptable.

44. Antitopoisomerase antibody positivity predicts nailfold capillary-
scopic changes in scleroderma
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Background: At the time of clinical presentation, antinuclear antibody is usually present in high titre and nailfold capillaroscopy is classically abnormal in patients with scleroderma. Indeed, the presence of antinuclear positivity in conjunction with abnormal nailfold capillaroscopy is often used to delineate primary from secondary Raynaud’s in patients with evolving connective tissue disease.

The temporal evolution of scleroderma before disease diagnosis – including the tem-
poral relationship between autoantibody positivity and abnormal nailfold capillaro-
scopy – remains incompletely determined. The preclinical staging of the disease is also undefined.

We wish to present a patient’s history in which the temporal relationship between an-
titopoisomerase antibody positivity and nailfold capillaroscopy changes were fortui-
tously observed. We wish to tentatively propose a temporal staging of the prediagnostic phase of scleroderma.

Materials and Methods: Case Report: A 25 year old female first presented in May 2005 with a history of increased fatigue, weight loss, and stiff, swollen painful upper and lower extremities. Antitopoisomerase antibody was noted. Nailfold capillaroscopy was normal. The patient then developed bismarck Raynaud’s, symptomatic synovitis and finger clawing. Scleroderma was diagnosed in December 2005 by which time she had had sclerodactyly, slowly pitting skin oedema over the fingers, periangual erythema, and mild neck flexor weakness. The remainder of the clinical examination was normal. Repeat nailfold capillaroscopy on this occasion showed dilatation of capillaries with an obvious degree of capillary irregularity with increased intravascular red cell ag-
gregation.

Conclusions: In this patient with early scleroderma, the serological abnormality of antitopoisomerase antibody positivity preceded vessel wall changes as evidenced by nailfold capillaroscopy changes. Whether a similar temporal pattern of autoantibody positivity preceding microvascular pathology noted on nailfold capillaroscopy is borne out by further observations remains to be determined.

We tentatively propose 5 prediagnostic stages for this disease of multifactorial aetiology.

Stage 1: Conception. Genetic predisposition. Raynaud’s symptoms.

Stage 2: Phase after initial environmental exposure – with no perturbation of body

Stage 3: Phase after initial environmental exposure – with no perturbation of body

Stage 4: Phase characterized by asymptomatic clinical changes.

Stage 5: Symptomatic stage.
46. Anti-endothelial cell antibody levels are low in scleroderma internal organ vasculopathies.

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Introduction: Systemic sclerosis is associated with fibrosis and vasculopathy. Sclerodermatous renal crisis (SRC) and scleroderma-associated pulmonary hypertension (PAH) are exemplars of vasculopathy in scleroderma. Anti-endothelial cell antibodies (AECA) have been found at high titres in SSC and a variety of other connective tissue diseases. They have been associated with disease activity and subsets and subsets of disease.

Methods: Serum samples diluted 1:100 from 18 age-matched controls, 26 cases of SSC without SRC or PAH, 17 PAH, 27 SRC, 12 prior to onset of SRC, and 48 samples >2 months after SRC were assayed for AECA on 3rd passage HUVEC. Results were expressed as an index where pooled serum is 1, and negative control is 0. The upper limit of normal was taken as mean of controls +2SD.

Results: AECA index was elevated in SSC compared with controls (index 2.35 vs 1.23, 77% vs 0% above ULN, p<0.001). Levels were lower in PAH than SSC controls (mean 1.64, 35% above ULN, p=0.07). Levels were lower in SRC than SSC controls (mean 1.56, 26% above ULN, p=0.001). Levels before SRC are not different to SSC controls, and rise after SRC (mean 2.09, 71% above ULN). There is a negative correlation with skin score, but no difference in those with or without significant pulmonary fibrosis.

Conclusions: AECA are high in SSC. The levels drop in cases with internal organ vasculopathy and rise when vasculopathy recovers. This phenomenon may be due to increased binding of the antibodies to activated endothelium.

47. Proteasome inhibitor bortezomib overrides TGF-beta effect in human fibroblasts

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Background: Extracellular matrix (ECM) provides a controlled environment for cellular differentiation and tissue development. Its integrity is maintained through a balance between ECM components deposition and degradation. Excessive ECM deposition is observed in fibrotic diseases. One of the predominant ECM components found in fibrotic lesions is type I collagen (COL1A2 and COL1A1). ECM accumulation is controlled by relative expression levels of collagens, ECM degrading enzymes such as matrix metalloproteinases (MMPs) and their inhibitors, named Tissue Inhibitor of MMPs (TIMPs). We previously published that bortezomib, a proteasome inhibitor, exerts an in vitro anti-fibrotic activity, dominant over the pro-fibrotic phenotype induced by TGFbeta. We report here an extensive study of the transcriptional regulation of ECM genes in human dermal fibroblasts.

Materials and methods: Variation in ECM mRNA and protein levels was determined by RT-PCR, enzyme-linked immunosorbent assay (ELISA) and Western blotting. Promoter activity of COL1A1 and MMP-1 genes was measured by reporter gene assay. Increase in binding of various transcription factors to specific promoter region of ECM genes was performed in vivo via chromatin immunoprecipitation (ChIP) and in vitro via electrophoretic mobility shift assay (EMSA).

Results: Bortezomib activated transcription of MMP-1 via increased binding to AP-1 site. Analogous response to bortezomib treatment was observed for MMP-13, whereas TGFbeta activated transcription of COL1A1 and COL1A2 via increased binding to AP-2 or SPI sites, respectively. While bortezomib did not affect TGFbeta-induced binding of AP-2 to COL1A1 promoter, it completely abolished TGFbeta-induced binding of SPI to COL1A2 promoter.

Conclusions: We identified elements of MMP-1 and COL1A1 promoters in fibroblasts, essential for bortezomib- or TGFbeta-mediated activation. Bortezomib treatment targets converging signals: activation of MMP-1 and MMP-13 transcription, due to increased occupancy of AP-1 site and repression of TGFbeta-mediated induction of COL1A2 transcription on the SPI site. These signals result in an in vitro anti-fibrotic phenotype in human fibroblasts.

48. Detection of possible risk factors for digital ulcers in systemic sclerosis

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Background: Digital ulcers (DU) are a major complication in the course of systemic sclerosis (SSc). In recent years, efficacious, but expensive vasoactive therapies (e.g. iloprost, sildenafil or bosentan) have shown to improve healing or to reduce recurrence of DU. In order to deliberate timely or even prophylactic treatment it would be useful to identify potential risk factors for DU in patients with SSc. Such statistical analyses have been rare, because they require sufficiently high numbers of patients.

Materials and methods: The German Network for Systemic Sclerosis (DNSS) encompasses a nation-wide patient registry of patients with SSc. We evaluated data of 1881 patients included by August 2007. We assessed potential risk factors for DU by comparing patients with (n=408), and without active DU at time of their entry into the network.

Results: Multivariate analysis revealed that male gender, presence of pulmonary arterial hypertension (PAH), involvement of oesophagus, diffuse skin sclerosis (only when PAH was present), anti-Scl70 antibodies, young age at onset of Raynaud’s phenomenon (RP), and elevated sedimentation rate (ESR) present independent factors associated with DU.

Conclusions: Certain combinations increase the patients’ probability to present with DU, with the highest probability (88%) for male patients with early onset of RP, ESR above 30, anti-Scl70 antibodies and PAH. Patients with DU developed RP, skin sclerosis and organ involvement approximately 2 to 3 years earlier than patients without DU.

Certain combinations increase the probability of DU occurrence in SSc. Since these DU are prone for local complications, it may be justified to consider prophylactic vasoactive treatment for these patients.
49. Microarray analysis of murine cGVHD as an animal model for human systemic sclerosis

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Background: The relevance of mouse models to systemic sclerosis (SSc) has been highly debated. Some aspects of chronic graft versus host disease (cGVHD), including skin thickening, presence of autoantibodies and visceral organ involvement, have been observed. We have reported that the heterogeneity in SSc can be captured and quantitatively measured at the level of gene expression level and that these gene expression signatures can be used to sub-divide the patients. Here, we have used DNA microarrays to directly compare the extent to which a murine model of cGVHD resembles the human SSc at the level of gene expression. We specifically tested the hypotheses that the pro-fibrotic cytokines IL13 and TGFB are involved in the pathogenesis of both SSc disease and murine cGVHD.

Methods: Total RNA was isolated from lesional back skin of murine cGVHD and control mice at 2 weeks and 5 weeks. Samples were hybridized to Agilent 44,000 element mouse microarrays. Significance Analysis of Microarrays (SAM) was used to identify differentially expressed genes in both the murine and human gene expression datasets. Interspecies comparisons were drawn by extraction of mouse and human orthologs from the Mouse Genome Informatics site at Jackson Laboratories.

Results: We identified 819 genes differentially expressed in cGVHD mice compared to the control group. The expression of these 819 genes was examined in a library of gene expression measured in skin biopsies from patients with SSc, morphea and healthy controls (Milano et al. submitted). We report that the cGVHD mice show striking similarity to the “Inflammatory” subset of SSC. An IL13 gene expression signature derived from the literature (Fulkerson et al. 2006) was differentially expressed in cGVHD mice compared to controls, where as expression of genes associated with TGFβ signaling was heterogeneous.

Conclusions: At the level of gene expression, the murine cGVHD model most closely approximates the inflammatory subgroup of SSC. This suggests that this model may be appropriate to study and test therapies for this subgroup of patients. We present evidence that IL13 but not TGFB is involved in driving pathogenesis of murine cGVHD skin, and in a subset of SSC patients.

50. Nitric oxide metabolism and antioxidant expression in the tight skin mouse model of fibrosis

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Background: Nitric oxide (NO) is an important physiological signalling molecule and potent vasodilator. We have previously demonstrated abnormal NO metabolism and increased levels of nitrated proteins in the plasma of patients with systemic sclerosis (SSc; scleroderma), a disease that features excessive collagen overproduction as well as large and small vessel dysfunction. The aim was to examine NO metabolism and the role of antioxidants in the tight-skin 1 (TSK-1/) mice, an experimental animal model of fibrosis.

Methods: Skin and lung tissues, or plasma, were taken from fifteen TSK-1/ and twelve wild-type (WT) littermate mice fed on a diet low in nitrates. Type 1 collagen, endothelial nitric oxide synthase (eNOS), haemoxgenase (HO-1) and Nr2 transription factor expression was determined by Western blot. RT-PCR was also used to determine eNOS, HO-1 and Nr2 gene expression. NOS activity was evaluated by conversion of [14C] L-arginine to [14C] L-citrulline and levels of circulating plasma nitrite/nitrate (NOx) were measured to establish the production of NO. Total antioxidant activity was evaluated by ABTS+ production.

Results: Type I collagen protein expression was significantly elevated in TSK-1/ skin tissue, while eNOS protein and gene expression were reduced compared to WT. Furthermore, there was decreased NOS activity in TSK-1/ skin tissue; however, there was no difference in plasma NOx. Correspondingly, the protective antioxidant enzyme HO-1 and Nr2 showed reduced protein and gene expression levels in TSK-1/ skin; there was also less total antioxidant activity. In the TSK-1/ lung tissue, however, we observed no difference in either collagen protein expression or NO metabolism, HO-1 expression and antioxidant activity compared to WT.

Conclusion: The findings suggest abnormal nitric oxide metabolism in the TSK-1/ mouse model of fibrosis, particularly in the skin, while expression and activity of protective antioxidants are reduced. This highlights the importance of NO and reactive oxygen species in the regulation of collagen biosynthesis.

51. Course of modified Rodnan skin score (MRSS) in diffuse systemic sclerosis (dcSSc) clinical trials: analysis of 3 large multicenter, randomized clinical trials

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Background: It is generally accepted that MRSS tends to worsen in early disease and improve in late disease although time of peak involvement remains poorly defined. Based on natural history cohorts, the ACR Committee on Clinical Trials in SSc recommended patients with disease duration <2 years from the onset of first symptom suitable for drug efficacy trials.

Objective: To assess whether the course of MRSS in patients in clinical trials follow a course different than that noted in natural history cohorts with early (defined as <2 years from first non-Raynaud’s phenomenon symptom) and late (>2 years) dcSSc.

Methods: Data from 3 large RCTs of patients with dcSSc were analyzed – D-Penicillamine, recombinant human Relaxin, and oral Bovine Collagen. Patients were divided into 3 groups for D-Pen (<6, 6-12, and 12-18 months) and 4 groups for Relaxin and Collagen (<12, 12-24, 24-48 and >48 months) at baseline. All patients with disease duration ≤2 years were termed as “early disease” group and greater than 2 years as “late disease” group. Linear mixed effects model for correlated data was used to model MRSS as a function of time (in months), baseline disease duration, and the interaction of time and baseline disease duration.

Results: At entry, mean MRSS was 21.04 in D-Pen, 27.34 in Relaxin, and 26.14 in Bovine Collagen, respectively. The skin score improved in patients with both “early disease” and “late disease.” There was a significant drop in skin score per month in D-Pen and Relaxin trials (i.e. time was a significant predictor of decline in MRSS, P-value <.0001), starting from date of entry. The rate of change in MRSS for patients with “early disease” was significantly different to “late disease” in Relaxin and Collagen studies.

Conclusion: Our study suggests that patients entered in dcSSc clinical trials do not follow the same trend in natural history of skin thickening as seen in the general dcSSc population. This needs to be taken into account when designing future drug efficacy trials and in studies where “prevention of worsening” is the main objective.