

Prevalence and Classification of Meniscal Calcifications in the Human Knee

Bijay Ratna Shakya¹, Ville-Pauli Karjalainen¹, Iida Hellberg¹, Mikko A.J. Finnilä¹, Khaled Elkhoully¹, Amanda Sjogren², Aleksandra Turkiewicz², Patrik Önerfjord³, Velocity Hughes², Jon Tjörnstrand⁴, Martin Englund², Simo Saarakkala^{1,5}

¹Research Unit of Health Sciences and Technology, University of Oulu, Oulu, Finland, ²Lund University, Orthopaedics, Clinical, Epidemiology Unit, Lund, Sweden, ³Lund University, Rheumatology and Molecular Skeletal Biology, Lund, Sweden, ⁴Lund University, Skane University Hospital, Department of Clinical Sciences Lund, Orthopaedics, Lund, Sweden, ⁵Department of Diagnostic Radiology, Oulu University Hospital, Oulu, Finland
bijay.shakya@oulu.fi

Disclosure: BRS (N), V-PK (N), IH (N), MAJF (N), KE (N), AS (N), AT (N), PÖ (N), VH (N), JT (N), ME (Board member of the Osteoarthritis Research Society International), SS (Associate Editor of Osteoarthritis and Cartilage Open)

INTRODUCTION: The meniscus plays a vital role in maintaining the proper function of a knee joint, and degeneration of the meniscus is strongly associated with knee osteoarthritis (OA). It has been reported that calcification within joint soft tissues is linked to pathogenesis of OA. Two main types of calcium crystals, i.e., basic calcium phosphate (BCP) and calcium pyrophosphate dihydrate (CPPD), have been observed in articular cartilage. Interestingly, meniscal cells tend to calcify more than cartilage cells in OA, which significantly impacts the biomechanics of the meniscus and may contribute to the development and progression of OA. Despite calcification can be often found even in individuals without joint disorders, limited research has focused on identifying meniscal calcifications and analysing its prevalence in knee OA. Therefore, this study aims to investigate the presence of calcification in the meniscus of patients with and without OA, as well as to identify the specific type of calcification present in each group.

MATERIALS AND METHODS: This study utilized 82 meniscus samples from 41 subjects collected from the knee tissue biobank (MENIX, Lund, Sweden): 40 samples from patients with medial compartment knee OA, who underwent total knee replacement surgery, and 42 samples from deceased adult donors without known knee OA. Posterior horns from both medial and lateral meniscus from the selected knee were studied for each subject. Inclusion criteria for OA patients involved surgeon's Outerbridge classification of medial grade IV and lateral grade 0 or 1, while the donor menisci were macroscopically intact (no meniscal tears but mild degeneration/fibrillation allowed). The posterior horns were fixed in 4% saline-buffered formaldehyde and subsequently dissected into 5-10 mm thick sections. One of the sections was embedded into a paraffin and multiple slices were cut. Slices were then stained with Alizarin red and Eosin Y for histology, and adjacent sections went for Raman measurements after dewaxing. For Raman measurements, a 10x/0.25NA air objective and a high-range grating were used. Raman scattering was initiated using a 785 nm laser at 25 mW, passed through a 50 µm confocal pinhole aperture. Each Raman spectrum was collected for 0.25 seconds and averaged over 8 scans to enhance the signal quality. Specific regions of interest with visible calcifications were manually selected for Raman mapping using a step size of 1.2 µm. For the analysis, we utilized a logistic regression model with robust standard errors that took into consideration the clustering of menisci within subjects. The model included Pauli score as main exposure, presence of calcifications as outcome and was adjusted for side (medial or lateral), age, BMI and sex. This study was approved by the regional ethical review board at Lund University (Dnr 2015/39 and Dnr 2016/865).

RESULT: Calcifications were histologically observed in both the medial and lateral menisci of 15 knee OA patients using Alizarin red staining, while 5 patients had calcifications solely in the medial meniscus. We were also able to detect calcifications in either medial or lateral side of all the OA patients with Raman spectroscopy. For donor samples, 4 samples from two donors demonstrated calcification with an Alizarin red stain which was also detected with Raman spectroscopy. A comparison of Alizarin red and Eosin Y staining of the same samples suggested the presence of both BCP and CPPD calcification in menisci. This was further confirmed by Raman spectroscopy, which revealed a characteristic marker band for BCP and CPPD at 960 cm⁻¹ and 1049 cm⁻¹, respectively. Among 35 OA samples with calcification, 28 had BCP calcifications, 5 had CPPD calcifications, and 2 had both types. In 4 donor samples, 3 had CPPD while 1 had both. We observed a strong association between Pauli score and the presence of BCP calcifications, with odds ratio of 1.9 per 1 Pauli score increase (95%CI 1.3 to 2.8). Results for CPPD were more inconclusive with an odds ratio of 1.2 (95%CI 0.9 to 1.6) (Figure 2).

DISCUSSION: Our study suggested that meniscal calcifications are highly prevalent in patients with end-stage medial compartment knee OA. Interestingly, we also found that meniscal calcifications can be present in subjects without OA. Among the two menisci present in the knee joint, the medial meniscus has a higher prevalence of calcifications, which is in line with patients having medial side knee OA. This could be attributed to the greater mechanical stress it typically endures in knees with varus alignment. Additionally, distinct variations in vascular supply between the medial and lateral menisci could contribute to the observed discrepancies in calcification prevalence. BCP was the most prevalent calcification in menisci affected by OA as it is associated with inflammation and is more commonly found in degenerated tissues. We report strong positive associations between Pauli score and BCP, as well as with CPPD calcifications (although potentially to lesser extent) which underscores the relevance of calcifications in assessing disease severity.

SIGNIFICANCE: By providing insights on the prevalence and type of meniscal calcification occurring in both end-stage knee OA patients and donors without known knee OA, this research holds the potential to expand our understanding of the pathogenesis of OA and its potential implications for patient management and treatment strategies.

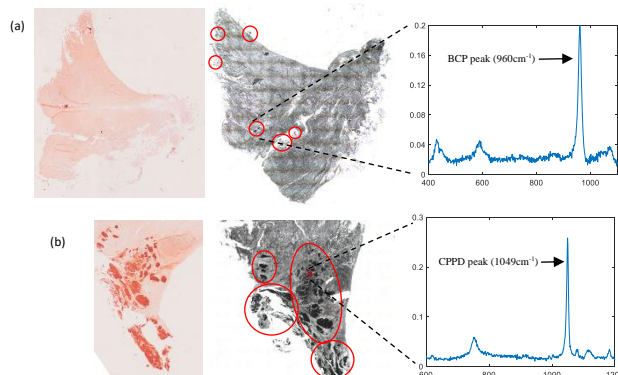


Figure 1: Alizarin Red – stained histological (left) and Raman (middle) images of the samples demonstrating calcifications, accompanied by corresponding Raman spectra (right) of (a) BCP and (b) CPPD. Calcifications are indicated by red circles.

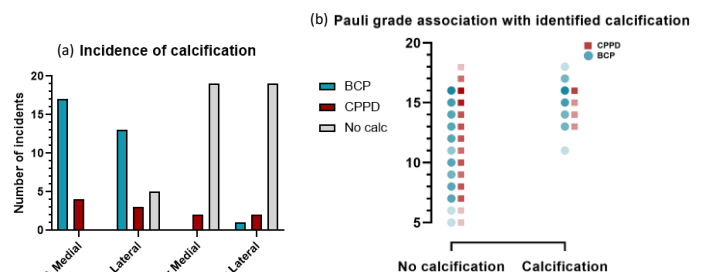


Figure 2: Images showing (a) incidence of calcification among OA patients and donors, as well as its association with (b) Pauli score.