Articular cartilage degeneration after acute subchondral bone damage

An experimental study in dogs with histopathological grading

Andreas Lahm[1], Markus Uhl[2], Christoph Erggelet[1], Jörg Haberstroh[3] and Eike Mrosek[1]

Departments of [1]Orthopaedic Surgery, [2]Diagnostic Radiology and [3]Surgical Research, University Hospital, Hugstetter Str. 55, DE-79106, Freiburg, Germany
Correspondence AL: lahm@ch11.ukl.uni-freiburg.de
Submitted 02-12-02. Accepted 04-03-26

Background Subchondral fracture patterns and bone bruises have been described and some clinical studies have shown alterations in the initially healthy cartilage after such lesions.

Methods and results After having performed cadaver studies, we created an animal model to produce pure subchondral damage without affecting the articular cartilage, under MRI control. We used 12 beagle dogs. For quantification of different degrees of staining, we used a grading of the sections by means of the HHGS (Histological-Histochemical Grading System) or Mankin score.

Results In all cases, FLASH 3D sequences revealed intact cartilage in MRI after impact. The best detection of subchondral fractures was achieved in fat-suppressed TIRM sequences. Image analysis based on the HHGS showed changes in 10 of 12 samples, with a high degree of significance 6 months after the initial trauma. Correlation analysis showed loss of the physiological distribution of proteoglycans and glycoproteins in the different zones of articular cartilage. Subcategories "Structure", "Cells" and "Safranin-O Staining" also showed high significance, and the category "Tidemark Integrity" showed a tendency.

Interpretation Our findings indicate that acute subchondral fractures are a predictor of degenerative changes within 6 months. Modifications and supplements to rehabilitation might be needed in cases with accompanying subchondral lesions, e.g. in ACL tears.

■

In recent years, increasing attention has been paid to the role of subchondral bone in the pathogenesis of osteoarthrotic changes (Burr 1998, Imhof et al. 2000, Kawak et al. 2001). Mechanical factors especially, such as a steep stiffness gradient in the underlying subchondral bone, have been suspected to be important in the initiation and progression of cartilage damage (Radin and Rose 1986, Burr and Schaffler 1997).

Acute subchondral lesions such as bone bruises, sub- and osteochondral fractures were described by Mink and Deutsch (1989). Since then, MRI has become the accepted method for detection of these lesions (Potter at al. 1998, Uhl et al. 1998, Irie et al. 2000, Lahm et al. 2000). Others have described these lesions to be associated with anterior cruciate ligament injuries in up to 80% of cases (Stein et al. 1995, Lahm et al. 1998, Lynch et al. 1998, Faber et al. 1999). They are often termed occult lesions because they may be hidden from view by the overlying cartilage on direct arthroscopic evaluation, and are usually not visible on standard radiographs.

Histopathology and cryosections of human bone bruise injuries of the knee have shown microfractures of cancellous bone and weight-bearing trabeculae, edema and bleeding of fatty marrow which correlate with the lesions detected on MRI (Rangger et al. 1998).

Animal models have been used to investigate the pathogenesis of osteoarthrosis and cartilage reconstruction without opening of the knee joint, but

DOI 10.1080/00016470410004166

the effect of subchondral damage on the healthy cartilage has yet to be determined experimentally (Donohue et al. 1983, Thompson et al. 1991, Borelli et al. 1997, Panula et al. 1998, Lahm et al. in press). After developing an animal model to induce subchondral damage without changes in the overlying cartilage and without invading the joint surgically (Lahm et al. in press), we have examined the medium-term effect on cartilage using a scoring system.

Animals and methods

In a previous study (Lahm et al. in press), an animal model was developed to produce subchondral lesions of the knee joint. The forces necessary to produce microscopic or macroscopic cartilage damage and pure subchondral bone damage were determined in cadaver studies and adult beagle dogs. The reproducibility of the forces applied under standardized conditions with intact articular structures including the hyaline cartilage was determined in that study.

In the present study, 12 adult beagle dogs (10 female) underwent impact to the right knee joint with a force that was able to produce a subchondral fracture without cartilage damage. The nonimpactedleft knee was examined as a control knee in every animal. The 12 healthy adult beagle dogs weighed 14.8 (SD 1.3) kg on average, and had an average age of 15.6 (SD 1.1) months.

The transarticular force was applied by a weight which was dropped onto the patella of a rigidly held knee joint of an anesthetized dog. The construction consisted of a drop-tower, a drop weight of 2.1 kg with a force-transmitting rod tip with a diameter of 1.9 cm (adapted to the patella of the dog), a load cell (Kistler Swiss Type 5001) and a force transducer (Kistler Quarz). The forces transmitted were recorded on an oscilloscope with settings of 0.5 msec/div. and 0.5 V/DN (1V/1000 N). After the animal had been anesthetized, it was placed in lateral recumbency with the hip abducted and flexed 90°. The tibia was held at 100° of flexion in the knee joint, and the whole lower extremity was secured rigidly with the thigh positioned in a frame Pre-impact radiographs in sagittal and axial views were performed for a correct perpendicular impact. The force is induced by the fall of the weight released over a prescribed distance.

The dogs were housed in groups of 2 in kennels with an outdoor area. The animals were fed a standard pelleted diet and water was freely available. One day before anesthesia, the animals were not fed but given free access to water. General anesthesia was induced with 0.3 mg/kg midazolam i.v. followed by 5–6 mg/kg propofol i.v. and, after endotracheal intubation, maintained by the i.v. infusion of 0.3 mg/kg/min propofol and repeated i.v. bolus of 0.05 mg/kg fentanyl. At the end of anesthesia and for the next 3 days, they received caprofen subcutaneously, 4 mg/kg once daily, for analgesia. Both knee joints were examined by MRI using a 1.5 Tesla imaging unit (Vision, Siemens, Erlangen, Germany) using a head coil and a small surface ring coil. The first MRI examination took place 4 hours after impact and consisted of the following sequences: 1) Fast T1-weighted SE (Spin Echo) sequences (TR/TE 550/14 ms) in 3 perpendicular planes; 2) T1-weighted SE sequence (TR/TE 420-535/12–17 ms) with a slice thickness of 3 mm and a 240 × 256 matrix (sagittal); 3) TSE (Turbo Spin Echo) with spectral fat saturation T2-weighted (Turbo factor = 15, TR/TE 2900/120 msec), slice thickness 3 mm (sagittal); 4) fat-suppressed TIRM sequences (Turbo Inversion Recovery Magnetitude) with a slice thickness of 4 mm, TR/TE 6194/60 msec (sagittal); 5) 3D-FLASH (fast low-angle shot) fat-suppressed sequence, TR/TE 48/11 msec. Flip angle = 40°, 336 × 512 matrix, slice thickness 1.5–2 mm (sagittal).

6 months after the trauma, a second MRI was performed and osteochondral sections were taken from both the area above the former subchondral lesion and the corresponding area of the unaffected knee joint. The animals were not killed; the defects were treated with microfracturing. The sections were anonymized coded, fixed, decalcified in EDTA, dehydrated and embedded in paraffin. Hematoxylin-eosin, van Gieson, safranin-O, PAS and Alcian blue staining was performed using standard methods. The sections were graded according to the Mankin Score (Mankin 1982, Pannula et al. 1994, 1998, Ostergaard et al. 1999). Statistical analysis was performed using signed rank test (Wilcoxon) and sign test (McNemar).

The study was performed in accordance with the German law for animal protection and had been approved by the “Review Board for the Care of

Figure 1. Fat-suppressed 3D-FLASH sequence using the head coil, showing intact articular cartilage (thin bright line) immediately after trauma causing a subchondral fracture.

Figure 2. Subchondral fracture in the lateral aspect of the trochlea on T1-weighted image, 4 hours after impact (same knee as in Figure 1).

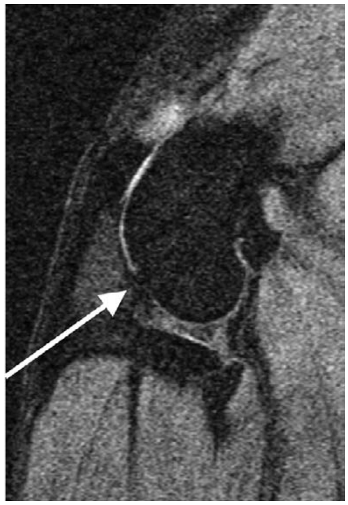

Figure 3. Defect of articular cartilage contour at the lateral femoral condyle, taken at follow-up 6 months after subchondral fracture in the same knee as in Figure 1.

Animal Subjects" at the "Regierungspräsidium", Freiburg, Germany.

Results

Physiological articular cartilage could be demonstrated in the FLASH 3D sequences in combination with the T2-weighted TSE sequences in all 12 cases of injured knees, hours after impact (Figure 1). Subchondral fractures (microfractures) with bleeding were indicated by a decrease in signal intensity on TIRM and T1-weighted images due to transformation of hemoglobin to deoxyhemoglobin, or even methemoglobin (Figure 2). The signal intensity usually increased after some hours with a higher proportion of methemoglobin, an effect that could also be identified on T1-weighted images. These changes correlated with microfractures of cancellous bone and weight-bearing trabeculae. In no case was any intraarticular structure affected by the impact.

Follow-up

On follow-up, MRI and histology showed articular cartilage changes in 10 of the 12 knees with former subchondral injury (Figure 3). In the very thin superficial zone, we found absence of safranin-O staining in both groups. The transitional zone and (even more) the deep radial zone showed a substantial amount of proteoglycans in the samples of the nonimpacted knee joints, and a significant loss in the joints after subchondral fractures in both regions combined with destruction of the cartilage surface (Figures 4 and 5). In the zone of calcified cartilage, there was an increase in safranin-O staining of the pericellular matrix. In 7 of the 12 cases, we found vascular invasion in this area.

Image analysis based on the HHGS or Mankin score showed changes in 10 of 12 samples with a high degree of significance ($p < 0.001$) (Table). These had not shown any evidence of damage during the initial examination (Figure 6).

Subcategories "Structure", "Cells" and "Safranin-O staining" also showed high degree of significance ($p < 0.002$), and the category "Tidemark integrity" showed a tendency ($p < 0.0156$) in signed rank test (Wilcoxon) and sign test (McNemar). Chondrocyte cloning was visible in the articular cartilage itself, sclerosis and a decrease in trabecular number in the subchondral bone. The distribution of proteoglycans and glycoproteins in the different zones of the articular cartilage had changed, indicated especially by a loss of proteoglycans in the radial zone, as shown by a decrease in safranin-O-staining.

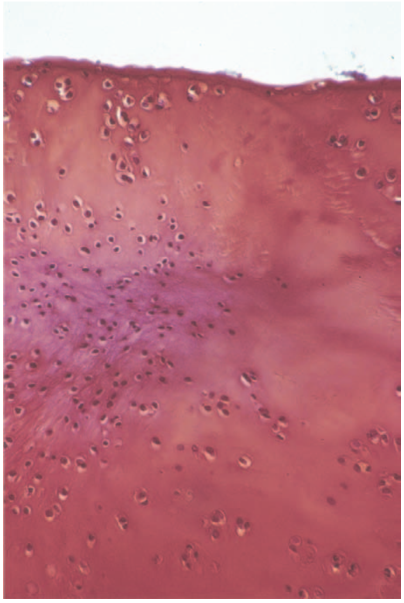

Figure 4. Safranin-O staining (× 200) of articular cartilage 6 months after subchondral fracture with focal pannus, granulation tissue, hypocellularity and decrease in staining.

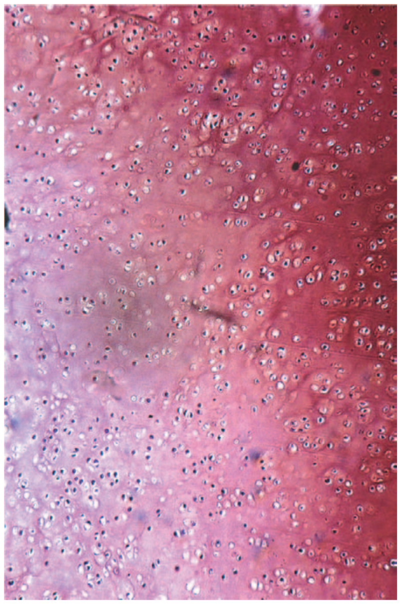

Figure 5. Safranin-O staining (× 200) from the radial zone, with transition from mild (right side) to severe (left side) decrease in staining as a sign of decrease in amount of proteoglycans above former subchondral fracture.

Table. Mankin scores from the articular cartilage from both knee joints of beagles (A: 6 months after impact with subchondral damage; B: contralateral control knee without former subchondral damage) and analysis of Mankin score regarding median and interquartile (Q1 and Q3)

	Group A	Group B
Dog 1	11	1
Dog 2	6	0
Dog 3	4	0
Dog 4	0	0
Dog 5	5	0
Dog 6	5	0
Dog 7	8	0
Dog 8	8	1
Dog 9	4	0
Dog 10	8	0
Dog 11	0	0
Dog 12	5	0
Median	5	0
Q1	4	0
Q3	8	0

Discussion

The prevailing view of the role of subchondral bone in the pathogenesis of osteoarthrosis has changed in the past few years (Burr 1998, Imhof et al. 2000, Kawak et al. 2001). Recent studies have shown the relative importance of subchondral nutrition of the cartilage in autologous transplants (Kawak et al. 2001) and physiological cartilage (Imhof et al. 2000). Other studies have suggested that the search for significant changes in the metabolism of articular cartilage has failed to yield consistent data (Radin and Rose 1986). Although several studies suggest a molecular degradation of the subchondral tissue that leads to a loss of mechanical integrity during the development of osteoarthrosis, almost all animal models for investigation of the role of cartilage repair techniques, including chondrocyte transplantation, do not take into consideration the existence of concomitant subchondral damage at the original lesion.

In 1986, Radin and Rose focused on the role of subchondral bone as an effective shock absorber. They found shear stress in the articular cartilage which always occurred whenever there was a discontinuity or substantial gradient in stiffness of the subchondral plate. In their studies, finite element analysis showed increasing stress in the cartilage subsequent to subchondral plate stiffening. These changes occurred without any evidence of meta-

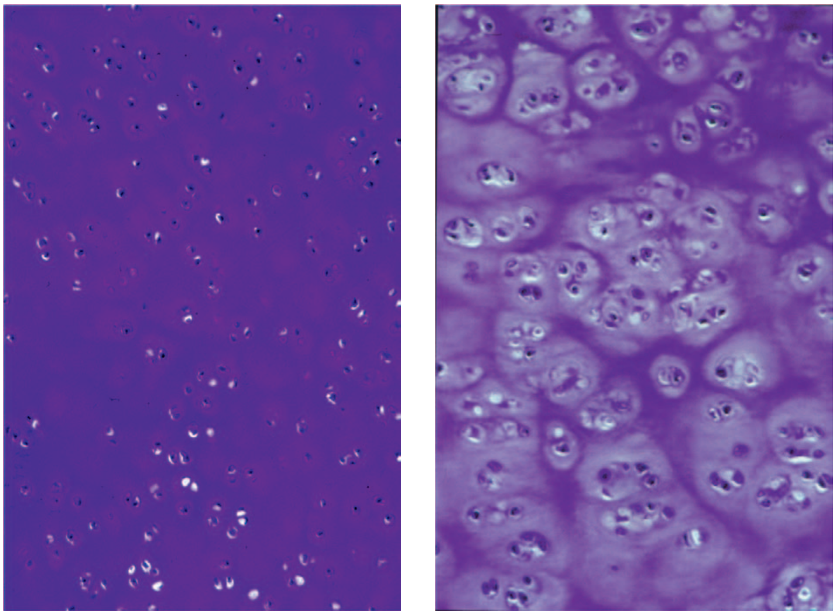

Figure 6. PAS staining (× 200) representing amount of glycoprotein, with very intensive colouring of healthy cartilage (left) and cell clustering with significant decrease in pericellular staining as a result of abnormal cellular glycoprotein production (right).

bolic or inflammatory changes, which implied that the latter follow the mechanical changes, first in the bone and then in the cartilage.

Clinical studies by Johnson et al. (1998) and Lahm et al. (1998) have shown that osseous lesions such as subchondral fractures may heal into stiffer constructions such as subchondral sclerosis, compared with the previous normal bone. In these cases, follow-up MRI or arthroscopy after some months also revealed alterations of the hyaline articular cartilage which had not shown any evidence of damage during the initial examination. In a more recent study, Johnson et al. (2000) also reported an increased loss of joint hemostasis in patients who had sustained subchondral damage in addition to an ACL injury, as well as a higher complication rate in such patients after ACL reconstruction. The present study—and also the fact that often no correlation exists between knee stability and the development of degenerative changes—show that concomitant damage such as subchondral fractures contribute significantly to the final outcome of the articular cartilage after knee trauma. Lesions associated with medial collateral ligament injuries are less severe than those associated with ACL tears because more force is transmitted with ACL tears. Also, the lesions are not directly subchondral, more diffuse and less localized in combination with MCL-tears.

Our study shows clearly that acute subchondral fractures are a factor for future degenerative changes in the cartilage, even in the absence of any initial intraarticular damage. Modifications and supplements of rehabilitation, such as open kinetic chain in isokinetic treatment or orthosis with relief of affected compartments might be required, as some studies confirm high axial forces in closed kinetic chain rehabilitation programs (Irrgang and Pezullo 1998).

For cartilage evaluation, we used the Histological-Histochemical Grading System (HHGS) (Mankin 1982, Ostergaard et al. 1999) or Mankin score, which was developed for the grading of cartilage damage in humans. The system has been widely used in animal studies on osteoarthrosis (Panula et al. 1994, 1998). The capability of the system has been demonstrated, and it has been found useful for systematic assessment of articular cartilage, as the categories included in the scoring system encompass highly relevant histological and histochemical variables. The capacity of the HHGS to differentiate between moderate and mild arthrotic changes is still questionable, however (Ostergaard et al. 1999).

This study was supported generously by the Wissenschaftliche Gesellschaft Freiburg i. Br.

Borelli J Jr, Torzilli P A, Grigiene R, Helfet D L. Effect of impact load on articular cartilage: development of an intra-articular fracture model. J Orthop Trauma 1997; 11 (5): 319-26.

Burr D B. The importance of subchondral bone in osteoarthrosis. Curr Opin Rheumatol 1998; 10 (3): 256-62.

Burr D B, Schaffler M B. The involvement of subchondral mineralized tissues in osteoarthrosis: quantitative microscopic evidence. Microsc Res Tech 1997; 37 (4): 343-57.

Donohue J M, Buss D, Oegema T R, Thompson R C. The effect of indirect blunt trauma on adult canine articular cartilage. J Bone Joint Surg (Am) 1983; 65: 948-57.

Faber K J, Dill J R, Amendola A, Thain L, Spouge A, Fowler P J. Occult osteochondral lesions after anterior cruciate ligament rupture. Six-year magnetic resonance imaging follow-up study. Am J Sports Med 1999; 27: 489-94.

Imhof H, Sulzbacher I, Grampp S, Czerny C, Youssefzadeh S, Kainberger F. Subchondral bone and cartilage disease: a rediscovered functional unit. Invest Radiol 2000; 35: 581-8.

Irie K, Yamada T, Inove K. A comparison of magnetic resonance imaging and arthroscopic evaluation of chondral lesions of the knee. Orthopaedics 2000; 23: 561-4.

Irrgang J J, Pezullo D. Rehabilitation following surgical procedures to adress articular cartilage lesions in the knee. J Orthop Sports Phys Ther 1998; 28: 232-40.

Johnson D L, Urban W P Jr., Caborn D N M, Vanarthos W J, Carlson C S. Articular cartilage changes seen with magnetic resonance imaging. Detected bone bruises associated with acute anterior cruciate ligament rupture. Am J Sports Med 1998; 26: 409-14.

Johnson D L, Bealle D P, Jefferson C B Jr, Nyland J, David N M C. The effect of a geographic lateral bone bruise on knee inflammation after acute anterior cruciate ligament rupture. Am J Sports Med 2000; 28: 152-5.

Kawak C E, Mc Ilwraith C W, Norrdin R W, Park R D, James S P. The role of subchondral bone in joint disease: a review. Equine Vet J 2001; 33: 120-6.

Lahm A, Erggelet C, Steinwachs M R, Reichelt A. Articular and osseous lesions in recent ligament tears: Arthroscopic changes compared with magnetic resonance imaging findings. Arthroscopy 1998; 14: 597-604.

Lahm, A, Erggelet C, Steinwachs M R, Reichelt A. Arthroscopic management of osteochondral lesions of the talus: results of drilling and usefulness of magnetic resonance imaging before and after treatment. Arthroscopy 2000; 16: 299-304.

Lahm A, Uhl M, Edlich M, Erggelet C, Haberstroh J, Kreuz P C. An experimental canine model for subchondral lesions of the knee joint. The Knee (in print)

Lynch T C, Crues J V 3rd, Morgan F W, Sheehan W E, Harter L P, Ryu R. Bone abnormalities of the knee: prevalance and significance at MR imaging. Radiology 1998; 171: 761-6.

Mankin H J. The response of articular cartilage to mechanical injury. J Bone Joint Surg (Am) 1982; 64: 460-6.

Mink J H, Deutsch A L. Occult cartilage and bone injuries of the knee: Detection, classification and assesment with MR imaging. Radiology 1989; 170: 823-9.

Ostergaard K, Andersen C B, Petersen J, Bendtzen K, Salter D M. Validity of histopathological grading of articular cartilage from osteoarthritic knee joints. Ann Rheum Dis 1999; 58: 208-3.

Panula H E, Helminen J H, Kiviranta I. Slowly progressive osteoarthritis after tibial valgus osteotomy in young beagle dogs. Clin Orthop 1994; (302): 11-6.

Panula H E, Nieminen J, Parkkinen J J, Arnala I, Kröger H, Alhava E. Subchondral bone remodelling increases in early experimental osteoarthritis in young beagle dogs. Acta Orthop Scand 1998; 69: 627-32.

Potter H G, Linklater J M, Allen A A, Hannafin J A, Haas S B. Magnetic resonance imaging of articular cartilage in the knee. An evaluation with use of fast-spin-echo imaging. J Bone Joint Surg (Am) 1998; 80: 1276-84.

Radin E L, Rose R M. Role of subchondral bone in the initiation and progression of cartilage damage. Clin Orthop 1986; (213): 34-40.

Rangger C, Kathrein A, Freund M C, Klestil T, Kreczy A. Bone bruise of the knee: histology and cryosections in 5 cases. Acta Orthop Scand 1998; 69: 291-4.

Stein L N, Fischer D A, Fritts H M, Quick D C. Occult osseous lesions associated with anterior cruciate ligament tears. Clin Orthop 1995; (313): 187-93.

Thompson R C Jr., Oegema T R Jr., Lewis J L, Wallace L. Osteoarthrotic changes after acute transarticular load. J Bone Joint Surg (Am) 1991; 73: 990-1001.

Uhl M, Ihling C, Allmann K H, Laubenberger J, Tauer U, Adler C P, Langer M. Human articular cartilage: in vitro correlation of MRI and histologic findings. Eur Radiol 1998; 8: 1123.