Conclusions: 1. Detectable contrast peak by CEUS was associated with lower KOOS indices
2. Detectable arrival time correlated with number of synovial blood vessels
3. In some patients CEUS and PDU can offer discrepant results. Further investigation is needed in cases of longer AT (> 30 sec) when CEUS might be more informative than PDU in detection of low blood flow.
4. Using CEUS and performing time/intensity analysis enables to differentiate states of increased vascularity in the KOA.

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025 SENSITIVITY TO CHANGE OF KNEE IMAGES DIGITAL ANALYSIS COMPARED TO ALTMAN GRADING FOR CLASSIFICATION OF RADIOGRAPHIC PROGRESSION IN EARLY OSTEOARTHRITIS (CHECK)

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Purpose: Grading radiographic osteoarthritis (OA) on an ordinal scale commonly results in low sensitivity to change. Measurement of separate radiographic OA features on a continuous scale using Knee Images Digital Analysis (KIDA) theoretically enables more precise measurement and greater sensitivity to change. The longitudinal Cohort Hip & Cohort Knee (CHECK) evaluates radiographic progression early in OA. The objective of this study was to determine whether the sensitivity to change was greater by using KIDA than by using ordinal grading according to the Altman atlas.

Methods: Sensitivity to change was determined by calculation of the smallest detectable difference (SDD), which is a measure for the variability of the radiographic procedure and digital analysis. SDD was determined for the KIDA measures of the joint space narrowing (JSN; lateral and medial), osteophyte area (lateral and medial femur, lateral and medial tibia), and bone density (BD; lateral femur and medial tibia). Since no repeated radiographs were available, subsets of unchanged radiographic pairs from baseline to 2 year follow-up were selected based on an Altman grade of 0 at both time points. Radiographic pairs were selected from 1002 CHECK participants (2004 knees) for the JSN, osteophyte, and BD parameters separately (313, 303, and 213 pairs respectively). Next, of all CHECK participants available baseline and follow-up radiographic pairs were evaluated. The percentage of pairs changing on the radiographic parameters was determined for KIDA, defined as a change larger than the SDD, and for the Altman atlas defined as a change of at least one grade. Further, for each parameter radiographic pairs with a change on KIDA and/or Altman were evaluated using cross-tabulations.

Results: The SDD was on average 1.8mm for JSN, 3.4mm2 for osteophyte area, and 11.6mm/Al for BD parameters. Using KIDA, an average increase in OA features was found in 6.4% (JSN), 7.6% (osteophyte), and 3.5% (BD) of radiographic pairs. Using Altman, an increase in OA severity was found in 7.4% (JSN), 7.5% (osteophyte), and 0.8% (BD) of pairs. Interestingly, of the radiographic pairs with a change, only a small percentage changed both on KIDA and Altman grading, and a substantial percentage changed on either KIDA or Altman. E.g. for medial JSN 17% of radiographic pairs changed on KIDA and Altman, 38% changed only on KIDA, and 44% changed on Altman only. Agreement was 10% for osteophyte, and 0% for BD parameters.

Conclusion: Sensitivity to change of KIDA is comparable to Altman grading in participants with early OA and relatively short follow-up, despite the measurement on a continuous scale with KIDA. Importantly, KIDA and Altman grading identified different OA progressors. The latter needs thorough evaluation and might involve the quality of radiographic procedures leading to non-comparative radiographs over time.

Sponsor: CHECK

Disclosuare Statement: No conflict of interest.

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026 RESORPTIVE BONE REMODELING IS A PROMINENT FEATURE OF BONE MARROW LESIONS DETECTED BY MAGNETIC RESONANCE IMAGING (MRI) IN OSTEOARTHRITIS

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Purpose: Bone marrow lesions (BMLs) are an important source of symptoms and risk for disease progression in osteoarthritis (OA). Prior studies have suggested increased bone remodeling within these lesions. The objective of this study was to begin to test the hypothesis that BMLs in OA knees result from microfracture of pathologic bone with subsequent remodeling associated with secondary bone healing. Specifically, bone remodeling in BMLs was characterized histologically and presence and activity of osteoclasts in BMLs were histochemically evaluated.

Methods: Magnetic resonance imaging (MRI): Fourteen patients with predominantly medial compartment osteoarthritis scheduled for total knee replacement (TKR) underwent MRI. TKR outcomes were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Knee injury and Osteoarthritis Outcome Score (KOOS). MRI grading of lesions was performed by using KIDA than by using ordinal grading according to the Altman atlas. Sensitivity to change was determined by calculation of the small-detectable difference (SDD), which is a measure for the variability of the measurement.

Results: MRI identified osteoclast activity of bone remodeling. Specifically, bone remodeling in BMLs was characterized histologically and presence and activity of osteoclasts in BMLs were histochemically evaluated.

Conclusions: MRI grading of lesions was performed by using KIDA than by using ordinal grading according to the Altman atlas. Sensitivity to change was determined by calculation of the small-detectable difference (SDD), which is a measure for the variability of the measurement.

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and nerve growth in osteoarthritic human menisci, providing a mechanism for the genesis of pain in knee OA.

**Methods:** Cases from whom menisci were obtained post mortem (PM) were screened based on displaying either high or low macroscopic tibiofemoral chondropathy scores (possible range 0 to 400) as a measure of the presence and degree of OA. Forty cases (20 per group) were selected for study of meniscal variability and 16 cases (8 per group) for study of meniscal innervation. Antibodies directed against alpha-actin (vascular smooth muscle and pericytes) and calcitonin gene-related peptide (CGRP) (sensory nerves) were used to localise blood vessels and nerves by immunohistochemistry. Computer-assisted image analysis was used to compare vascular densities and nerve growth between menisci from high and low chondropathy groups. Vascular density was measured as the percentage of tissue section area occupied by alpha-actin immunoreactivity (1) in synovium adjacent to inner meniscal region (two fields of highest vascular densities), (2) in all fields along the sagital fibrocartilage junction between inner and outer meniscal regions, and (3) within 8 consecutive fields along the entire transverse midline from the inner tip to the periphery. Inter-group comparison of vascular densities within each field along the midline was used as a measure of vascular distribution. Nerve densities were measured as the number of nerve profiles within 6 most densely innervated fields of view in the outer region of the meniscus. Each individual field of view comprised an area of 1.68mm² for the vascular study and 16800μm² for the nerve growth study. Comparison between groups was made using Mann Whitney U test and data are presented as median [Interquartile Range (IQR)].

**Results:** Patients in the high chondropathy group had chondropathy scores of 203 (198 to 245) or 199 (181 to 240) and those in low chondropathy group had chondropathy scores of 24 (15 to 27) or 23 (19 to 46) for the vascular or nerve studies respectively (both P<0.0005). Vascular densities were greater in the high than in the low chondropathy group both in the synovium [3.8% (2.6 to 5.2), 2.0% (1.4 to 2.9), P=0.002] and at the fibrocartilage junction [2.3% (1.7 to 3.1), 1.1% (0.8 to 1.9), P=0.003]. The outer meniscal region was more vascular than the inner region, with an abrupt drop in vascularity in the fourth field along the midline from the tip of the menisci, which corresponded to the region of the fibrocartilage junction. Vascular densities in the 4th field were greater in the high than in the low chondropathy group [1.0% (0.3 to 1.5), 0.3% (0.0 to 0.8), P=0.015] indicating a greater degree of vascular penetration in the high chondropathy group. Menisci from the high chondropathy group had greater number of perivascular sensory nerve profiles in the outer region [144 nerve profiles/mm² (134 to 189)] compared to menisci from the low chondropathy group [119 nerve profiles/mm²] (104 to 1144), P=0.049.

**Conclusions:** Vascular penetration into the meniscus is increased in OA and is associated with increased sensory nerve growth. Vascularisation and the associated innervation in menisci may contribute to pain in knee OA.

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**028 INFRAFATELLAR FAT PAD FROM LATE OSTEARTHRITIS PATIENTS HAS AN ANABOLIC PHENOTYPE**

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**Introduction:** Onset and progression of osteoarthritis (OA) are associated with obesity. Since not only knee OA but also hand OA is associated with obesity, systemic metabolic factors next to mechanical factors might influence this process. Recent studies showed that adipose tissue is not only able to secrete adipokines but also cytokines such as IL-1βeta and IL-6. In this study, we investigated whether infrapatellar fat pad (IPFP) is able to influence cartilage biology and contribute to the onset of OA.

**Methods:** Explants of Hoffa’s fat pad obtained during total knee replacement of 29 OA patients were cultured separately in medium for 24 hours in a concentration of 50 mg/mL, resulting in 29 different batches of OA conditioned medium (FCM). Pro-inflammatory (IL-1β, IL-6, IL-12) and anti-inflammatory cytokines (IL-10) and growth factors (FGF2 and PAI1) content (LumineX) of the FCM were measured and the presence of CD68+ (macrophages), CD86+ (classically activated macrophages) and CD206+ (alternatively activated macrophages) cells in the explants of IPFP (immunohistochemistry) were determined.

**Results:** Explants of healthy cartilage were cultured in FCM (1:1 with fresh medium) for 48 hours. We evaluated Nitric Oxide (NO) production and glycosaminoglycan (GAG) release by the cartilage, gene expression of MMP1, MMP13, ADAMTS4, ADAMTS5 and COMP in the cartilage.

**Results:** All cytokines were measurable in the 29 batches of FCM. High numbers of CD68+ and CD206+ cells were present in the IPFPs, but large variety was seen between the patients. Samples of Hoffa’s fat pad with high numbers of CD68+ cells seemed to produce more PAI1 than samples with low numbers of CD68+ cells. When high numbers of CD206+ cells were present, a trend towards high levels of FGF2 was seen in the FCM. No association was found between CD68+ cells and production of cytokines. Culturing cartilage in FCM resulted in lower NO production and GAG release than the control condition. MMP1 gene expression was also lower in response to the FCM, whereas MMP13, ADAMTS4 and ADAMTS5 remained unchanged. COMP gene expression increased in response to FCM.

**Discussion:** Medium conditioned by infrapatellar fat pad from late osteoarthritic patients had mainly anabolic effects on cartilage. High numbers of macrophages (CD68+ cells) and alternatively activated macrophages (CD206+ cells) were present in the IPFPs used for making the fat conditioned medium. These cells might have contributed to the production of anti-inflammatory cytokines and growth factors and thus the anabolic/repair effect seen on cartilage.

**029 THE ANTERIOR CRUCIATE LIGAMENT IN AGING AND OSTEOARTHRITIS**

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**Purpose:** Joint instability caused by anterior cruciate ligament (ACL) deficiency is a known risk factor for early onset of osteoarthritis (OA), but aging-related changes in the ACL and their relationship to changes in articular cartilage and onset of OA are not well characterized. The purpose of this study was to correlate histological and cellular changes in ACL with cartilage lesion patterns and severity in human knee joint samples from autopsy.

**Methods:** Human knee joints were obtained at autopsy (n=95; age 24-92 yr), and cartilage and ACL were graded macroscopically and histologically. Macrosopic grading of all cartilages was performed using a modified Outerbridge scoring system and the ICRS knee map. The microscopic appearance of ACL degeneration was classified as normal, abnormal and ruptured (Alline et al 2001 Acta Orthop Scand). For histology ACLs were resected at the insertion sites on the femur and tibia. Approximately 1 cm thick transverse and longitudinal sections were harvested from subchondral and femur attachment side where ACL tears frequently occur. ACL sections were stained with hematoxylin and eosin, and graded histologically as follows: 1) orientation and collagen fibers; 2) formation of new blood vessels and inflammatory cell infiltrates; 3) change in the size of fascicles. Both sagittal and axial sections of each ACL were scored. ACL inflammation was analyzed separately as the extent of synovial hyperplasia surrounding the ACL, and changes within the ligament itself. Immunohistochemistry and western blotting was also performed with antibodies to MMP-3 and CD45 antibody.