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NLRP3 INFLAMMASOME PLAYS A CRITICAL ROLE IN THE PATHOGENESIS OF HYDROXYAPATITE-ASSOCIATED ARTHROPATHY

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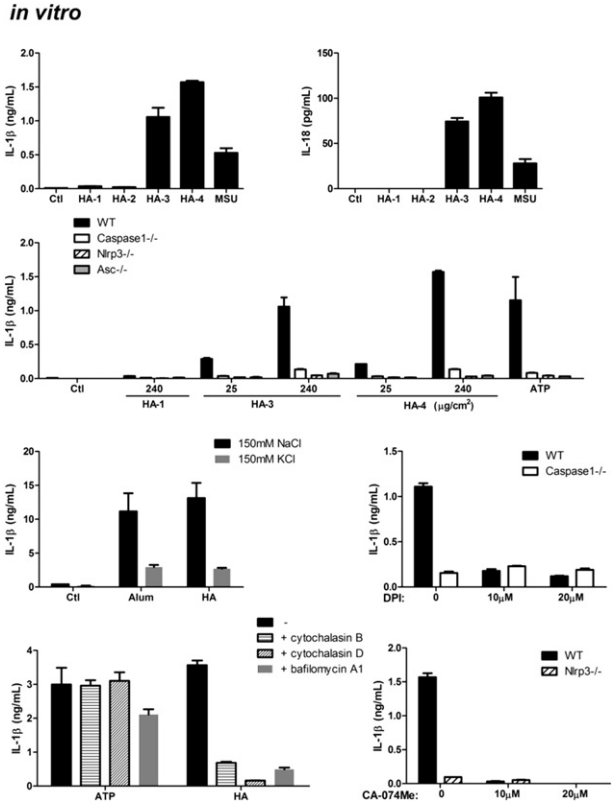
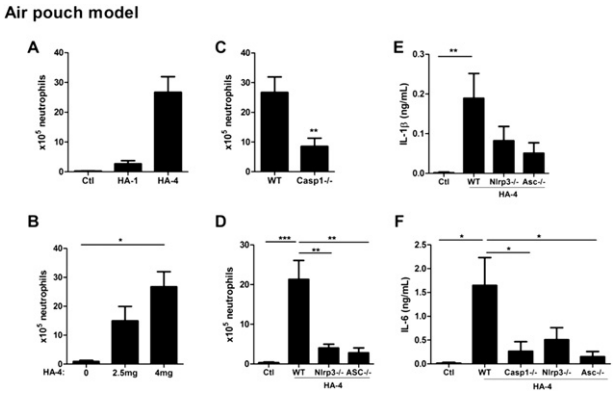
Purpose: The pro-inflammatory and catabolic cytokine IL-1β has been implicated in the pathogenesis of osteoarthritis (OA) by mediating synovial inflammation and cartilage degeneration. The level of IL-1β is elevated in synovial fluids and the cartilage of OA patients. Genomic studies have identified the association of genes encoding IL-1α, IL-1β, and IL-1 receptor antagonist (IL1RN) with the development of human OA, and IL-1β antagonists have shown some efficacy in the treatment of OA in animal models. Although synovial macrophages are suggested to be the source of IL-1β, the mechanism remains unclear. Ectopic deposition of hydroxyapatite (HA) crystals in joints is closely associated with OA and other arthropathies, but the precise role of HA in arthritis pathogenesis has not been clearly demonstrated. As IL-1β secretion is tightly controlled by inflammasomes, and the crystalline nature of HA is reminiscent of a number of identified agonists of the NLRP3 inflammasome, we aimed to test the hypothesis that the NLRP3 inflammasome might mediate the pathological effect of HA crystals in OA.
Methods: To characterize the inflammatory property of HA crystals, LPS-primed primary murine macrophages were stimulated with different forms of synthetic HA crystals and the secretion of pro-inflammatory cytokines TNF-α, IL-1β and IL-18 were analyzed. To understand the underlying molecular mechanism, we further examined such HA-induced response from macrophages derived from mice lacking different inflammasome components including NLRP3, ASC, caspase-1 and NLRC4, and under conditions in which potassium efflux, phagocytosis or ROS production was inhibited. To verify the physiological relevance of our in vitro findings in the context of joint inflammation, we applied the established air pouch model and compared the HA-induced neutrophil influx and pro-inflammatory cytokine production in the pouch exudates between inflammasome-deficient mice and WTs. Furthermore, we generated NLRP3/ANK and caspase-1/ANK double knockout mice to examine whether the lack of NLRP3 inflammasome could suppress the development of joint destruction induced by the ANK-deficiency-associated spontaneous HA deposition.





Results: Although the HA crystals we tested all had the same chemical composition, their pro-inflammatory activities varied greatly with their physical parameters, and only the crystals of size and shape similar to those found in diseased joints stimulated robust secretion of the proinflammatory cytokines IL-1β and IL-18 from murine macrophages in a NLRP3 inflammasome-dependent manner. We found further that activation of the inflammasome by HA was dependent on potassium efflux, generation of reactive oxygen species (ROS), phagocytosis and lysosomal damage, but independent of cell death. Mice lacking the inflammasome components were protected against HA-induced neutrophilic inflammation in the air pouch model of synovitis. Furthermore, lack of NLRP3 or caspase-1 attenuated the arthropathy associated with spontaneous HA deposition in ANK deficient mice. Finally, calcium-crystal-positive synovial fluids from some OA patients exhibited inflammasome-stimulatory activity in vitro.
Conclusions: Our results demonstrate that the NLRP3 inflammasome mediates the pathological effect of HA crystals both in vitro and in vivo and suggest a critical role for the inflammasome in the pathogenesis of OA. Targeting NLRP3 inflammasome might have beneficial effects on osteoarthritis treatment.

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ELECTROARTHROGRAPHY: ELECTRICAL POTENTIALS MEASURED AT THE SURFACE OF THE KNEE DURING MECHANICAL LOADING OF THE JOINT ORIGINATE FROM THE ARTICULAR CARTILAGE AND CAN ASSESS CARTILAGE DEGRADATION IN PATIENTS WITH OSTEOARTHRITIS

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Purpose: A new technique called electroarthrography (EAG) which measures the electrical potentials on the knee surface during mechanical loading of the knee joint was recently developed. The purpose of this study was to determine the origin of the EAG signals and to evaluate the effectiveness of EAG signals to assess joint cartilage degeneration.
Methods: The study comprised two groups of subjects: 20 asymptomatic subjects (Control group) and 20 patients diagnosed with bilateral knee osteoarthritis (OA group), all of whom had had a unilateral total knee replacement. The EAG signals were recorded over both knees for the OA group, and over the dominant knee for the Control group. The EAG potentials were recorded using a wireless recording system with 8 electrodes pasted on the surface of the knee. The loading of articular cartilage was performed by the standing subject with a simple transfer of weight from one leg to another. This weight transfer was repeated during two minutes to reduce the noise level by computing average EAG values. The recorded signals were low-pass filtered, corrected for the drift of the baseline and averaged during the multiple loading episodes. Finally, in order to examine the electrical sources of EAG signals, a realistic finite element model of the knee was developed which included the articular cartilage layers as electrical sources and accounted for the influence of the conductivity of tissues

between this source and the sites of EAG potential measurements on the skin.

Results: The EAG repeatability was assessed with a test-retest protocol for 14 normal subjects. This protocol showed statistically significant high Intraclass Correlation Coefficients between the first and second tests for four electrode sites overlying the joint line ($p < 0.05$). These sites also showed the highest mean EAG values. In the control group, the mean amplitude of the EAG signals was statistically larger over the medial side than over the lateral side of the knee (0.231 ± 0.017 mV vs. 0.126 ± 0.042 mV for two sites over the joint line, $p < 0.001$), reflecting joint forces that are known to be higher on the medial side (mean $\pm$ standard error). For EAG signals recorded over the total knee replacement, their mean amplitude was statistically not different than zero, reflecting the absence of cartilage. For signals collected over the arthritic knees, their mean amplitude was statistically lower than in healthy subjects (0.057 ± 0.043 mV vs. 0.231 ± 0.017 mV for a medial site over the joint line, $p < 0.001$), reflecting the deterioration of cartilage caused by osteoarthritis. In the control group, the correlation coefficients between the amplitude of the EAG at each electrode site, and anthropometric variables such as age, weight, body mass index and height were not statistically significant; similarly, no significant differences were observed between men and women for the mean EAG values at each site. Finally, the finite element model computed electric potential distributions with maximum potential values located over the joint line, corresponding well to the maximum values that were measured experimentally in the Control subjects.

Conclusions: Further analyses indicate that EAG signals arise from the 'streaming potentials' that can be measured directly on the surface of the articular cartilage during compression. Cartilage degradation, produced by a reduction in the concentration of glycosaminoglycans or collagen content, is known to decrease the amplitude of the streaming potentials. Therefore, electroarthrography constitutes a non-invasive method for measuring streaming potentials and for assessing cartilage degradation. The non-invasive nature of the EAG, its sensitivity to cartilage degradation, as well as its low cost make this approach very interesting for the early diagnosis of osteoarthritis and to monitor patients during the assessment of new treatments.

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EXPLORATORY ANALYSIS OF A WIDE SPECTRUM OF BIOCHEMICAL MARKERS IN A LARGE COHORT OF INDIVIDUALS WITH SYMPTOMS OF VERY EARLY KNEE AND HIP OSTEOARTHRITIS: DATA FROM CHECK

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Purpose: Firstly, to assess a wide spectrum of biochemical markers (biomarkers) in a large cohort of individuals with symptoms of (very) early osteoarthritis (OA), as opposed to literature mainly reporting on small studies of individual or limited numbers of biomarkers in more advanced OA. Secondly, to investigate interrelationships between these biomarkers and with demographics to demonstrate validity of the obtained dataset and extend hypotheses on the backgrounds of these biomarkers.

Methods: Fourteen biomarkers (uCTX-II, uCTX-I, uNTX-I, sCOMP, sPIIANP, sCS846, sC1,2C, sOC, sPINP, sHA, sPIIINP, pLeptin, pAdiponectin, pResistin) were assessed by ELISA or RIA in CHECK (Cohort Hip & Cohort Knee), a 10-year prospective cohort of 1002 individuals with symptoms of early knee and/or hip OA. Associations between biomarkers and demographics were investigated through multiple linear regression analysis. Clusters of interrelated biomarkers within the assessed biomarker spectrum were identified through principal component analysis.

Results: Quality controls revealed that gathered data were technically reliable. Principal component analysis identified six clusters, consecutively designated as 'bone-CTX-II', 'inflammation', 'synovium', 'C1,2C-adipokines', 'PIIANP', and 'CS846' cluster. Notably, uCTX-II clustered with biomarkers of bone metabolism, while sCOMP clustered with biomarkers of synovial activity. Furthermore, pResistin and pAdiponectin contributed inversely to one cluster together with sC1,2C. All biomarkers, except for sCS846, were statistically significantly associated with demographics.

Conclusions: This is the first time that a wide spectrum of biomarkers was assessed in such a large longitudinal cohort of early-OA subjects followed in detail for 10 years. Data were generally considered of good technical quality. Associations between biomarkers and demographics were conform hypotheses based on established OA risk factors and literature data. sCOMP was mainly associated with (markers of) synovial activity. Indeed, sCOMP is (also) produced by synoviocytes, is present in synovial tissue, and has been associated with clinical and ultrasonographically detected signs of synovitis and/or effusion. Furthermore, uCTX-II was mainly associated with (markers of) bone metabolism. Other authors have also suggested this association and uCTX-II has been linked to osteoclastic resorption of calcified cartilage by some. In summary, our unique biomarker data were considered valid since exploratory analysis of our data extended knowledge on individual or limited numbers of biomarkers from, mostly, smaller cohorts.

This study was funded by CHECK (Cohort Hip & Cohort Knee), an initiative of the Dutch Arthritis Association.

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LARGE BONE MARROW LESIONS AND WORSENING OF BONE MARROW LESIONS IN THE MEDIAL TIBIO-FEMORAL COMPARTMENT ARE ASSOCIATED WITH KNEES UNDERGOING TOTAL KNEE REPLACEMENT : DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: Total knee joint replacement (TKR) is a cost-effective procedure with good long-term outcomes. However, at present there is no clear consensus on indications for TKR. Imaging biomarkers may be able to aid in the decision making process on a patient level as well as in clinical studies and trials.

Subchondral bone marrow lesions (BMLs) have been identified as important disease features relevant not only for clinical disease manifestations such as pain but also for structural progression. Incidence and increase in BMLs was found to be associated with cartilage loss. Thus, BMLs are promising biomarkers for structural progression to important clinical outcomes such as TKR.

The aims of this study were therefore to test whether presence and size of BMLs were associated with increased odds of TKR, and if worsening of BMLs over time is also associated with TKR.

Methods: We studied knees from 121 Osteoarthritis Initiative (OAI) participants that underwent TKR before the 48 month visit for the time point prior to TKR, i.e. "T0" (e.g. for a TKR reported at the 48 month (M) visit, T0 = 36M); and 121 control knees that did not undergo TKR that were matched for radiographic disease stage, gender, and age within 5 years and were assessed at the same T0 follow-up visit. MR images were acquired at four OAI clinical centers using dedicated Siemens Trio 3 T scanners. The coronal IW 2D TSE, the sagittal 3D DESS sequence, coronal and axial multiplanar reformations of the 3D DESS and a sagittal IW fat suppressed TSE sequence were used for semiquantitative assessment.

MRIs were read for subchondral BMLs in 14 articular subregions using the semiquantitative MOAKS system, which scores BMLs in three categories: size of BMLs, % of BML that is cystic and number of BMLs per subregion. Only BML size, which is scored from 0-3, was considered in the analyses.

Analyses were performed on a plate (medial tibia, medial femur, lateral tibia, lateral femur, trochlea, patella) and compartmental level (medial tibio-femoral joint [TFJ], lateral TFJ and patello-femoral joint - [PFJ]).

Conditional logistic regression was applied to assess if knees that undergo TKR are more likely to have large BMLs than controls using a per-plate analytic approach at T0. In addition, we assessed if knees undergoing TKR have more subregions per compartment that show BML worsening from the time point prior T0 (=T-1) to T0.

Results: Subjects were on average 65.3 years old (SD $\pm$ 8.6), predominantly female (58.1%) and overweight (mean BMI 29.6 SD $\pm$ 4.9). In the cross-sectional analysis, TKR knees were more likely to have large (i.e., grade 3) BMLs in the medial compartment when compared to matched non-TKR knees (unadjusted OR 2.62, 95% confidence interval [CI] 1.16-5.91 for the medial tibia, and 2.35, 95% CI 1.10-5.00 for the medial femur, respectively). In regard to the number of BMLs that exhibited increase in size from T-1 to T0, knees with $\geq$3 subregions increasing in size in the medial TF compartment had increased odds for TKR than knees with no