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ORIGINAL PAPER

Differences in type II collagen turnover of osteoarthritic human knee and ankle joints

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Abstract

Purpose We analysed hyaline cartilage of human knee and ankle joints for collagen and proteoglycan turnover in order to find differences in the metabolism and biochemical content of the extracellular matrix that could explain the higher prevalence of osteoarthritis (OA) in the knee joint, compared to the ankle joint. *Methods* Cartilage tissue from ankle and knee joints of OA patients were assessed for total collagen and proteoglycan content. For turnover, the aggrecan 846-epitope (CS 846), the type II collagen C-propeptide (CP2) and the collagenase-generated intrahelical cleavage neoepitope (C2C) were quantified. *Results* Molecular analyses showed that type II collagen turnover (CP2 and C2C) was significantly elevated in the ankle, whereas aggrecan turnover (CS 846), total proteoglycan and total collagen were comparable between both joints. Analysis of the inter-relationships in the components of cartilage matrix turnover showed a significant positive correlation of C2C vs CP2.

Conclusions The data suggest an increased type II collagen turnover in ankle vs knee OA cartilage but a comparable aggrecan turnover and comparable contents of type II collagen and proteoglycan. These findings point towards a focused attempt in advanced OA cartilage to structurally repair the collagen network that was more pronounced in the ankle joint and may explain in part the higher prevalence of OA in the knee as compared to the ankle joint.

Keywords Ankle · Cartilage · Human · Knee · Metabolism · Osteoarthritis

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Introduction

Symptomatic osteoarthritis (OA) of the knee affects approximately 6% of the adult population [1]. This increases to almost 10% in people aged over 65 years [2]. Symptomatic ankle OA is seen in less than 1% of the population and occurs only infrequently with advanced age [3]. The underlying reasons are not entirely understood.

In articular cartilage, early OA is characterized by the gradual loss of matrix, an increased biosynthesis of matrix molecules and degradative enzymes [4]. This increase in metabolic activity reflects the response of the chondrocyte to changes in the extracellular matrix and/or the synovial fluid and indicates a repair attempt [5].

More specifically, Aigner and Dudhia (1997) [6] proposed the following steps in the pathogenesis of OA: 1. the synthesis of type II collagen (Col 2) and the proteoglycan (PG) aggrecan increases; 2. the chondrocyte phenotype changes, perhaps due to contact with Col 2 fibrils [7] and atypical matrix molecules such as type III collagen are produced, resulting in a fibrogenic phenotype [8]; 3. the synthesis of aggrecan and Col 2 significantly decreases. It is assumed that there is no general shift in the cellular phenotype during OA, but rather a cyclic sequence of events in different areas of the diseased tissue [9].

Col 2 and aggrecan are two of the components of articular cartilage with major functional relevance. The content of the C-terminal propeptide of Col 2 (CP2) in cartilage matrix directly reflects synthesis of this molecule [10], whereas the 846 epitope (CS 846) is a marker of aggrecan turnover [11, 12]. Degeneration of Col 2 involves cleavage of the triple helix by collagenases which produces a larger ¾ (TCA) and a smaller ¼ (TCB) fragment. This cleavage creates a neoepitope (C2C) that is located on the carboxy-terminus of the TCA (¾) fragment [13].

In previous studies, the synthesis and degradation of Col 2 and aggrecan in human articular cartilage has been investigated [14]. Comparing knee and ankle joints, similar turnover rates in normal, healthy cartilages were found. Interestingly, an upregulation of matrix synthesis in the ankle vs. an upregulated matrix degradation in the knee was detected in non-OA joints in early low grade focal cartilage lesions [15, 16]. This difference between the two joints was also present upon stimulation of healthy, intact cartilage with the catabolic cytokine interleukin-1 [17]. These important molecular differences between the knee vs. ankle joint may contribute to the known difference in their OA frequency. However, it is not known whether these differences between the two joints are typical for intact and non-OA lesional cartilage tissue, or whether these differences are also present in OA. Thus, this study investigated whether differences in Col 2 and aggrecan metabolism of knee and ankle joints are present in end stage OA. Such knowledge is important, as it would help us to understand whether compositional and metabolic differences between large human joints are maintained or lost in advanced stages of this disease.

Materials and methods

Human articular cartilage

Articular cartilage from the talar dome of ten OA ankle joints (five male, five female; age 68 years ± 5.52, range 59 to 76) and the femoral condyles of ten OA knee joints (five male, five female; age 71.7 years ± 4.62, range 63 to 78) were obtained during surgery with institutional approval and written informed consent. All patients suffered end stage OA based on the classification criteria developed by the American College of Rheumatology [18]. All knee OA patients and four ankle OA patients were treated with total joint replacement. In six ankle OA patients, joint fusion was performed. Immediately after surgery, the tissue was prepared as follows: Full thickness cartilage plugs (5 × 5 mm each) of the remaining hyaline cartilage of the lateral posterior femoral condyle of the knee and the talar dome of the ankle were manually dissected down to the calcified zone and prepared for histology, biochemical and immunoassays.

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent was obtained from all individual participants included in the study.

Histology

The cartilage plugs were frozen in embedding compound (Tissue Freezing Medium, Triangle Biomedical Sciences, Durham, NC) and cryo-sectioned with 8 μm thickness. The staining was performed as a double stain with safranin-O and fast green as described previously [19]. The sections were then graded according to the scale developed by Mankin et al. [20].

Biochemical analyses and immunoassays

The cartilage was stored at −80 ° C until assayed. Glycosaminoglycan (GAG) content (reflecting PG content) was measured in combined alpha-chymotrypsin and proteinase K digests using the dimethylene blue (DMB) assay [21]. Total collagen content was measured using the OH-proline assay as described by Huszar et al. [22]. DNA content was measured with the Hoechst 33258 fluorescence-assay [23] and the Col 2 and aggrecan turnover were detected using the ELISA kits for CP2, C2C and CS 846 (IBEX, Montreal). To extract the aggrecan bearing the CS 846 epitope, CP2 and any extractable newly synthesized collagen, the explants were treated for 48 h at 4 °C with 4 M guanidinium hydrochloride (GuHCl) containing the proteinase inhibitors 1 mM EDTA, 1 mM iodoacetamide, 1 mM phenylmethyl sulfonyl fluoride and 5 ug/ml pepstatin A, in 50 mM sodium acetate, pH 5.8 and 1% 3-(3-[cholamidopropyl dimethylaminonio]-1-propanesulfonate) (CHAPS). The extract was exhaustively dialyzed against 50 mM sodium acetate, pH 6.3 [12] and assayed for CP2 [10] and the CS 846 epitope [12] as described. Separate cartilages were extracted with alpha-chymotrypsin. The collagenase generated cleavage neoepitope (C2C) was measured in alpha-chymotrypsin extracts [13].

Statistical analyses

Analyses were performed using SigmaPlot 11.0 (SPSS Inc., Chicago, USA). Data is presented as mean ± standard deviation (SD), minimum (min) and maximum (max) value and the median. The Kolmogorov-Smirnoff test was performed to check for normal distribution (normality test). The Student's t-test (parametric test) or the rank-sum test (non-parametric test) was subsequently used. Correlations were assessed using Spearman's rank correlation coefficient; p values less than or equal to 0.05 were considered significant.

Results

Based on histological sections stained with hematoxylin/eosin and safranin O (Fig. 1), the Mankin-grades were determined for OA

knee and ankle cartilages. The average Mankin grade of explants from knee joints was 5.6 ± 2.59 with a range of 3 to 10. Ankle joint cartilage had a Mankin score of 5.5 ± 2.95 with a range of 2 to 10. There was no statistically significant difference ($p = 0.94$).

A detailed summary of the metabolic data is shown in Table 1. Most relevant is the expression on the basis of wet weight for the matrix components and on a DNA basis for synthesis and turnover, since changes in the matrix content relate to matrix mass, but synthesis and turnover relates to cellularity.

Total collagen and PG contents of knee joint cartilage (220.33 ± 66.58; and 34.76 ± 15.15 μg/mg wet weight (ww), respectively) was similar to the ankle (198.09 ± 51.25; and 32.31 ± 18.64 μg/mg ww, respectively) ($p = 0.414$ and $p = 0.751$). No significant difference could be detected for DNA content (knee: 188.11 ± 36.38 ng/mg ww; ankle: 165.16 ± 49.62 ng/mg ww) ($p = 0.253$, Fig. 2).

Immunoassays (ELISA)

There is a higher turnover of Col 2 in ankle cartilage compared with the knee joint, which is statistically significant. Based on DNA content, the amount of C2C in ankle cartilage is 4.2 times higher compared with the knee. For CP2, the ratio is 3.1 to 1 (Fig. 3). The ratio is 3.2 to 1 and 2.5 to 1 (normalized to wet weight), as well as 3.4 to 1 and 2.85 to 1 (normalized to total content) for C2C and CP2, respectively. No difference could be detected for CS 846. The individual values are shown in Table 1.

Inter-relationships in components of cartilage matrix turnover

The correlation coefficients for the inter-relationships in the components of cartilage matrix turnover are

Fig. 1 Hematoxylin/eosin staining (**a** and **b**) as well as Safranin-O staining (**c** and **d**) of hyaline cartilage from a representative osteoarthritic knee (**a** and **c**) and ankle (**b** and **d**). Note the different heights of the cartilage of the knee (**a** and **c**) and ankle (**b** and **d**) at the same magnification (original magnification ×12.5). The cartilage of the knee joint (**a** and **c**) is derived from the lateral posterior condyle. The cartilage of the ankle is from the central part of the talar dome
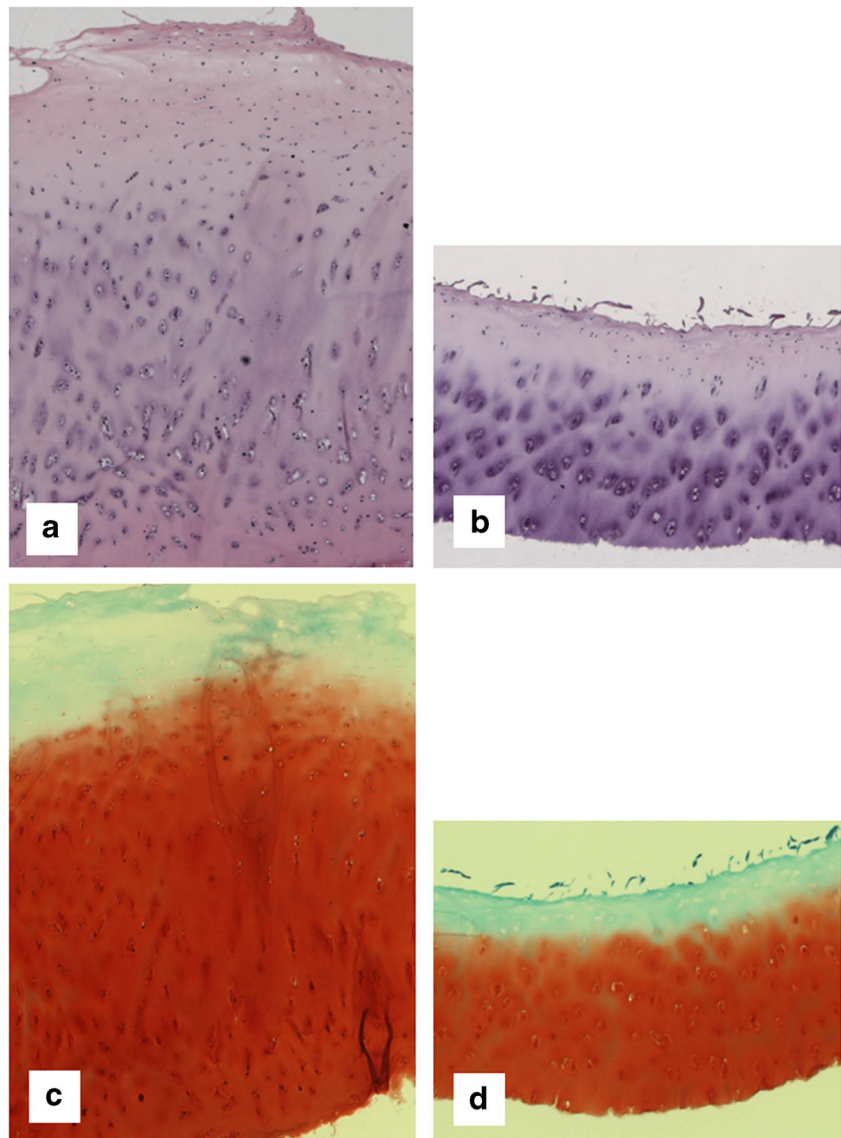
Table 1 Summary of the data of the biochemical assays and the immunoassays for osteoarthritic knee and ankle cartilage samples

	OA knee (mean ± SD)	Min ↔Max (median)	OA ankle (mean ± SD)	Min ↔Max (median)	p-value
Mankin-grade	5.60 ± 2.59	3 ↔ 10 (5.0)	5.50 ± 2.95	2 ↔ 10 (4.5)	0.788
DNA (ng/mg ww)	188.11 ± 36.38	152.42 ↔ 253.85 (247.54)	165.16 ± 49.62	89.56 ↔ 242.94 (166.53)	0.253
Collagen (μg/mg ww)	220.33 ± 66.58	150.55 ↔364.3 (198.8)	198.09 ± 51.25	117.45 ↔ 291.55 (195.43)	0.414
Proteoglycan (μg/mg ww)	34.76 ± 15.15	12.8 ↔ 60.9 (38.49)	32.31 ± 18.64	6.81 ↔ 55.38 (29.77)	0.751
CP2 (ng/μg DNA)	104.44 ± 82.18	18.77 ↔ 242.32 (73.52)	332.8 ± 300.62	77.47 ↔ 949.67 (184.49)	0.031 (*)
CP2 (ng/mg ww)	19.37 ± 15.04	4.77 ↔ 41.59 (14.42)	49.76 ± 39.05	16.52 ↔ 115.86 (28.69)	0.034 (*)
CP2 (ng/mg Collagen)	100.95 ± 90.26	21.07 ↔ 247.74 (51.94)	278.93 ± 253.89	57.55 ↔ 782.44 (142.28)	0.064
C2C (ng/μg DNA)	4.70 ± 3.50	0.66 ↔11.09 (3.84)	19.9 ± 14.73	2.26 ↔ 43.06 (17.31)	0.011 (*)
C2C (ng/mg ww)	0.91 ± 0.75	0.15 ↔ 2.29 (0.64)	2.91 ± 1.91	0.37 ↔ 6.12 (3.40)	0.014 (*)
C2C (ng/mg Collagen)	4.91 ± 4.54	0.51 ↔ 13.75 (3.58)	17.06 ± 13.02	1.74 ↔35.82 (16.33)	0.021 (*)
CS 846 (μg/μg DNA)	3.63 ± 4.16	0.09 ↔ 11.51 (1.54)	3.61 ± 4.18	0.09↔12.43 (2.20)	0.970
CS 846 (μg/mg ww)	0.62 ± 0.70	0.02 ↔ 1.75 (0.29)	0.56 ± 0.69	0.01 ↔2.08 (0.28)	0.910
CS 846 (μg/mg PG)	21.96 ± 28.57	0.25 ↔83.34 (6.44)	16.60 ± 15.39	0.61 ↔ 41.95 (12.61)	0.970

Individual values are expressed as mean ± standard deviation (SD), minimum (min) and maximum (max) values and the median

PG proteoglycan, *ww* wet weight

summarized in Table 2. On analysis of C2C, CP2 and CS 846 epitope, proteoglycan, collagen and DNA content as well as the Mankin grades of all knee and ankle samples together ($n = 20$), a direct relationship was observed for C2C with CP2 epitope ($r = 0.537$; $P = 0.0147$). There was an indirect correlation between the proteoglycan content and Mankin grades ($r = -0.493$; $P = 0.0269$), as well as between the C2C epitope and the collagen content ($r = -0.559$; $P = 0.0104$).
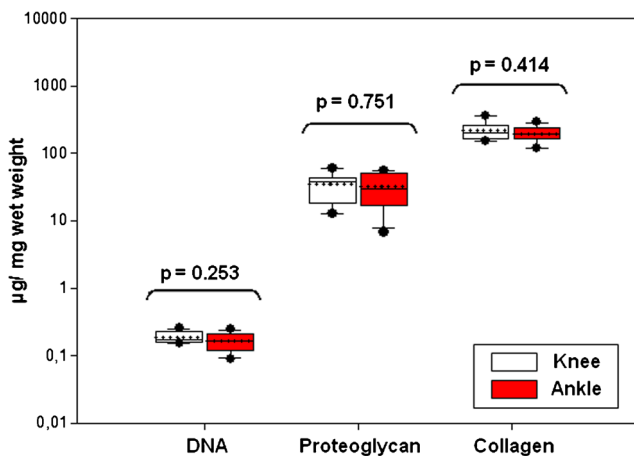
Discussion

Previously, important differences in the metabolic characteristics between the knee and ankle joints of human donors have been uncovered in early focal degenerative cartilage lesions of the knee and ankle joints. It was assumed that cartilage of the ankle joint can react significantly better to an injury or damage than the knee and that this is due to a more efficient synthetic
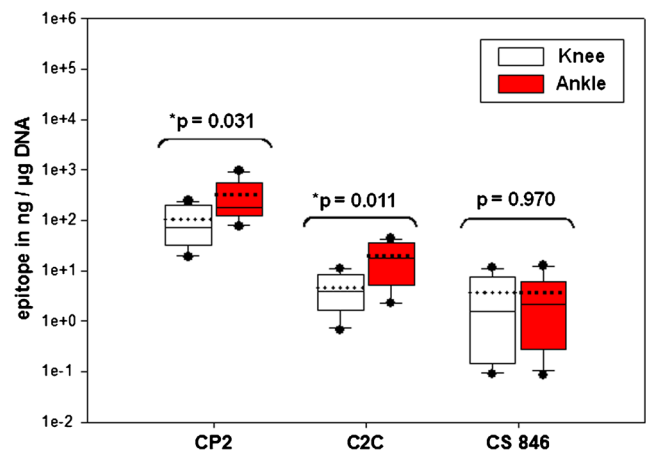

Fig. 2 Biochemical parameter for DNA content, total proteoglycan content and total collagen content of hyaline cartilage of osteoarthritic knee and ankle joints. The data is presented as BOX plots. Each box represents the values between the 25th and the 75th percentile. Lines outside the boxes represent the 10th and the 90th percentile. The points represent values below the 10th or above the 90th percentile. *Solid lines* within the boxes represent the median, *dotted lines* the mean value. *statistical significance with $p < 0.05$

Fig. 3 Epitopes of collagen and proteoglycan metabolism of hyaline cartilage of osteoarthritic knee and ankle joints. Values are based on the DNA content for type II collagen synthesis (CP2), type II collagen cleavage (C2C) and the epitope CS 846, which represents the turnover of the proteoglycan aggrecan. The data is presented as BOX plots. Each box represents the values between the 25th and the 75th percentile. Lines outside the boxes represent the 10th and the 90th percentile. The points represent values below the 10th or above the 90th percentile. *Solid lines* within the boxes represent the Median, *dotted lines* the mean value. *statistical significance with $p < 0.05$

Table 2 Spearman's rank correlation analysis for the inter-relationships in the components of cartilage matrix turnover

	Correlation coefficient (r)	P value	n
PG vs Mankin-grade	−0.493	0.0269	20
Col vs Mankin-grade	NS	NS	20
DNA vs Mankin-grade	NS	NS	20
PG vs DNA-content	NS	NS	20
Col vs DNA-content	NS	NS	20
PG vs Col	NS	NS	20
C2C vs CP2	0.537	0.0147	20
C2C vs CS 846	NS	NS	20
CS 846 vs CP2	NS	NS	20
C2C vs DNA-content	NS	NS	20
CP2 vs DNA-content	NS	NS	20
CS 846 vs DNA-content	NS	NS	20
C2C vs Col	−0.559	0.0104	20
CP2 vs Col	NS	NS	20
CS 846 vs Col	NS	NS	20
C2C vs PG	NS	NS	20
CP2 vs PG	NS	NS	20
CS 846 vs PG	NS	NS	20

The data is expressed on the basis of wet weight for the matrix components (PG, Col, DNA) and on a DNA basis for synthesis and turnover (C2C, CP2, CS 846). The data of knee and ankle were combined (n = 20). The individual analysis of the knee (n = 10) and the ankle (n = 10) did not show any correlation. The pairs of variables with positive correlation coefficients and P values below 0.05 tend to increase together (direct correlation). For the pairs with negative correlation coefficients and P values below 0.05, one variable tends to decrease while the other increases (indirect correlation). For pairs with P values greater than 0.05, there is no significant relationship between the two variables

NS not significant, n number of knees/ankles

capacity of the ankle cartilage [15, 16]. In the present study, the question was whether comparable molecular differences between the two joints may be present in advanced OA. In particular, we investigated whether differences in Col 2 metabolism, aggrecan synthesis and/or PG content between knee and ankle joints were present in the remaining hyaline cartilage of knee and ankle joints from patients with advanced OA undergoing arthroplasty or joint fusion. A direct comparison of cartilage of osteoarthritic knee and ankle joints in terms of collagen and PG turnover has not been published.

The present study demonstrated the presence of an increased Col 2 turnover in OA cartilages of ankle vs knee joints, which is in accordance with previous studies on human donor cartilage [16]. Interestingly, the present study also demonstrated that aggrecan turnover and Col 2 and PG contents were not significantly different in cartilage from human knee and ankle joints with advanced OA. These results point towards the presence of a reparative attempt in OA ankle cartilage with the focus on repairing the collagen network rather than maintaining the aggrecan pool, as

aggrecan turnover and proteoglycan content were not different between the two joints. Thus, differences in the metabolic and compositional characteristics between the two joints were in advanced OA largely based on collagen metabolism but not aggrecan turnover or proteoglycan content. Taken together, these findings may explain, in part, the higher prevalence of knee joint OA compared to ankle joint OA.

Comparing our data to the literature, the turnover of Col 2 and aggrecan was investigated. The values for C2C (0.91 ng/mg wet weight for knee and 2.91 ng/mg wet weight for ankle) were only slightly lower than that described by Billinghurst et al. [13], who determined values of 4.86 and 10.94 ng/mg wet weight for normal and OA knee cartilages. The values measured for CS 846 (0.62 and 0.56 ng/mg wet weight for knee and ankle) are within the range measured by Rizkalla et al. [12] (0.052 ng/mg wet weight for normal knee joints, or 0.255 ng/mg wet weight for OA knee cartilage at a Mankin-grade of 7 to 13). However, our values for CP2 (19.37 and 49.76 ng/mg wet weight for knee and ankle) are significantly higher than those determined by Nelson et al. [10] (0.45 and 3.43 ng/mg wet weight for normal and OA knee cartilage). The authors report an inhomogeneous distribution of CP2 within the cartilage tissue. According to their observation, CP2 is scarcely present in the upper layers but accumulates in deeper layers of the cartilage near the tide mark. This is an indication that in our study of OA cartilage the deeper layers are relatively overrepresented. In a previous study on low grade lesional cartilage [16], the CS 846 in the ankle is significantly higher than in the knee. In the present study on OA cartilage there are no significant differences between knee and ankle with regards to CS 846. The upregulation of aggrecan turnover, which was detected in lesional ankle cartilage, is obviously absent in the state of OA. In contrast, there is a statistically significant increase in the turnover of Col 2 in OA ankle, compared to OA knee cartilage for both the anabolic (CP2) and catabolic (C2C) site. One possible explanation is that in low grade damaged cartilage with focal lesions, proteoglycan turnover is increased in the ankle, while there is a shift of the repair mechanisms towards an increase of Col 2 in OA cartilage with high grade cartilage lesions and advanced OA.

Another interesting observation is the increased DNA content of knee cartilage compared to the ankle, which indicates an increased number of cells in knee cartilage. This is supported by our histological observation that in OA knee cartilage, the upper layers showed deep lacerations and cell clustering. In contrast, the surface of OA ankle cartilages appeared mostly smooth and without cell clustering (data not shown). This could partially explain a higher cell number in osteoarthritic knee cartilage compared to the ankle. However, these histological observations could also be an indication of fundamental differences in cartilage degradation mechanisms between the knee and ankle joint.

One approach would be to quantify hydroxypyridinium crosslinks between the collagen fibres and the comparison of these

values between knee and ankle cartilage. Poole et al. [24] found that due to the crosslinks, cleaved Col 2 fibres remain within the extracellular matrix (ECM). If the number of crosslinks in ankle cartilage is significantly higher than in the knee, this would be a possible explanation for the increase of the C2C epitope found in our study. Possible differences in the degradation of knee and ankle cartilage could also explain the difference between the knee and ankle joint with respect to the CP2. Nelson et al. [10] report on the accumulation of CP2 in the deeper layers of cartilage. Differential structural degradation may lead to a relative over-representation of the deeper layers in ankle cartilage tissue. This could be an explanation for the increase of CP2 in ankle compared to knee cartilage.

Taken together, this study demonstrated that a reparative attempt was present in advanced OA, and that this attempt was more pronounced in ankle vs the knee joint. Moreover, it appeared to be focused on the collagen network, whereas aggrecan pool maintenance was not significantly different between the ankle and knee joints of patients with advanced OA.

Compliance with ethical standards

Conflict of interest   The authors declare that they have no conflict of interest.

Ethical approval   The study has been approved by the local ethics committee (approval #0981-10/02 from the Ethics Committee of the Medical Faculty at the University of Jena, Jena, Germany) and was performed in concordance with the 1964 Declaration of Helsinki and according to the German Data Protection Act. Before the start of the investigation, written informed consent was obtained. The legal requirements concerning confidential medical communication were met. At any time, the patients had the right to withdraw consent without giving reasons and without disadvantages regarding further medical treatment. The medical therapy of the patients during hospitalization was performed independently of this research project. Patient data was stored exclusively in the Hospital according to current data protection laws. Third persons were not provided insight into source data. Any patient has the right to have his data deleted.

Informed consent   Informed consent was obtained from all individual participants included in the study.

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