Further work will be done to study the role of the ratio in predicting fracture risk in patients with other conditions.

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Cartilage, synovium and bone

FRI0504 TOCILIZUMAB CONTROLS BONE TURNOVER IN EARLY POLYMYALGIA RHEUMATICA

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Background: Tocilizumab has been proved to be an alternative to corticosteroids in treating polymyalgia rheumatica. Considering the action on interleukin-6 on bone turnover an effect of tocilizumab is supposed. Few data are available about bone turnover in rheumatoid arthritis patients treated with tocilizumab but no data are available in polymyalgia rheumatica patients.

Objectives: This study explores changes in the bone homeostasis by testing the N-terminal collagen type I extension propeptide (PINP) marker for osteo-formation and the carboxy-terminal region of collagen type I (CTX-I) marker for osteo-resorption in patients taking tocilizumab for polymyalgia rheumatica (PMR).

Methods: Twenty patients were included in the prospective open-label TENOR study (Clinicaltrials.gov NCT01713842) and received three monthly tocilizumab infusions, followed by corticosteroids starting at week (W)12. PINP and CTX-I were tested at inclusion (W0), after tocilizumab but before steroid initiation (W12), at the end of the protocol (W24) and were compared to healthy controls. Information regarding disease activity, inflammatory parameters and interleukin (IL)-6 levels were collected during the follow-up of the patients.

Results: Polymyalgia rheumatica patients were characterized by higher levels of CTX-I relative to healthy controls matched in age and sex at baseline. PINP levels increased at W12 ($p=0.0008$, versus W0) following tocilizumab introduction and CTX-I levels decreased at W24 and after steroid initiation ($p=0.001$, versus W0) (figure 1). Such modifications explain the altered correlation observed between PINP and CTX-I at W0 ($r=0.255$ at W0 versus $r=0.641$ in healthy controls) and its correction after treatment ($r=0.760$ at W12 and $r=0.767$ at W24). Finally, greater changes in PINP were observed in patients whose circulating IL-6 levels decreased after tocilizumab therapy.

Figure 1. Evolution of PINP (A) and CTX-I (B) in polymyalgia rheumatica patients under tocilizumab therapy (W0-W12) and under corticosteroids (W12-W24). Correlation between CTX-I and PINP in polymyalgia rheumatica patients at week 12 (C) and 24 (D)

Conclusion: Control of bone turnover, in part through the inhibition of the IL-6 axis, is observed during tocilizumab and subsequent steroid treatment of polymyalgia rheumatica.

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FRI0505 CORRELATIONS BETWEEN CARTILAGE MOLECULAR COMPOSITION DETERMINED BY RAMAN SPECTROSCOPY AND MANKIN SCORE: IMPACT OF INTER AND INTRA-VARIABILITY

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Background: Osteoarthritis (OA) is an incurable disease and methods for its early diagnosis are still an unmet clinical need. Raman spectroscopy (RS) presents potential as a diagnosis technique based on the detection of peaks that can be assigned to cartilage components and molecular rearrangements during disease progression.¹ Mankin score (MS) is the main validated method to evaluate severity of cartilage degradation considering structure, cell distribution, Safranin-O staining and tidemark integrity.²

Objectives: To evaluate the correlations between OA cartilage RS assigned peaks and MS, considering the inter- and intra-variability of different observers.

Methods: MS analysis (Subscore-I, structure: 0-6; -II, cellularity: 0-3; and -III, safranin-O staining: 0-4; Total Score: 0-13) of human OA cartilage explants from 22 donors (age range: 32-92), obtained from lesion (n=22) and/or adjacent tissues (n=14), was performed by 3 blinded observers (O1, 2 and 3). Moreover, one of the observers performed the scoring in triplicate, with at least one month between observations. Inter- and intra-observer variability was determined by kappa and intraclass correlation (ICC) coefficients. Raman spectra were obtained with a FT-Raman Bruker RFS100 ($\lambda=1064\text{nm}$) and main peaks assigned (6 ratios related to proteoglycans, collagen, lipid index or calcium phosphate). Spearman's non-parametric correlation coefficient rho was used to compare MS and RS assigned peaks.

Results: Inter-observers variability indicated good (ICC>0.74) or moderate agreement (ICC>0.5) for all scores in lesions, whilst only a good agreement (ICC=0.70) was found for subscore-I, in adjacent tissues, and no agreement for the remaining parameters (subscores -II, -III, and total scoring). However, when performing analysis using kappa coefficients, a simultaneous agreement between the 3 observers was not observed. Intra-observer variability revealed good concordance (ICC>0.6) for all subscores and total scoring in cartilage for both sites, except for subscore -III, in adjacent tissues. In this case, ICC results were confirmed by kappa coefficients. Spearman's correlation coefficient between cartilage main peaks assigned by RS and MS indicated significant differences between observers (Fig.1). Correlations were found for a greater number of MS subscores in O1 (6) regarding O2 (4) or O3 (3) which could be related to the observers' experience (being O1>O2>O3). These correlations were mostly found in lesions (5, 3 and 1 for O1, 2 and 3, respectively) in comparison to adjacent tissues (2, 1 and 2 for O1, 2 and 3, respectively).

Conclusion: Eventhough inter-observation correlations for MS were in the moderate- good range, when analyzing kappa coefficients (categorical variables) these were not maintained. In addition, inter- and intra-observer variability results for adjacent tissues revealed possible limitations when characterizing early to mild OA. In view of MS-RS correlations, a reader dependency is underlined, indicating MS subjectivity and further limitations in the validation of RS using MS.

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Figure 1. A: Raman spectra (RS) for lesion and adjacent cartilage tissue and assigned peaks; B: Histological stainings (Hematoxylin-eosin (H-E), Safranin-O/Fast-Green (SO-FG) and Toluidin blue (TB)) used for MS analysis from the adjacent and lesion sites from a selected donor; C: rho Spearman's correlation coefficients and significance between RS and MS (total scoring) in lesion and adjacent sites for Observer 1, 2 and 3 (O1, 2 and 3).

FRI0506 ROLE OF MITOCHONDRIA FROM PATIENTS WITH OA IN CELLULAR SENESCENCE

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Background: Chondrorescence, chondroptosis and autophagy contribute to cell death and tissue damage in OA. The mitochondria are related with these three process implicated in the cartilage degeneration. Mitochondrial dysfunction is well documented in OA and has the capacity to promote abnormalities in chondrocyte viability contributing to cartilage degeneration. Cybrids are optimal cellular models to study the mitochondrial function, since they carry different mitochondrial variants with the same nuclear background, therefore, excluding the variations because of nuclear genome.

Objectives: The aim of this work is to study the role of human OA mitochondria in the cellular senescence, apoptosis and autophagy.

Methods: Cybrids were developed using 143B.TK⁺ Rho-0 cell line (nuclear donor) and platelets (mitochondrial donors) from healthy (N) and knee OA donors. Senescence level was measured by real-time PCR method. The percentage of mitochondrial depolarized was evaluated incubating cells with DiIC1(5) in presence of FCCP 1μM using Flow Cytometer. The percentage of apoptotic cells was measured by Flow Cytometry using Annexin-V. Autophagy was evaluated through the developed of Microtubule-associated protein 1A/1B-light chain 3 (LC3) WB. Appropriate statistical analyses were performed with GraphPad Prism v6.

Results: The gene expression corresponding to senescence marker protein (SMP30) showed higher levels in OA cybrids than in N (4.535±1.63; 1.21±0.42 respectively, p<0.0005). OA cybrids showed higher increment depolarized mitochondria under negative stimuli in comparison with basal condition than N (2.57±1.20; p<0.0005; 1.76±0.99; p<0.05 respectively). The analysis of apoptotic levels, when the cells were submitted for a positive stimuli with staurosporine (2 μM) and an inflammatory environment with IL-1β (10 ng/ml), OA cybrids reflected a statistically significant increase in positive cells for Annexin-V in comparison with N cybrids in both conditions (staurosporine 15.68±6.39; 6.41±4.88 respectively, p<0.05. IL-1β 0.924±0.19; 0.47±0.24 respectively, p<0.05). Autophagy was analyzed studying LC3 a marker for autophagosome formation and the results showed that LC3 activation was reduced in OA cybrids (1.19 ±0.24; 1.41±0.21 respectively, p<0.05).

Conclusion: Mitochondria from OA patients is involved in cellular senescence, apoptosis and autophagy (three relevant processes involved in OA).

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FRI0507 THE HUMAN-BASED IN VITRO 3D ARTHRITIC JOINT MODEL

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Background: Our ultimate goal is to develop a valid human *in vitro* 3D joint model to simulate the pathogenesis of arthritis. The model consists of different components including an osteogenic and chondrogenic part, the joint space with synovial fluid and the synovial membrane. It contains all involved cell types and thus, to allow interactions between cells by cell contacts and signaling molecules. The arthritic joint is simulated by the application of sorted immune cells and typical cytokines. As an alternative experimental setup for animal models, our *in vitro* 3D joint model will enable us to study the influence and efficacy of potential drug candidates. Currently, there is no valid 3D model, which is able to mimic an arthritic joint.

Objectives: Here, we aimed to develop the single joint components, namely the osteogenic and chondrogenic part, the joint space with synovial fluid and the synovial membrane, separately and in co-culture without and with the application of neutrophils (PMNs) and cytokines.

Methods: We have used human bone marrow derived mesenchymal stromal cells (hMSCs) to develop the different 3D tissue components that are characterized in detail (e.g. cell vitality, structural integrity) using histological, biochemical and molecular biological methods as well as μCT and scanning electron microscope (SEM). For the osteogenic component, we populated β-tricalcium phosphate (TCP) – mimicking the mineral bony part – with hMSCs, while the scaffold-free 3D cartilage component was generated from a marrow tissue exclusively consisting of hMSC under intermittent mechanical stimulation (fzmb GmbH). Simulating the synovial fluid, non-animal stabilized hyaluronic acid was applied to the osteochondral model. In order to model the synovial membrane, a confluent hMSC layer was formed on a polycarbonate membrane. Subsequently, we applied sorted CD15⁺ cells to the synovial membrane and cytokines (e.g. TNF, MIF, IL6) into the synovial fluid, analyzed using qPCR, multiplex immunoassay and flow cytometry.

Results: We developed an *in vitro* 3D bone model by successfully seeding hMSC on a β-TCP scaffold. Cells consistently adhere onto the scaffold for up to 6 weeks as observed by SEM. The analysis of cell viability via LDH detection and LIVE/DEAD staining showed no toxic effects on the cells even after 3 weeks of incubation as compared to the corresponding control. mRNA expression of bone-related genes such as *RUNX2*, *SPP1* and *COL1A1* as well as μCT analysis confirmed the osteogenic phenotype of hMSC grown in 3D. Mimicking the articular cartilage component, we verified its chondrogenic phenotype by HE and Alcian Blue staining as well as by the reduced mRNA expression of *COL1A1* and an abundant expression of *COL2A1*. Interestingly, co-cultivation of the osteogenic and chondrogenic part looks promising showing a close alignment and cell interaction between both components. Modelling the synovial membrane, we successfully and reproducibly created a confluent hMSC cell layer, with a high cell viability even after 3 weeks of incubation. In addition, we injected cytokines into the synovial fluid. In response, the simulated synovial membrane showed an upregulation of *IL6*, *HIF* and *MMP13* expression on mRNA level. Furthermore, under co-cultural conditions the hMSCs enhanced the survival of the PMNs.

Conclusion: Results from the analysis of the single components confirmed viability, integrity and morphology pointing towards an ultimately successful development of the anticipated *in vitro* 3D model. By combining the different components in a standard 96 well format, we aim to provide a mid-throughput system for preclinical drug testing as well as a valid *in vitro* human-based 3D disease model to study the pathogenesis of arthritis.