Results: LncMKLN1, dominantly located in nucleus, was up-regulated in lupus patients compared to healthy donors, and could be induced by IFNα and TLR ligands in HRMC. Silencing lncMKLN1 significantly reduced the expression of a group of interferon-inducible genes, including IFIT3, OAS1, CXCL10, etc. We used lose-of-function and gain-of-function strategy through CRSIPR system to confirm that lncMKLN1 positively regulated type I interferon pathway. Furthermore, it was identified the involvement of lncMKLN1 in interferon signalling pathway was through regulating the expression of STAT1, IRF9 and phosphorylation of IRF9 and STAT1 although its mechanism is also needed to investigate.
Conclusions: Upregulated lncMKLN1 expression contributed to abnormal activation of interferon pathway of SLE.
Disclosure of Interest: None declared
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AB0181 ENHANCED THERAPEUTIC EFFICACY OF APOPTOTIC CELL TREATED MESENCHYMAL STEM CELLS IN LUPUS PRONE MRL/LPR MICE

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Background: Mesenchymal stem cells (MSCs) exhibit promising therapeutic potential in systemic lupus erythematosus (SLE). Increased apoptotic cells (ACs) were observed in SLE and our previous study showed MSC transplantation reduced AC levels in patients with SLE. Yet the effects of ACs on MSCs are not clear.
Objectives: This study aims to investigate the effects of ACs on MSCs and efficacy of AC treated MSCs (AC-MSCs) in SLE.
Methods: Jurkat T cells were irradiated by ultraviolet ray to induce apoptosis and then co-cultured with umbilical cord derived MSCs. Then, ACs were removed and MSCs were further co-cultured with human peripheral blood mononuclear cells (PBMCs). The inhibition of MSCs on PBMC proliferation was detected by flow cytometry. MSCs and AC-MSCs were infused into lupus prone MRL/lpr mice respectively to compare their therapeutic effects.
Results: The suppression of MSCs on PBMC proliferation was significantly enhanced after co-culture with ACs. *In vivo* study showed that AC-MSCs significantly increased the survival rate of MRL/lpr mice and decreased urine protein as early as one week after treatment, while MSCs decreased urine protein eight weeks post infusion. Moreover, AC-MSCs remarkably reduced the number of splenic plasma cells and serum anti-dsDNA levels, whereas MSCs only showed decreased tendency.
Conclusions: ACs enhanced therapeutic effects of MSC transplantation in lupus mice, which provides new insights into MSC modification in the treatment of SLE.
Disclosure of Interest: None declared
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Systemic sclerosis, myositis and related syndromes – etiology, pathogenesis and animal models

AB0182 MOLECULAR MECHANISMS MEDIATING ANTIOXIDANT EFFECT OF EPIGALLOCATECHIN-3-GALLATE IN EXPERIMENTAL SCLERODERMA MODEL

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Background: Scleroderma (SSc) is an autoimmune multisystemic connective tissue disease characterised by skin and internal organ fibrosis(.[1]Underlying mechanism is still unclear for SSc. Besides there is no specific treatment for SSc, various treatments may alleviate symptoms and improve the quality of life. Epigallocatechin-3-gallate (EGCG) is a phenol with antioxidant effects in many disease processes.[2]In this disease, oxidative stress may play a role for pathogenesis(.[3–4]Recent studies showed a relationship between oxidative/antioxidative markers and SSc.[5]
Objectives: The aim of this study was to investigate the antioxidant effects of epigallocatechin-3-gallate in the scleroderma process in experimental mouse model with bleomycin.
Methods: Thirty-two healthy female Balb-c mouse species were used and randomly divided into four groups:control, bleomycin, bleomycin +EGC, EGCG. At the end of the experiment, skin tissues were collected. Sodium dismutase enzyme (SOD) and malondialdehyde (MDA) levels have been analysed for oxidative stress. High performance liquid chromatography (HPLC) was used for MDA measurements. Colorimetric kit was used for SOD analysis. Furthermore, the ratio of phosphorylated p-38/total p-38 protein, and phosphorylated-Akt/total Akt protein and NF-kappa B were measured by western blotting. Immunohistochemistry (α-SMA), histochemistry (masson trichrome-hematoxylin and eosin) studies were also performed on FFPE skin samples.
Results: When the experimental and control groups were compared, the degree of fibrosis in the connective tissue of the dermis areas stained with masson trichrome decreased in the EGCG groups.SOD activity was increased in the EGCG groups compared to the positive control group, and MDA was significantly decreased in the EGCG groups. According to Western blotting results, pp-38 MAPK and NF-κB were found to decrease significantly in the EGCG groups compared with the controls. Parallel to these findings, phosphorylated Akt protein was found to increase in the EGCG groups compared with the control groups.
Conclusions: It has been shown that EGCG can antioxidative effect in scleroderma.

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AB0183 VITAMIN D AND VITAMIN D RECEPTOR IN PATIENTS WITH SCLERODERMA SUBTYPES

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Background: The aim of this study was to compare the expression of Vitamin D receptor (VDR) and vitamin D in scleroderma subtypes.
Objectives: VDR is a member of the nuclear localised hormone receptor family. 1,25- (OH) 2D, a form of metabolically active Vitamin D3, is the ligand of VDR. When VDR and 1,25-(OH) 2D are linked, many genes initiate molecular interaction reactions that will modulate the transcription.[1] VDR has been shown to be a negative regulator of the TGF-β/Smad signalling pathway, which is important in the pathogenesis of scleroderma.[2, 3] Thus, reduced expression of VDR and decreased ligand levels may contribute to hyperactivity of the TGF-beta pathway in SSC and abnormal fibroblast activation. Also, Vitamin D has pleiotropic effects including immunomodulatory and antifibrotic properties in scleroderma pathogenesis.
Methods: 19 SSC patients and 6 healthy controls were included in the study and they were classified according to the 2013 ACR/EULAR criteria. They were applied to Dokuz Eylul University, Faculty of Medicine, Department of Rheumatology-Immunology, between 2015–2017. Rodnan scores were calculated of all scleroderma patients. 11 were of the limited type and 8 were of the diffuse type of scleroderma. Informed consent was obtained from all participants. 1 ml of total blood was collected. Vitamin D levels were determined in serum.
VDR gene expression was determined by quantitative PCR in isolated RNAs from the blood. Changes in mRNA levels were analysed according to the ΔΔCT method and beta-actin was used as the housekeeping gene. Student-t-test was used as a statistic. In addition, Pearson correlation test was used to determine the relationship between Rodnan score and VDR gene expression.
Results: VDR gene expressions in diffuse type scleroderma patients were statistically significantly decreased compared to the control ($p<0.01$).
It was found that VDR gene expression in limited type scleroderma patients did not show any significant difference when compared to control (p: 0.16).
Also, Vitamin D levels and vitamin D expressions were no correlation in scleroderma subtypes (p:0.2)
Conclusions: VDR gene expression decreased in patients with diffuse type scleroderma and showed negative correlation with Rodnan score. Further studies are planned to increase the number of samples to obtain more information.

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AB0184 DIAGNOSTIC IMPACT IN THE CLINICAL SETTING OF NAILFOLD VIEOCAPILLAROSCOPY ON CONNECTIVE DISEASES

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Background: Nailfold video-capillaroscopy (NVC) is a non-invasive technique that allows visualisation of structure and distribution of capillaries at the nailfold level[1], altered somehow in some connective diseases, specially in the Scleroderma (Scl) disease spectrum. The main indication of this technique is the investigation of Raynaud's phenomenon (RF).
Objectives: Our objective is to investigate the diagnostic impact of NVC in the daily clinical practice.
Methods: The design is an observational, longitudinal, retrospective and descriptive study, which included patients with at least one NVC between June 2012 and December 2016 from our Rheumatology register of patients. We collected demographic data (age, gender, indication, autoantibodies, etc.), number of explorations performed and their result. We also collected in a dichotomist fashion if the NVC contributed in the diagnostic workup, between one consultation and the other after the NVC realisation.
Results: 437 patients were included with a total of 637 explorations. Of these 437 patients, 115 (24.1%) had a second NVC, 39 (8.2%) a third one, 9 (1.9%) a fourth and only two with a fifth NVC (both with diagnostic of Scl). We noticed a diagnostic change between the first consultation and the next one in 35 cases (5.49%). In 14/35 (40%) of these cases, the NVC played an important role in the diagnostic change, with changes in the NVC pattern, from normal or unspecific to Scl pattern (table 1). These changes, occurred after the first NVC in 10 patients (71.4%), 3 (21.4%) after the second, and 1 (7.1%) after the third exploration in addition of new disease manifestations, diagnostic tests and other image techniques. Of these 14 patients, 100% had positive ANA, 5 (35.7%) Anticentromere Antibodies (Ab), 1 (7.1%) anti-Ro Ab and 1 (7.1) Antiphospholipid Ab.

Abstract AB0184 – Table 1

Change	n:14 (%)	Pattern
RF to Scl	11 (78.5%)	Early 7/11 (63.6%) Active 2/11 (18.1%) Late 2/11 (18.1%)
UC to Scl	2 (14.2%)	Early 2 (100%)
RF to UC	1 (7.1%)	Early 1 (100%)

Raynaud phenomenon (RF); Scleroderma (Scl); Undifferentiated connective disease (UC); Mixed connective tissue disease (MCTD); Systemic Lupus Erythematosus (SLE); primary Sjögren syndrome (pSS).
Of the 21 patients with a diagnostic change who did not developed a Scl pattern we have:
Normal pattern: 1 RF to possible Scl and 1 RF to UC.
Limit of normality: 1 SLE to MCTD, 4 RF to UC, 1 RF to pSS, 1 RF to MCTD and 1 UC to MCTD.
Unspecific (mild): 3 RF to UC, 2 RF to Scl and 1 UC to overlap syndrome.
Unspecific (moderate): 1 RF to UC, 1 RF to Scl, 1 UC to MCTD and 1 RF to MCTD.

Conclusions: The NVC in our centre had a limited but important impact in the diagnostic process of connective diseases. This impact was specially relevant in patients diagnosed with Scleroderma. The probability of having a diagnostic change diminishes with successive explorations.

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AB0185 ALTERED EXPRESSION OF RELAXIN RECEPTOR RXFP1/LGR7 IN DERMAL FIBROBLASTS CONTRIBUTES TO THE INEFFICACY OF RELAXIN-BASED ANTI-FIBROTIC TREATMENTS IN SYSTEMIC SCLEROSIS

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Background: Systemic Sclerosis (SSc) is an autoimmune disease characterised by progressive fibrosis of the skin and internal organs, coupled to widespread vascular pathology.[1] The pathogenesis is still poorly understood and there is no effective treatment for the fibrotic process.[1] Relaxin is a potent anti-fibrotic hormone that has been tested in the past to ameliorate skin, lung and kidney fibrosis in SSc, but the results remain controversial.[2]
Objectives: The aim of the study is to evaluate the presence of mutations in RXFP1 gene (encoding the relaxin receptor RXFP1/LGR7), and to assess mRNA and protein levels of the receptor in dermal fibroblasts of SSc patients, in order to understand the clinical inefficacy of relaxin-based anti-fibrotic treatments in the disease.
Methods: Fibroblasts were isolated from unaffected and affected skin samples of (n=20) of limited cutaneous SSc (LcSSc) and from (n=20) affected skin of diffuse cutaneous SSc (DcSSc) patients. Fibroblasts derived from healthy subjects were used as controls. Sequencing of exonic target regions of interest for gene RXFP1 was performed coupled with mRNA transcript variant analysis. RXFP1/LGR7 mRNA and protein levels were assessed by quantitative-real-time-PCR (qPCR) and by immunocitochemistry (ICC) in cultured SSc and healthy fibroblasts. Finally, synthesis of collagen and alpha-smooth-muscle actin (a-SMA) of transforming-growth-factor-beta-1 (TGF-β1) induced fibroblasts were assessed after 24 hours pre-treatment with serelaxin (a recombinant form of human relaxin-2 targeting the relaxin receptor RXFP1/LGR7).
Results: Sequencing of RXFP1 gene showed no relevant (single nucleotide polymorphisms) SNPs or small insertions and deletions (InDels) in affected LcSSc/DcSSc fibroblasts. No relevant mutations were found in unaffected LcSSc and healthy fibroblasts as well. However, alternatively spliced transcript variants encoding multiple isoforms were observed for this gene in all the fibroblast populations. The total RXFP1 mRNA levels resulted upregulated ($p<0.05$) in the affected LcSSc/DcSSc fibroblasts compared to unaffected LcSSc ($p<0.05$) and to healthy ones ($p<0.05$). On the contrary, ICC demonstrated the absence of RXFP1/LGR7 receptor in affected LcSSc/DcSSc fibroblasts and the regular expression in unaffected LcSSc and healthy fibroblasts. In fact, serelaxin pre-incubation was unable to counteract the TGF-β1 driven upregulation of collagen and a-SMA in affected LcSSc/DcSSc fibroblasts only, but not in unaffected LcSSc and healthy ones.
Conclusions: The absence/altered expression of relaxin receptor RXFP1/LGR7 in the affected fibroblasts of SSc patients could explain the inefficacy of relaxin-based anti-fibrotic treatments in the disease. The exclusion of RXFP1 gene mutations could lead to the hypothesis that the presence of receptor splice variants could exert a dominant negative effect on the wild type isoform in terms of maturation, and subsequent trafficking to the cell surface, resulting in loss of function.

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