

Terminal Complement Complex Formation is Increased in Degenerated Human Menisci

Graciosa Q. Teixeira, Michael Gross, Jonas Schwer, Luisa de Roy, Anita Ignatius, Andreas M. Seitz
Institute of Orthopaedic Research and Biomechanics, Ulm University, Ulm, Germany
graciosa.teixeira@uni-ulm.de

Disclosures: The authors have nothing to disclose.

INTRODUCTION: The knee joint menisci contribute essentially to the knee joint homeostasis. Traumatic and age-related changes in the menisci are directly correlated with the onset of knee joint osteoarthritis (OA). Inflammation is known to contribute to meniscus degeneration, but there is limited knowledge regarding a possible involvement of the innate immunity, particularly the complement system, which is our body's first line of defense. Hence, the present study investigated whether the production of catabolic and inflammatory mediators, as well as complement activation products such as the terminal complement complex (TCC/C5b-9) are associated with changes in the structural composition of the meniscus with aging/degeneration.

METHODS: Based on the Pauli classification, 12 mildly (age: 47±11 years) and 12 severely (age: 82±9 years) degenerated human menisci were investigated¹. Confined compression Stress-relaxation tests were performed on axial explants from pars anterior (PA), pars intermedia (PI) and pars posterior (PP) regions of lateral and medial menisci to assess the permeability (k) and the equilibrium modulus (E_{eq}). Tensile tests were performed on circumferential explants to determine the Young's modulus (E) and the failure load (F_{max}). DNA, sulphated glycosaminoglycans (sGAG) and collagen were quantified in enzymatically digested samples by biochemical assays. Picrosirius red staining was used to investigate collagen fiber maturity and distribution. Collagen type I (COL1), COL2, aggrecan (ACAN), matrix metalloproteinase-3 (MMP-3), interleukin-6 (IL-6), TCC/C5b-9 and its inhibitor CD59 were analyzed by immunohistochemistry. PA, PI and PP regions were investigated (Fig. 1A), as well as the white-white zone (zone 1, Fig. 1B), the surface layer (zone 2) and the inner region of the red-white zone (zone 3). Statistics: Mann-Whitney U or Kruskal-Wallis test (significance, $p < 0.05$).

RESULTS: The compression-relaxation tests showed an increase of E_{eq} with degeneration, particularly for the lateral meniscus ($p < 0.05$), without affecting k ($p > 0.76$). The meniscus tensile properties were not altered with degeneration ($p > 0.16$). While the sGAG content increased, the percentage of collagen decreased with degeneration, both in lateral ($p < 0.05$) and medial ($p = 0.06$) menisci. A progressive destruction of the otherwise hierarchically arranged fibrous collagen structure was observed without significantly affecting COL1 or COL2 ($p > 0.20$). Interestingly, the percentage of COL1 and COL2 was lower in zone 3 compared to zones 1 and 2 in both mildly and severely degenerated menisci ($p < 0.05$). With degeneration, higher percentage of ACAN, particularly in the lateral meniscus, was observed ($p < 0.05$, Fig. 1C). Less percentage of ACAN was identified in zones 2 and 3 compared to zone 1, in mildly and severely degenerated lateral and medial menisci ($p < 0.05$, Fig. 1D). Regarding catabolic and inflammation markers, no differences were observed in the percentage of MMP-3 and IL-6 staining area between mild and severe degeneration in PA/PI and PI/PP regions; however, higher percentage of MMP-3 and IL-6 were identified in the zone 2 of severely degenerated tissues ($p < 0.05$). Higher percentage of TCC/C5b-9 deposition was detected in degenerated menisci ($p < 0.05$, Fig. 2A,B), particularly in zone 2 of lateral menisci and zones 2 and 3 of medial menisci ($p < 0.01$, Fig. 2C). CD59 production was slower in degenerated menisci ($p < 0.05$, Fig. 2D,E), except in AH/PI of medial meniscus, in which it was higher ($p < 0.01$). Less CD59 was detected in zone 2 of severely *versus* mildly degenerated lateral menisci ($p < 0.001$, Fig. 2F).

DISCUSSION: The increase in E_{eq} might have resulted from changes in the matrix composition, namely the increase in sGAG accompanied by tissue calcification, particularly observed in lateral menisci. Furthermore, this work has investigated for the first time TCC and CD59 production in the meniscus. The increase of TCC and of catabolic and inflammatory mediators with degeneration underlines the role of complement activation in osteoarthritis². Overall, this combined investigation indicates that meniscus structural changes associated with inflammation and complement activation may be detectable at protein level before biomechanical alterations can be observed. Our data is valuable for the validation of *in silico* determination of meniscus material parameters and for the identification of new targets for age-related meniscal degeneration therapeutics.

SIGNIFICANCE/CLINICAL RELEVANCE: This project addresses age-mediated meniscus tissue damage which is described to be an OA trigger. OA patients will benefit from a more comprehensive characterization of degenerated menisci at both, biomechanical and structural levels. This knowledge is fundamental, for instance, for the development of effective regenerative approaches for the meniscus.

REFERENCES: 1. Pauli et al., *Osteoarthritis Cartilage*, 2011.
2. Silawal et al., *Clin Med Insights Arthritis Musculoskelet Disord*, 2018.

ACKNOWLEDGEMENTS: This research was funded by the German Research Foundation (AS3135/2-1) and the ON Foundation (21-340).

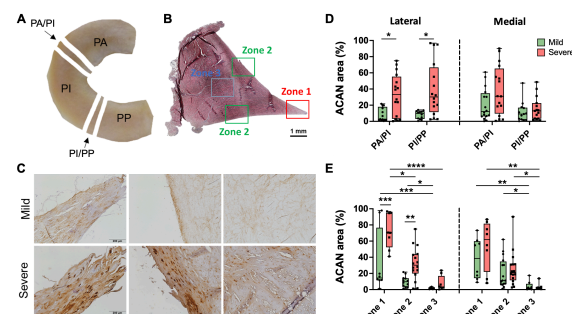


Figure 1. A) Sample collection for histological investigations from pars anterior (PA)/ pars intermedia (PI) and from PI/pars posterior (PP) regions. B) white-white zone (zone 1), surface layer (zone 2) and inner red-white zone (zone 3) investigated by immunohistochemistry. C) Representative images of aggrecan (ACAN) staining in mildly and severely degenerated menisci. D, E) Aggrecan area (%). n=8-16, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

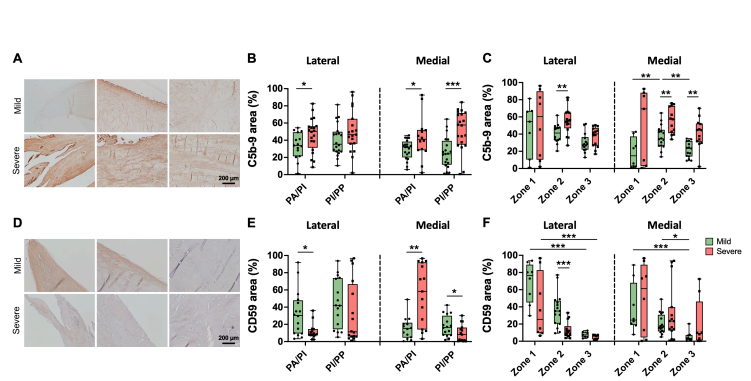


Figure 2. A) Representative images of TCC/C5b-9 staining in mildly and severely degenerated menisci. B, C) TCC/C5b-9 area (%). D) Representative images of CD59 staining in mildly and severely degenerated menisci. E, F) CD59 area (%). n=8-21, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.