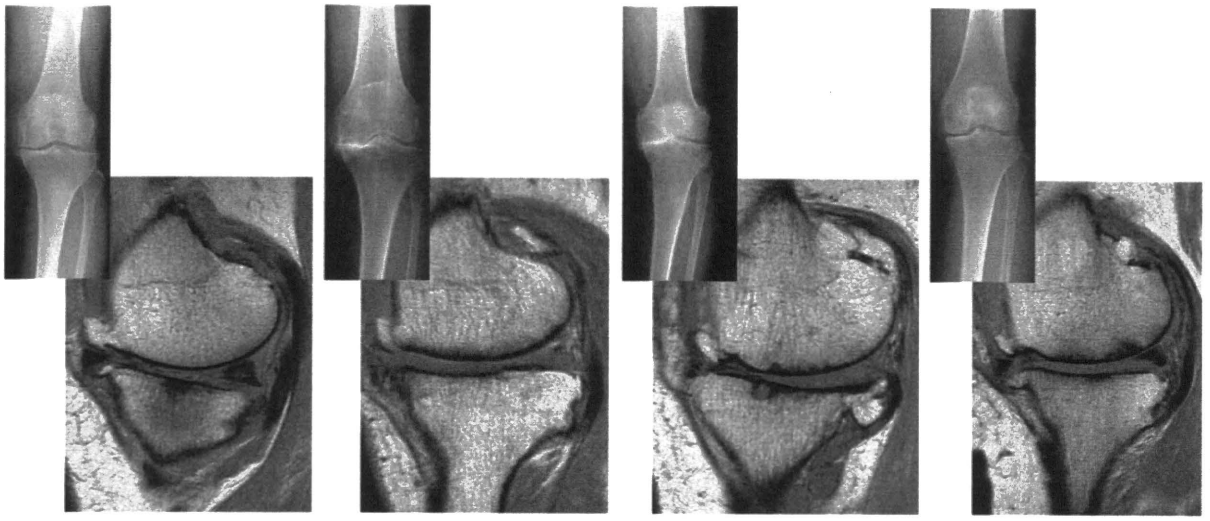

1.58
症例 1　72歳　女性

2.52
症例2　77歳　男性

2.77
症例3　76歳　女性

4.34
症例4　73歳　女性

図 4　不整度（SDS）と代表例の画像所見
代表症例を 4 例，左からSDSの少ない順に並べ提示した。いずれも内側型膝OAであるが，不整度の差異を肉眼的に判定することが困難であることがわかる。

症例 2，3，4 では不整度の大きさを肉眼的に判定するのは困難である。

考　　察

今回の検討の結果，大腿骨内側顆の不整度はTKA を適応する際の指標として，また関節鏡手術を適応とする際の指標となり得ることが示唆された。我々はこれまでに，不整度がJOA スコア，Lysholm スコア，JKOM と負の相関のあること，疼痛の強さのVisual analogue scale（VAS）スコアとは正の相関を持つことを示してきた[4]。すなわち，不整度が膝 OA の客観的な重症度の指標となることを示してきたのである。従って今回の結果はある程度までの重症度であれば関節鏡手術で対応でき，ある一定の段階を超えた場合はTKAを適応するのがよいのであるという，ある意味では当たり前のことであろうが，この事実を数字で示した新しい知見である。手術の適応に関しては医師の裁量に負うところが大きいのが現実であろうが，適切な治療体系の確立にはある程度の客観的な指標が必要であり，不整度はその一つの手段となり得ると考えられる。

今回は関節鏡手術と保存療法群の間には不整度の差がなかった。この理由として，ひとつには関節鏡手術が適応となるような症例では，変性断裂した半月板が症状の発現に関与している可能性があるが，不整度計測では半月板を無視していることが挙げられる。また，ふたつめとして保存療法を受けることとなった症例の中にはMRI 検査が必須の検査項目ではく，未実施の症例が存在する。今回の検討の保存療法群はMRI 検査が施行された症例に限られているためバイアスがかかっていることが考えられる。いずれにしろ不整度という単一の指標のみにて各種治療法の適応を決めることは困難ではあり，他の方法と組み合わせることがよりよい指標作りには必要となることが考えられる。Peterfy らが報告した Whole Organ Magnetic Resonance Imaging（WORMS）法は半月板，滑膜，靭帯などの要素も評価する方法であり，不整度計測の欠点を補うものである可能性がある[5]。

輪郭が不整となることについての病理学的な意義であるが，MRI での顆部の輪郭とは軟骨下骨領域に相当すると考えられる。われわれは

これまでにOA罹患膝の軟骨下骨の免疫組織学的検討を行っており，不整度の強い部位では，軟骨下骨領域にCOX-2，TNF-α，サブスタンスPなどが高率に存在することを示してきた[6,7]。Suriらも指摘するように軟骨下骨が疼痛の原因組織の一つと考えられるのである[8]。すなわち，不整度の高低は軟骨下骨が疼痛を生ずる組織へと変化している程度を表しているものと考えられる。

こうしたことからわれわれはTKAによる除痛のメカニズムとして，骨切りにより病的な状態に陥った軟骨下骨を取り去るデブリードマン効果と考えている。よって不整度の計測は骨を温存する治療法の限界を表しているのかもしれない。

ま と め

内側型OAの客観的な重症度を評価する方法である大腿骨内側顆の不整度計測法が治療法の適応を決定する際の指標となり得ることを示した。

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Ⅳ. MRI

変形性膝関節症に対するMRIを用いた重症度評価法に基づく治療法の選択

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変形性膝関節症（膝OA）は国内の患者数が1,000万人を超えると推定されるほど非常に頻度の高い疾患である。高齢者の日常生活動作に大きな影響をもつ疾患であるため，高齢社会においてますます重要性を増している。ありふれた疾患ともいえるが，わが国でも大規模コホートを用いた研究がなされるなど，本疾患に対する医療側の取り組みも盛んになってきた。一般的にも関心の高い疾患であり，膝によいとのうたい文句で多数の健康食品が宣伝されており，しばしばマスコミにも取り上げられている。

膝OAは長い目でみると徐々に進行していく疾患であるため，進行の過程をなんらかの形でステージ分けすることや，重症度をグレーディングすることなどが可能となるはずである。重症度を客観的に評価することは，疾患の状態を把握するために必要となるものであり，治療が必要な際には治療法を選択するための指標となりえるものと考えられる。

疼痛を主訴とすることが多い疾患であるために，症状の程度を患者自身が主観的に評価する方法も重要ではあるが，治療指針を確立していくためには客観的な重症度の評価法を確立することが必須となる。その方法として屈曲拘縮の程度や膝可動域といった身体所見，血液・尿・関節液を試料とするバイオマーカー，歩行分析，X線像を始めとする画像検査などが考えられる。

このなかで繁用されているのがX線検査に基づくグレーディング法である。グレーディング法にはいくつかの種類があるが，代表的なものはKellgren & Lawrenceのグレーディング法であり，国際的にも広く使われている。この方法は簡便な方法であるという大きな利点をもつが，重症度を評価する方法として信頼性があるかどうかとなると議論のあるところである。

より信頼度の高い画像評価法を確立することを目的に，著者らはこれまでにMRIを用いた重症度評価法の作成に取り組んできた。以下，この方法を紹介をするとともに，本法に基づく治療法の選択につき症例を提示し，また，今後の展望や問題点についても記す。

開発の経緯

膝OAに対して客観的な重症度の評価法が必要であるとの認識から，著者らがMRIを用いて膝OAの重症度評価法に取り組み始めたのは2000年からである[1)]。この検討により，内側型膝OAにおいて，大腿骨内側顆の軟骨下骨領域に現れる変化が重症度の指標となりえるという結論が得られた。この検討を施行するにあたり，コンピュータによる画像解析の手法を取り入れ始めた。また，鏡視下後内側解離術（postero-medial release；PMR）の術後成績を検討した際にも，MRIに基づく評価法が有用であることを示した[2)]。ここでも膝OAが進行してくると明らかになってくるMRI上の大腿骨内側顆の軟骨下骨領

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域に相当する部位にみられる変化に着目したのである。

こうした変化は換言すると大腿骨顆部の輪郭の不整像といえる。平たくいうと輪郭がどの程度ギザギザしてきているかということになる。この不整の程度はフィルムとなったMRIの画像から肉眼的にグレーディングすることも可能ではあったが，よりわかりやすくするために最も単純な画像処理をした。

すなわち最も低輝度にみえている部位のみを抽出したのである。こうすることで肉眼的には最も黒い(低輝度の)と認識される部位のみを取り出し，検者間での差異の低減や再現性を高めることができると考えられた。

しかし，こうした方法によっても検者間の差異や再現性の問題が皆無となるわけではない。

そこで，抽出した輪郭の不整の程度を自動的に計測し，数値として表現できる方法を模索していたところ，ほかの目的に開発されたソフトウエアが応用できそうであったため，試用することとなった。その結果，計測された不整度と膝機能スコアに負の相関関係が認められた[3]。しかし，この方法ではいくつかの問題点があったため，さらに精度の高い測定法を確立するために専用のソフトウエアを開発するに至った[4]。その後も改良を重ね現在に至っている。

撮像準備

不整度を測定するためのソフトウエアであるが，Matlab6.5(サイバーネットシステム社)を用いてプログラミングした。このソフトウエアが施行する計測法の概要を示す。

第1段階は撮像したデータ(DICOM data)をPCに取り込むことである。当初はMRIフィルムをスキャナーに通してからPCに取り込んでいたが，フィルムとなることで生じる誤差を低減するために，MRIのデータを直接取り込めるようにした。

第2段階では，取り込んだ画像を白黒画像に変換する(**図1**ⓐ→ⓑ)。そのためにMRIを構築するピクセルの濃淡の程度と頻度をヒストグラムに提示する。すると症例による差はあるものの主に3つのピークが現れる(**図2**)。これはMRIが低輝度，中間輝度，高輝度の3つの輝度のピクセルからなることを表すが，低輝度と中間輝度のピクセルを消去することで白黒画像が得られる(**図1**ⓑ)。得られた白黒画像では肉眼的に確認できる大腿骨の顆部の輪郭線と半月板などが残存する。

ここから，第3段階として輪郭のみを抽出し(**図1**ⓒ)，輪郭の上縁と下縁をトレースする。上縁を青色の線が下縁を赤色の線が示している(**図3**)。

その後に不整度に関係する4つのパラメータが計測される。つまり，輪郭の厚みの平均(ATS：これは青線と赤線の間の距離の平均ともいえる)，厚みのばらつき

図1　輪郭の不整度の測定法

DICOMデータから取り込んだMRIの元画像を白黒画像に変換し，大腿骨顆部の輪郭のみを抽出する過程を示した。

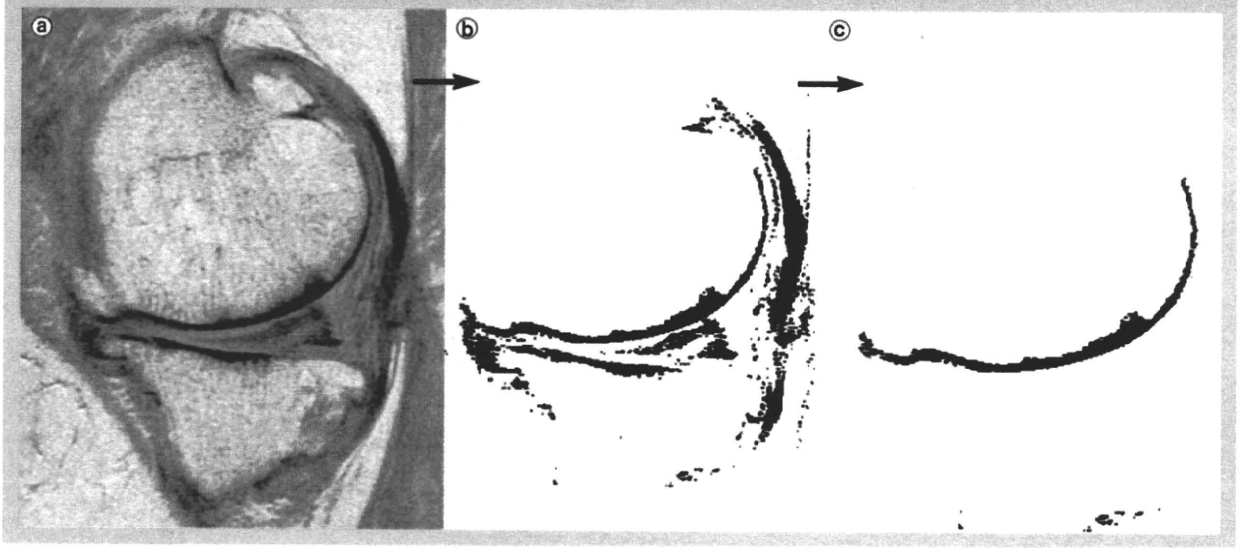


図2　MRIを構成するピクセルの分布(概念図)

図1で行った白黒画像への変換であるが，元画像を構成するピクセルの頻度を縦軸に輝度を横軸(右にいくほど明るい)とすると，典型的には3つのピークが現れる。このうち最も左のピークを残すようにして，ほかのピクセルを消去することで白黒画像への変換がなされる。必ずしもピークが3つに限るわけではないため，概念図とした。典型的な3つのピークとならない場合には最も左のピークのみ残すように画像処理をする。

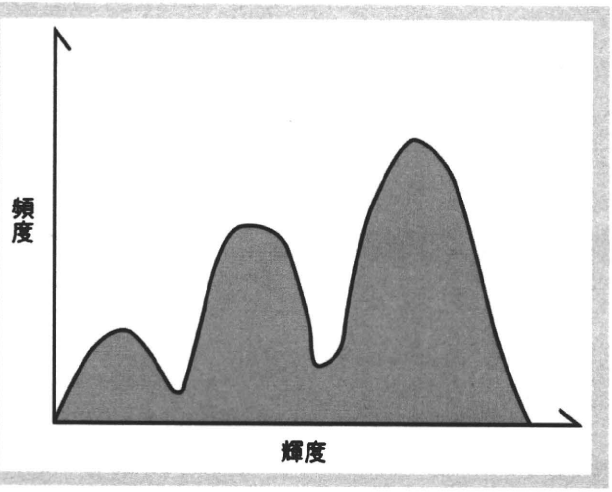
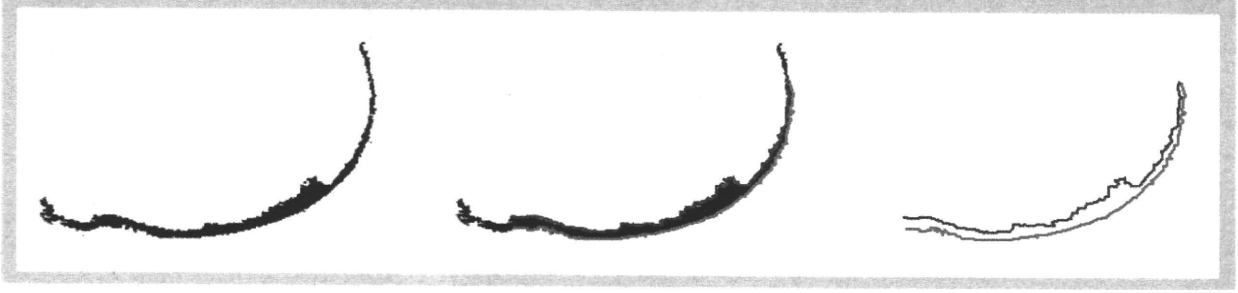


図3　抽出した輪郭の画像処理(概念図)

抽出した輪郭の上縁(赤線)と下縁(青線)は自動的にトレースされ，抽出される。実際にはより細い線での抽出であるが，ここでは理解しやすくするために太めの線で示した。



(SDS)，厚みの2乗の平均(ASTS)，輪郭の上縁の長さと下縁の長さの比(RUL：これは青線と赤線の長さの比である)などが計算されるのである。なお，輪郭の厚みはピクセルごとに測定される。

撮像

MRI撮像

MRI撮像に使用したのはシグナ1.5テスラ(GE medical system)である。プロトン密度強調矢状断像を検討に用いた。撮像に用いたパラメータは2,000/16ms(TR/TE)，FOV：14cm×14cm，matrix number：512×512，number of excitation：2，slice thickness：3mm，slice gap：0mm，である。この撮影法では内側コンパートメントは8枚程度となる(骨棘の存在や膝の大きさによる個体差はある)。

ここにワザあり！

各症例においてPCLの大腿骨側の付着部を捉えたスライスから内側に向かって2枚目からの3スライスを測定に用いた。こうすることで内側コンパートメントの中央に相当する部位を検討することが可能となる。

3スライスの平均値が当該膝の値となる。

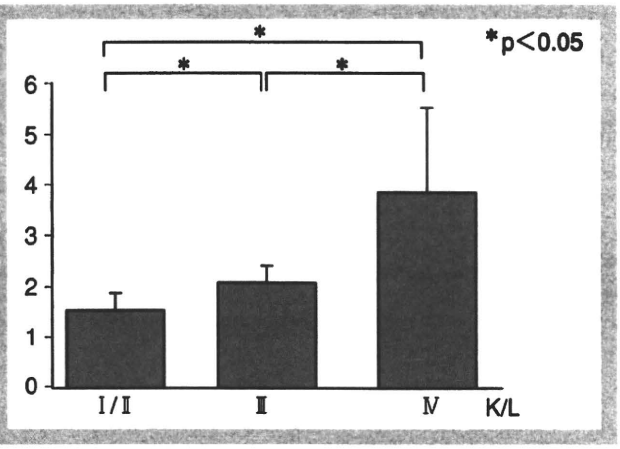
不整度による重症度評価

◆不整度と重症度の関係

こうして計測された不整度と膝機能スコアの関係であるが，4つのパラメータのいずれもがJOAスコア，JKOM[5]と負の相関のあることがわかっている。すなわち不整度が大きくなると膝機能スコアが低下する。痛みの程度を調べたVAS(visual analogue scale)の値においても不整度と負の相関のあることが確かめられている[4]。

さらにX線グレードとの関係であるが，X線のグレードが上昇するに従い，不整度が上昇することがわかっている（**図4**）。

図4 X線グレードと膝スコア
Kellgren & Lawrence (K/L)のX線グレードとSDSの関係を示した。グレードが上がるにつれSDSも上昇し，ばらつきも大きくなっていく。



◆不整度を測定する意義

不整度を測定することはある時点での重症度を評価する方法の1つである。したがって，得られた値は治療法を選択する際の目安となると考えられる。実際に鏡視下後内側解離術の適応としてSDSは有用であることがわかっている。すなわち，不整度がある数値以内であれば鏡視下手術により疼痛の改善が十分に期待できるが，ある値を超えた場合には人工膝関節置換術が望ましいということがわかったのである[6]。

また，経時的に測定することで膝OAの進行程度をモニターできるものと考えられる。とくに本法では連続する数値として重症度を評価測定できるため，経時的なモニタリングには適していると考えられる。

FINE SHOT

【代表症例と不整度の関係】

図5に不整度を表す指数の1つであるSDSとMRIの関係を示した。**【症例1，2】**のようにX線グレードはともにK/L Ⅳと同様であるが，不整度に大きな差のある症例が存在する。現在までにヒアルロン酸製剤を投与した成績や関節鏡手術の術後成績などから，SDSで3以上であることがTKAの適応と考えられ，2.5以下であることが鏡視下手術または保存療法の適応であると考えられる。ただし症例数がまだ十分ではなく暫定的な値ではある。

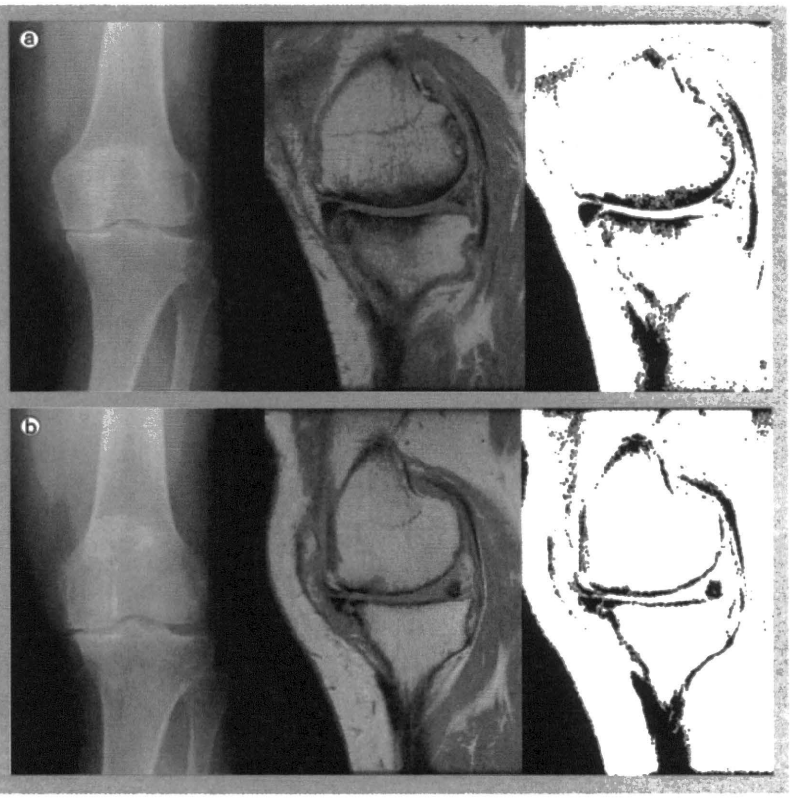
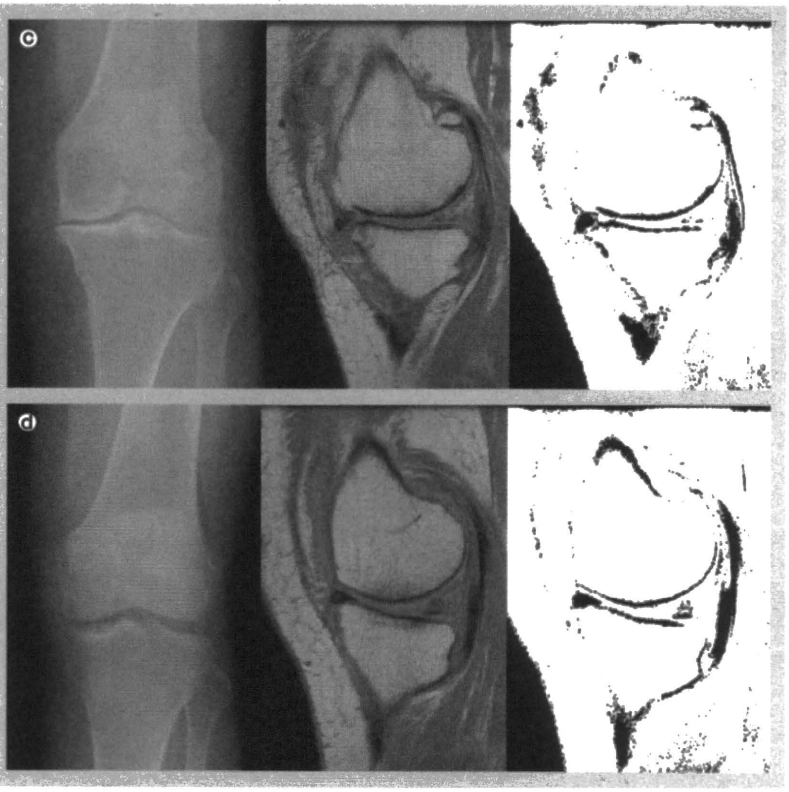


図5　X線グレードならびにMRIと不整度指数(SDS)

代表的な症例のX線像とMRIと計測された数値の関係を示した。
ⓐ：【症例1】75歳，男性。TKAとなった症例で，K/L Ⅳ，SDSは4.709と高値である。
ⓑ：【症例2】66歳，女性。K/L Ⅳ，SDSは2.476。

図5　つづき
ⓒ：【症例3】72歳，女性。K/L Ⅱ，SDSは1.985。
ⓓ：【症例4】76歳，女性。K/L Ⅱ，SDSは1.574。
ⓑ～ⓓはヒアルロン酸製剤の関節内注入で治療効果が得られた保存療法症例である。



● 今後の展望とリミテーション ●

まだまだ改善の余地の多い方法であるが，客観的な重症度評価法が必要とされるなかで臨床的にも有用な方法であると考えている。今後の展望として，現在は二次元での解析であるが，将来的には三次元的な解析ができるようなソフトウエアを開発する所存である。これにより詳細かつ全体的な不整度の程度を測定することが可能になると思われる。このためにはMRI撮像装置が進化する必要があると思われる。また，現在は大腿骨側のみの測定であるが，脛骨側も取り入れることを考慮している。

また本方法のリミテーションであるが，評価の対象となっているのは軟骨下骨のみであり，ほかの関節内の構成体が評価されていない。一方，最近評価法として使われ始めたWORMS法(Whole-Organ Magnetic Resonance Imaging Score of the knee in osteoarthritis)では，半月板，滑膜，骨棘などより広く関節内の組織を評価している[7]。どちらの方法が優れているか現時点では明らかではないが，比較検討する必要があり，現在検討中である。WORMS法は肉眼的なグレーディングが基本となっているが，自動的な計測という点では不整度法が優れている。

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THE JOURNAL OF BONE & JOINT SURGERY (BRITISH)

Histological evaluation of internally-fixed osteochondal lesions of the knee

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We evaluated the histological changes before and after fixation in ten knees of ten patients with osteochondritis dissecans who had undergone fixation of the unstable lesions. There were seven males and three females with a mean age of 15 years (11 to 22). The procedure was performed either using bio-absorbable pins only or in combination with an autologous osteochondral plug. A needle biopsy was done at the time of fixation and at the time of a second-look arthroscopy at a mean of 7.8 months (6 to 9) after surgery.

The biopsy specimens at the second-look arthroscopy showed significant improvement in the histological grading score compared with the pre-fixation scores ($p < 0.01$). In the specimens at the second-look arthroscopy, the extracellular matrix was stained more densely than at the time of fixation, especially in the middle to deep layers of the articular cartilage.

Our findings show that articular cartilage regenerates after fixation of an unstable lesion in osteochondritis dissecans.

Fixation of the osteochondral fragment to the osteochondral defect is a method of treatment for osteochondritis dissecans (OCD).[1-3] However, the outcome of this procedure remains controversial because the likelihood of healing of the osteochondral fragment largely depends upon its quality.[4] It is essential therefore that the histological changes which occur in the osteochondral lesion after fixation are well understood.

Internal fixation has been shown to be an effective method of treatment for unstable and displaced lesions in OCD.[5-10] Although this method has been evaluated in terms of the functional and radiological outcome using clinical analysis,[5-10] MRI and second-look arthroscopy, to date there have been no reports of the evaluation of the histological findings of the OCD lesion before and after fixation. Our aim therefore was to assess the histological changes before and after internal fixation of the OCD lesion. Our hypothesis was that an unstable osteochondral fragment might show regeneration of cartilage after internal fixation even if it had already deteriorated.

Patients and Methods

We obtained the approval of the Local ethical committee of our university and informed consent from all the patients and their parents.

Between 2003 and 2006, ten consecutive patients (ten knees) with unstable lesions of OCD were treated by open reduction and internal fixation. All the procedures were performed by one of two senior surgeons (MO or NA) involved in the study. There were seven males and three females with a mean age of 15 years (11 to 22). They were followed up for a mean of 23 months (12 to 30). Pre-operatively, all the patients had been restricted in their daily or sporting activities. Seven had experienced locking of the knee and six were unable to run because of pain in the knee. Two patients had limited range of movement and nine did not participate regularly in athletics. The mean duration of symptoms before surgery was 5.2 months (3 to 12). None of the knees had previous surgery. The operative details of the patients are given in Table I. Standing anteroposterior, lateral and skyline radiographs of the knee and CT and MR scans had been obtained pre-operatively. In six knees, bony fragments were noticed in the joint cavity separated from the site of the defect. Six lesions involved the medial femoral condyle, two the lateral femoral condyle and two the patellar groove. In one of the knees with a lesion in the patellar groove, repair of the medial patellofemoral ligament was also performed. In five patients, the distal femoral physis was open at the time of diagnosis. The operative details including the location of the lesion, the stability of the fragment, the number of pins used and associated procedures such as autogenous osteochondral grafting were recorded (Table I).

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doi:10.1302/0301-620X.91B6.20957 $2.00

J Bone Joint Surg [Br] 2009;91-B:823-9.
Received 25 February 2008; Accepted after revision 5 February 2009

Table I. Details of the ten patients and the surgical procedures

Case	Age (yrs)	Gender	Location	Size (mm)	Stability	Number of PLLA* pins used	Autologous osteochondral graft
1	14	F	Medial femoral condyle	30 × 20	Loose body	20 mm × 6	None
2	17	M	Medial femoral condyle	20 × 20	*In situ* detachment	20 mm × 1	Diameter 4.5 mm × 1
3	11	F	Patellar groove	30 × 30	Loose body	30 mm × 6	None
4	12	M	Medial femoral condyle	15 × 15	*In situ* detachment	20 mm × 3	None
5	12	F	Lateral femoral condyle	15 × 15	*In situ* detachment	30 mm × 2	None
6	16	M	Medial femoral condyle	20 × 20	Loose body	20 mm × 7	None
7	14	M	Patellar groove	10 × 8	Loose body	30 mm × 2	None
8	16	M	Medial femoral condyle	20 × 20	*In situ* detachment	30 mm × 3	None
9	22	M	Lateral femoral condyle	20 × 20	Loose body	30 mm × 7	Diameter 4.5 mm × 1
10	15	M	Medial femoral condyle	20 × 18	Loose body	30 mm × 2	Diameter 8.5 mm × 1

* PLLA, poly-L-lactide

Operative techniques. Standard anterior portals were created and a diagnostic arthroscopy was initially performed. Free fragments and their original beds were identified in detached cases, and the stability of the osteochondral fragments examined in cases of '*in situ* detachment'. The knee was then exposed through a small parapatellar arthrotomy. In four cases in which the articular cartilage was partially separated (*in situ* detachment) the fragment was hinged open. The bed was then debrided of fibrous tissue or calcified cartilage to expose the intact subchondral bone. If the subchondral bone was remarkably sclerotic, an autogenous osteochondral plug of diameter 4.5 mm or 8.5 mm (Mosaicplasty, Smith and Nephew, Andover, Massachusetts), obtained from the unloaded area of the femoral condyle was inserted through the centre of the lesion with the intention of encouraging the circulation of blood to the subchondral bone. Subsequently, poly-L-lactide (PLLA) pins of diameter 2 mm (Neofix; Gunze, Kyoto, Japan) were inserted around the periphery until a rigid and stable construct was obtained. In six knees with detached lesions, the free fragments were always larger than the original defects and had to be trimmed by approximately 2 mm to 3 mm around the periphery to fit the defect. The fragment was reduced and transfixed temporarily by two Kirschner (K)-wires of 1.0 mm diameter while compressing the fragment manually. Definitive fixation was achieved by PLLA pins of 2.0 mm diameter which were inserted into pre-drilled channels of 2.0 mm diameter through the fragment in divergent directions. A needle biopsy was performed with a 14.5 gauge biopsy needle at almost the centre of the fragment and a bio-absorbable pin was inserted into the remaining channel. The heads of the pins were placed at 2 mm below the level of the surrounding articular surface to avoid impingement. The K-wires were then removed and bio-absorbable pins inserted into the channels to promote stability of the fragment. The knee was immobilised in an above-knee cast at 30° of flexion for two to three weeks. The patients were encouraged to begin quadriceps strengthening exercises immediately after surgery. After removal of the cast, they then proceeded to passive and active range-of-movement exercises. Partial weight-bearing was allowed at three to four weeks, followed by full weight-bearing at eight weeks. Full participation in sports was restricted for up to six months depending on the clinical and radiological progress.

Follow-up examination. A second-look arthroscopy was performed to evaluate macroscopic healing at a mean of 7.8 months (6 to 9) after surgery. All the patients agreed to a second-look arthroscopy despite being asymptomatic. A needle biopsy was performed at a site as close as possible to the previous biopsy site and repaired tissues were evaluated histologically and immunohistochemically. At the final follow-up the knees were examined for range of movement, effusion and tenderness. Plain radiographs and MR scans were obtained to assess the union of the fragment. All the patients were asked to express an opinion on pain and function using the Lysholm scoring system.[11]

Histological examination. Each needle biopsy specimen was fixed with 10% buffered formalin for one day. Specimens were then decalcified with 0.25 methylenediaminetetraacetic acid in phosphate buffered saline (PBS) at a pH of 7.5, dehydrated in graded alchohol, and embedded in paraffin wax. They were cut sagittally into sections 5 μm thick and stained with Safranin O/Fast Green. Histological sections were graded according to the scale described by Mankin et al[12] (Table II) by a pathologist (KA) who was not provided with any information on the patients. In this grading system, the articular cartilage is evaluated for structure (0 to 6 points), cells (0 to 3 points), Safranin O staining (0 to 4 points), and tidemark integrity (0 to 1 points) (0 point for normal cartilage) (Table II).

Expression of type-II collagen within biopsy specimens was analysed immunohistochemically. Sections (5 μm) were cut, air-dried, deparaffinised, rehydrated, and incubated for three minutes in PBS. In order to abolish endogenous peroxidase activity, they were incubated with 0.3% H_2O_2 in methanol for ten minutes. After three washes in PBS with 0.2% Tween 20 at a pH of 7.4, the sections were enzymatically digested by testicular hyaluronidase type VIII (0.2% in PBS, pH 7.4) for 30 minutes at 37°C. They were then incubated overnight in a 1:100 dilution of type-II

Table II. The modified histological grading scale of Mankin et al[12]

	Score
Structure grade	
Normal	0
Surface irregularities	1
Pannus and surface irregularities	2
Clefts to transitional zone	3
Clefts to radial zone	4
Clefts to calcified zone	5
Complete disorganisation	6
Cells	
Normal	0
Diffuse hypercellularity	1
Cloning	2
Hypocellularity	3
Safranin O staining	
Normal	0
Slight reduction	1
Moderate reduction	2
Severe reduction	3
No dye noted	4
Tidemark integrity	
Intact	0
Crossed by blood vessels	1

collagen antibody (Oncogene Research Products, San Diego, California) at 4°C. Immunoreactivity was detected by the serial incubation of sections. The signal was developed as a brown reaction product with the use of peroxidase substrate 3, 3'-diaminobenzidine, and H_2O_2. The sections were then counterstained with Harris haematoxylin and dehydrated, cleared and mounted.

Statistical analysis. This was performed using Statview 5.0 (SAS Institute, Cary, North Carolina). All data were shown as the mean SEM. The histological scores relating to loose bodies and *in situ* detachment before and after fixation were assessed using analysis of variance (ANOVA) with Bonferroni/Dunn *post hoc* comparison. A p-value < 0.01 was taken to be statistically significant.

Results

Clinical and radiological evaluation. The mean size of the defect was 399 mm^2 (80 to 900). In three knees, the fragment was fixed by the combination of an osteochondral plug (4.5 mm or 8.5 mm in diameter) taken from the ipsilateral femoral trochlear and the use of bio-absorbable PLLA pins (mean 3.3 pins, 1 to 7). In the other seven knees, the fragment was transfixed by PLLA pins alone (mean 4.0 pins, 2 to 7). There was no intraoperative complications related to the insertion of the pins. Two patients (2 knees) were found to have a lateral discoid meniscus and underwent a partial lateral meniscectomy at the same time. Union of the fragment was obtained in all knees, with no pain or locking. Serial radiographs revealed no redisplacement of the fragment. None of the patients treated by bio-absorbable pins had synovitis during the follow-up period. At the final follow-up, all were satisfied and there was no swelling or limitation of movement of the knee. The mean Lysholm score improved from a pre-operative value of 70.5 points (58 to 89) to 98.3 points (90 to 100) at the final follow-up. MR scans obtained at the second-look arthroscopy showed that the image intensity of the fixed fragment was similar to that of the surrounding normal articular cartilage, although the channels of the PLLA pins could be seen (Figs 1 and 2).

Arthroscopic evaluation. At the second-look arthroscopy, in all ten knees, although the fragment was stable to probing and had an intact smooth surface, we could distinguish between the original lesion and the normal surrounding tissue or osteochondral plug because the border was slightly concave (Figs 1 and 2). In seven knees, there was complete integration of the border of the osteochondral fragment with the surrounding articular surface. In two knees with an intact smooth surface, the fragment had fibrillation on its surface. In one knee, the surface of the fragment was soft. There were no post-operative complications.

Histological evaluation. The loose bodies at the time of fixation showed a tendency towards breakdown of layered structure of normal cartilage. The extracellular matrix was stained weakly by safranin O and immunohistological staining showed the presence of type-II collagen. However, as regards the osteochondral lesions in *in situ* detachment, the extracellular matrix was well stained although there was breakdown of the normal cartilage. At the second-look arthroscopy, the extracellular matrix was stained more densely than at pre-fixation especially in the middle to deep layers. In addition, the layered structure showed improvement compared with that at pre-fixation (Figs 3 and 4).

The mean histological score in the loose-body group was 8.5 (SEM 1.0) before and 1.5 (SEM 0.8) after fixation indicating a statistically significant improvement ($p < 0.01$). The mean histological score in the *in situ* detachment group was 4.5 (SEM 0.6) before and 1.0 (SEM 0.8) after fixation which was also statistically significant ($p < 0.01$). Statistically significant differences were noted between both groups in the histological scores before fixation ($p < 0.01$), whereas the difference after fixation was not significant ($p = 0.44$; Fig. 5).

Discussion

There are several surgical techniques available for the treatment of unstable OCD lesions in the knee.[1-3,13] They include removal of the fragment and curettage of the crater, replacement and stabilisation of the fragment by internal fixation, allograft replacement, osteochondral autograft transplantation and autologous chondrocyte transplantation. The goal of these procedures is to produce a smooth articular surface of hyaline-like cartilage. We believe that if an unstable osteochondral fragment or

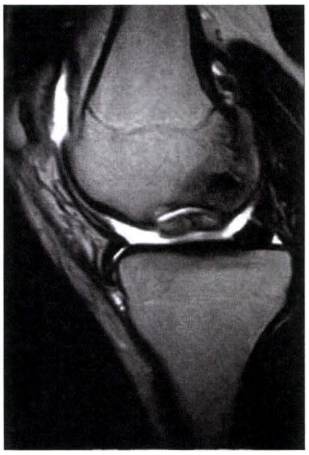

Fig. 1a

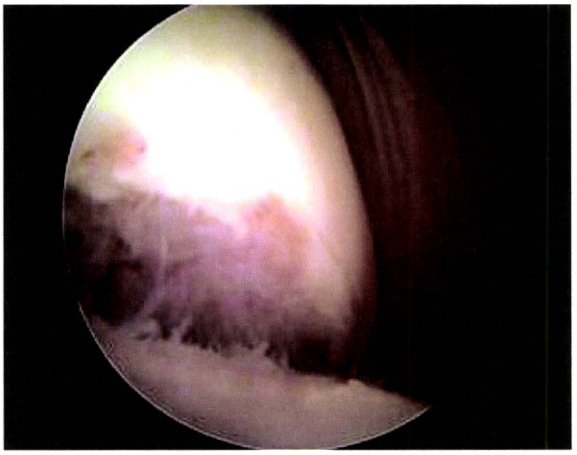

Fig. 1b

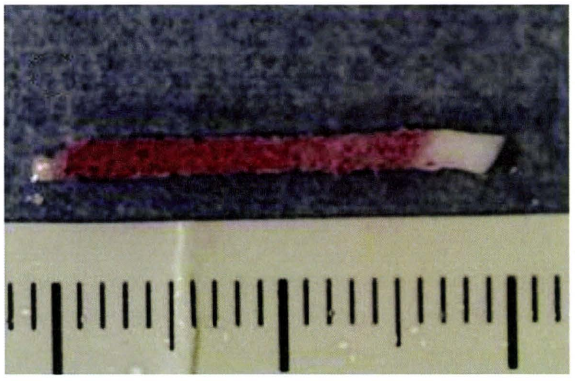

Fig. 1c

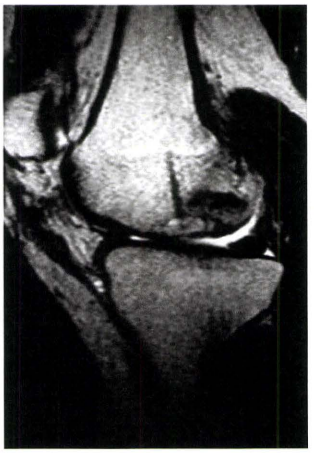

Fig. 1d

Case 8. A 16-year-old male with osteochondritis dissecans of the medial femoral condyle. a) Sagittal pre-operative MR scan showing a large lesion affecting the medial femoral condyle. b) Pre-fixation arthroscopic photograph showing complete detachment of the osteochondral lesion. c) Photograph of a biopsy specimen taken using a 14.5 gauge biopsy needle. d) Sagittal MR scan seven months after surgery showing consolidation of the fragment. The channel of the poly-L-lactide pin is still visible. e) Second-look arthroscopy seven months after surgery showing a smooth cartilage surface and no detachment.

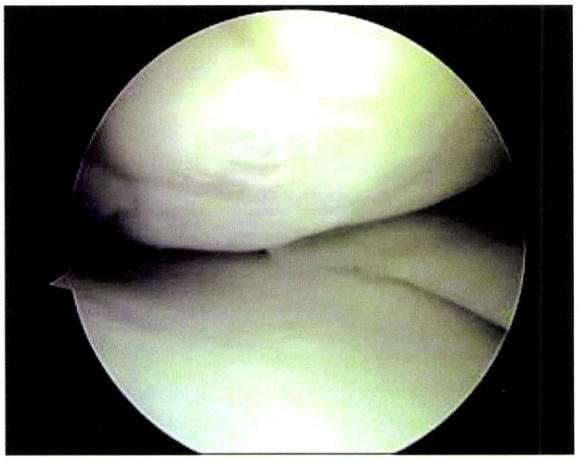

Fig. 1e

loose body is left in the joint with adequate conditions for fixation, internal fixation should be the preferred method of treatment because it preserves the natural contour of the articular surface.

The principles of internal fixation are the achievement of stable fixation, the promotion of a blood supply to the base of the osteochondral fragment and bone grafting at the base to promote healing with the articular cartilage over the

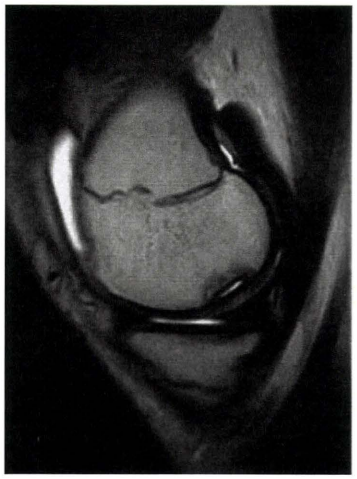

Fig. 2a

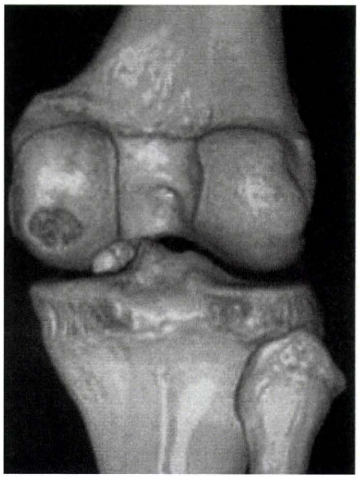

Fig. 2b

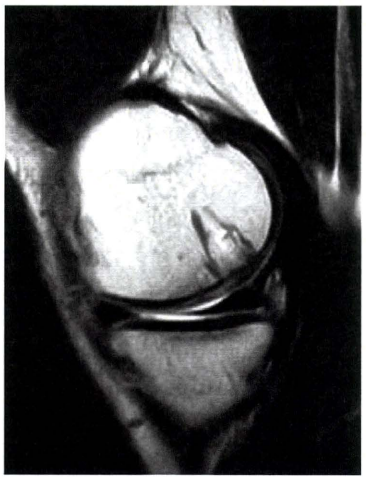

Fig. 2c

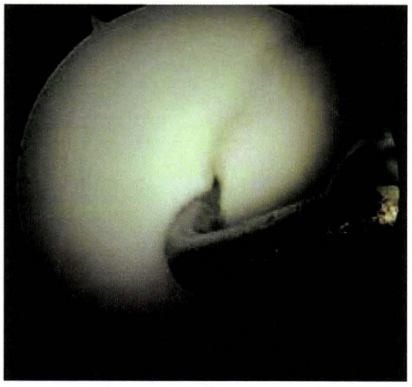

Fig. 2d

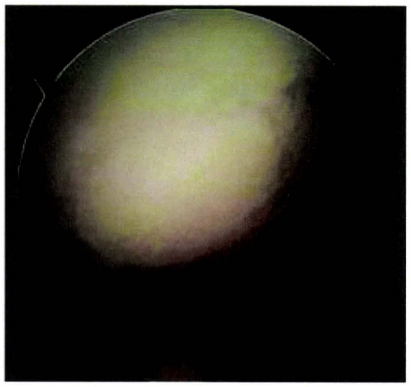

Fig. 2e

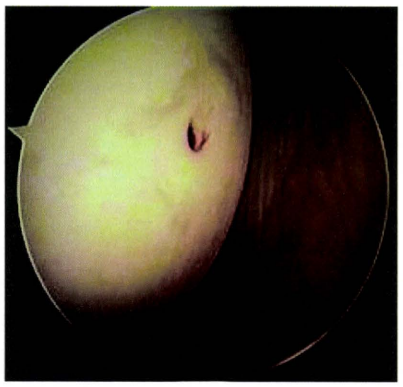

Fig. 2f

Case 10. A 15-year-old male. a) Sagittal pre-operative MR scan and b) pre-operative CT scan showing a large osteochondral defect affecting the medial femoral condyle and a loose body. c) Sagittal MR scan 11 months after surgery showing consolidation of the fragment. d) Arthroscopic photograph at the time of fixation showing that the loose body was stable on probing. e) Second-look arthroscopic photograph 11 months after surgery showing a smooth cartilage surface. f) Arthroscopic photograph showing hole after needle biopsy was performed.

osteochondral fragment being left as intact as possible. Recently, bio-absorbable pins or screws have been used for fixation of the osteochondral fragment with favourable results in unstable lesions.[1,2,14-16] Dervin et al[1] undertook internal fixation of fragments in nine skeletally mature OCD patients using PLLA rods, which resulted in radiologically stable lesions in eight. Din et al[9] described 12 knees with stable osteochondral lesions treated by early fixation using bio-absorbable pins and drilling. Within six months all the lesions had united on MRI and at a mean of 32.4 months all the patients were satisfied with the outcome with no swelling or restriction of movement of the knee.

Most studies on the surgical treatment of OCD have evaluated the findings clinically and radiologically and in most a second-look arthroscopy has been performed. Only a few have examined the histological findings after fixation of the osteochondral fragment. Prokop et al[17] studied 36 sheep with osteochondral fractures of the femoral condyle. After fixation using bio-absorbable pins, they were followed up radiologically and histologically for three years. Bone union was achieved in all knees and bio-absorbable pins did not lead to clinically significant inflammatory reactions in the joint. Touten et al[18] evaluated 18 rabbit knees histologically and immunohistochemically after internal fixation of loose bodies to the osteochondral defects using bio-absorbable pins. They found that although histologically there was deterioration in isolated osteochondral loose bodies and repaired tissue after fixation to the defect which was related to the duration of isolation of the fragment, some loose bodies showed regeneration of cartilage after fixation. However, to date there have been no reports of the histological findings before and after fixation in patients with OCD.

In our study we demonstrated some regeneration of cartilage after fixation, showing improvement in the layered structure of the cartilage and an abundant

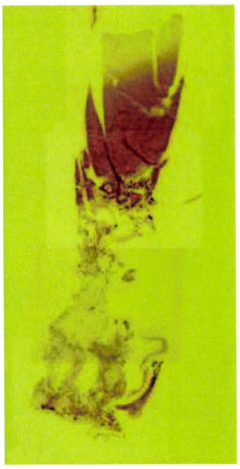

Fig. 3a

Fig. 3b

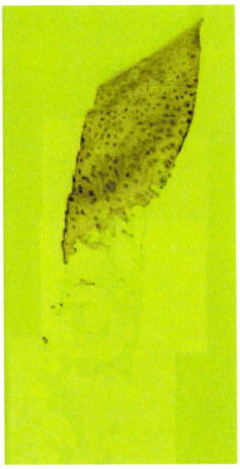

Fig. 3c

Case 8. Photomicrographs of the biopsy specimen. Safranin O staining (a) at the time of fixation showing fibrous tissue in the superficial layer of cartilage with deterioration in staining in the middle to deep layers and (b) seven months after surgery. c) Immunohistochemical staining for type-II collagen seven months after surgery. The extracellular matrix was stained more densely than at pre-fixation, especially in the deep layer of cartilage (× 50).

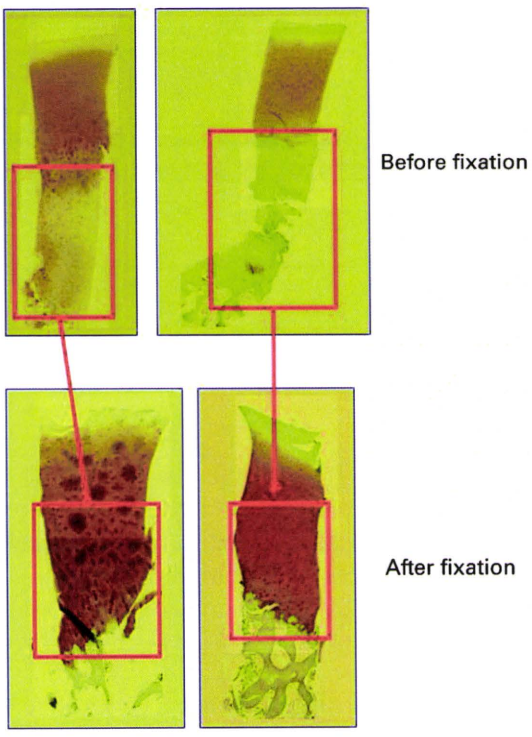

Fig. 4

Photomicrographs of a biopsy specimen showing the change after fixation. In the middle to deep layers (red square) of cartilage, the weak staining before fixation had improved markedly after fixation (Safranin O × 50).

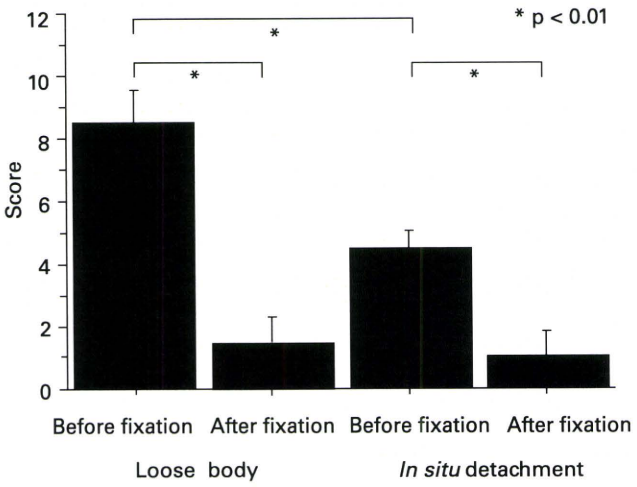

Fig. 5

Bar chart showing the histological grading scores of the loose-body group and *in situ* detachment groups.

extracellular matrix. To our knowledge, our study is the first demonstration of regenerative change in the articular cartilage layer within an OCD lesion after its fixation which has been supported by histological evidence. The second-look arthroscopy biopsy specimens of the loose bodies and the *in situ* detachment lesions showed an abundant extracellular matrix compared with that at pre-fixation, especially in the middle to deep layers near the subchondral bone. The layered structure of cartilage also improved compared with that at pre-fixation. This suggests the possibility that even a loose body whose cartilage has deteriorated may demonstrate some

regeneration if the fragment is fixed in a stable fashion. Although we have no obvious explanation for these findings, it is possible that stable fixation leads to improvement in the nutrition of the cartilage and the subchondral bone. The deteriorated cartilage within the osteochondral fragments could regenerate through normal biomechanical conditions, including movement of the joint and weight-bearing. Therefore, if loose bodies are identified in the joint, internal fixation should be the preferred method of treatment. By contrast, Milgram[4] reported in a study of 119 patients that degenerative calcification occurred in all osteochondral loose bodies which had been detached for more than three weeks.

There were several limitations to our study. First, the number of patients was too small to obtain definite conclusions. Although informed consent was obtained from all the patients and their parents, we limited the indications for biopsy according to the existence of loose bodies and the hope of an early return to sport, because the needle biopsy at the second-look arthroscopy is invasive. Secondly, the biopsies before and after internal fixation were not taken from exactly the same site, but from nearby. Therefore, differing biomechanical or biological conditions may have given rise to histological differences. Finally, although we mainly investigated the OCD lesions before and after internal fixation radiologically and histologically, biochemical evaluations were not performed. Additional biomechanical or biological studies, including quantitative analysis are therefore needed.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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Clin Rheumatol (2009) 28:1395–1402
DOI 10.1007/s10067-009-1258-0

ORIGINAL ARTICLE

Detection of pain-related molecules in the subchondral bone of osteoarthritic knees

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Received: 26 March 2009 / Revised: 1 July 2009 / Accepted: 7 August 2009 / Published online: 3 September 2009

Abstract Knee pain is predominant among osteoarthritis (OA) patients, but the mechanism is poorly understood. We investigated subchondral bone as a source of OA knee pain using immunohistochemistry. Fifteen medial-type OA knees with minimum involvement of the lateral compartment determined by X-ray as well as magnetic resonance imaging that received total knee arthroplasty (TKA) were involved. Each pair of the medial femoral condyle (MFC) and lateral femoral condyle (LFC) was compared obtained at the time of TKA. Osteocartilaginous MFC and LFC specimens were histologically examined and stained with antibodies against cyclooxygenase 1 (Cox-1), cyclooxygenase 2 (Cox-2), substance P, tumor necrosis factor-alpha (TNF-α), and neuron-specific class III beta-tubulin (TUJ1), a pan-neuronal marker. Formation of cystic lesions was more frequently seen in the MFC. The lesions were composed of vascular endothelial cells, osteoclasts, and mononuclear cells and were present in similar proportions between the MFC and the LFC. Four out of 15 MFC specimens were positive for Cox-1, 15 for Cox-2, and 13 for TNF-α. No LFC specimens were positive for any antibodies. Substance P-positive and TUJ1-positive fibers were found in the subchondral area of the MFC, but not in the LFC. Pathological changes in the subchondral bone can be a source of knee pain, which was detectable by the positive immunoreactivity of substance P, Cox-2, TNF-α, and TUJ1, in the subchondral bone of affected compartments. The relatively immediate reduction in pain obtained by TKA might account for the involvement of the subchondral bone in knee pain because most of the affected subchondral plate is excised in TKA (debridement effect of TKA).

Keywords Cox-2 · Knee osteoarthritis · Subchondral bone · Substance P · TNF-α · TUJ1

Introduction

The incidence of osteoarthritis (OA) of the knee has been increasing as society ages. OA of the knee is associated with substantial and persistent reduction of physical function in elderly people and can be disabling from the very onset [1, 2]. Therefore, establishment of an effective treatment for OA of the knee has become increasingly important in terms of social security as well as medical care. Among the many complaints that OA patients have, knee pain is the most common and predominant symptom. However, up until now, the mechanism of OA knee pain has been poorly understood.

It has been reported that OA knee pain originates from periarticular tissues and secondary synovitis [3, 4]. Some authors have reported that the joint pain mainly arises from free nerve endings that exist in the capsule or in the synovium [3, 5, 6]. Other joint components such as the periosteum, ligaments, menisci, muscle, and bone marrow have also been reported to be sources of OA knee pain [7–9]. A controversial area regarding the source of knee pain is

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when the cartilage and subchondral bone show drastic histological changes as OA progresses, since cartilage is aneural and subchondral bone is sparsely innervated [10, 11]. In 2007, Suri et al. reported on innervation of the osteochondral junction in human knee OA samples and indicated a possible contribution of the subchondral area to OA knee pain [12]. Our previous study showed that pathological changes in the subchondral plate were detectable by magnetic resonance imaging (MRI) as irregular contours of the femoral condyle [13]. We also showed that the irregularity became increasingly obvious as OA progressed and that an irregular change of the femoral condyle correlated with the knee score [14]. Taken together, these reports imply that the subchondral plate of the femoral condyle may be a potent source of pain in knee OA.

The purpose of the present study was to investigate the subchondral bone as a source of OA knee pain. We used immunohistochemical analysis to compare the medial femoral condyle (MFC) and the lateral femoral condyle (LFC) obtained from patients at the time of total knee arthroplasty (TKA). For this purpose, we selected patients with medial-type OA knees with minimal involvement of the lateral compartment.

Materials and methods

Patients

Included in this study were 15 medial-type OA knee patients who underwent TKA at our institution. At the time of operation, the patients' age and gender, as well as X-ray image grading of the medial and lateral tibiofemoral joints and MRI of the bone marrow edema (BME) of each compartment were recorded. For immunohistochemical analysis, patients with a lateral compartment less than grade II on the Kellgren and Lawrence (K/L) scale [15] and who displayed a lack of BME on MRI were selected. Patients with other arthritic diseases such as rheumatoid arthritis were excluded from the study. The study protocol was approved by the institutional ethics committee of Chiba University, and informed consent was obtained from all the patients.

X-ray and MR imaging

A standard anteroposterior X-ray was used to determine the K/L score for the medial and lateral compartments. Using MRI (Signa 1.5 T, GE Medical Systems), we assessed sagittal and coronal fat-suppressed T2-weighted images (TR 2,000 ms, TE 87 ms, field of view 13×13 cm, matrix 512×256, and 3 mm slice thickness with a 1-mm interslice gap) to detect BME in the affected knees

Specimens

At the time of TKA, the weight-bearing areas of the MFC and LFC were obtained (Fig. 1). Synovium was also obtained from the medial compartment. Specimens were immediately fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 24 h. The femoral condyle was demineralized in 20% ethylenediaminetetraacetic acid at room temperature for 6 weeks and then embedded in paraffin. Sagittal sections (6 μm) were cut and mounted on glass slides.

Immunohistochemical staining

The sections were deparaffinized using 80% xylene and ethyl alcohol, rinsed with PBS (pH7.4), and stained with Mayer's hematoxylin solution and 1% eosin alcohol solution (H&E staining). For immunohistochemistry, sections were washed with PBS, soaked in 0.3% methyl alcohol to remove endogenous peroxidase activity (from blood cells), then incubated with the following antibodies: anticyclooxygenase 1 (Cox-1, 1:200, Catalog No. 160109, Cayman Chemical, Ann Arbor, MI, USA), anticyclooxygenase 2 (Cox-2, 1:200, Catalog No. sc-1745, Santa Cruz Biotechnology, Santa Cruz, CA, USA), antitumor necrosis factor-alpha (TNF-α, 1:200, Catalog No. 654250, Calbiochem, San Diego, CA, USA), antihuman CD34 class II (CD34, 1:10, Catalog No. MCA547T, NC), antihuman substance P (1:200, Catalog No. sc-9758, Santa Cruz Biotechnology), and antineuron-specific class III beta-tubulin (TUJ1, Catalog No. MAB1195, R&D Systems, Minneapolis, MN, USA). The sections were then incubated with peroxidase-labeled streptavidin–biotin (Histofine, Nichirei, Tokyo, Japan). Localization of the antigens was visualized using 3,3′-diaminobenzidine tetrahydrochloride dehydrate (DAB). Sections were washed, dehydrated, and mounted under coverslips using Permount (Fisher Scientific Chemical Division, Fair Lawn, NJ, USA). Five serial slides from the center of the weight-bearing area of the condyles were evaluated to assess the corresponding antigens. A specimen that had immunoreactivity in any of the slides was considered a positive specimen. A specimen that had no immunoreactivity on five slides was considered a negative specimen.

In addition to the immunohistochemical examinations, tartrate-resistant acid phosphatase (TRAP) staining was performed to detect osteoclasts.

Histological evaluation

Following H&E staining, the numbers of cystic lesions that evaded the subchondral bone plate or calcified zone were counted in 10-mm-long sections to determine the density of

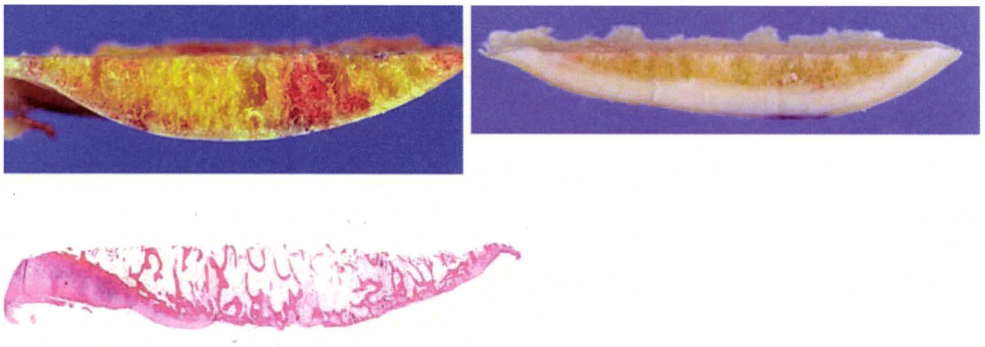

Fig. 1 Specimen obtained at the time of TKA. The *upper left* and *upper right* photos show representative examples of the MFC and LFC, respectively, which were obtained by a distal bone cut at the time of TKA. The specimens were then cut in half sagittally. The *lower photo* shows typical H&E staining of the MFC. Note that the majority of the surface of the MFC was denuded and partially covered with fibrous tissue, whereas the cartilage was preserved on the surface of the LFC

cystic lesions [16]. In addition, the cell populations forming the cystic lesions were analyzed. For this analysis, CD34 immunoreactive cells were identified as endothelial cells, polynuclear cells were identified as osteoclasts, and other cells were identified as mononuclear cells. The number of cells that was found in a cyst was also counted.

Statistics

Statistical analysis was done using the Mann–Whitney *U* test. A p value <0.01 was considered statistically significant.

Results

Patients

Of the 15 subjects examined, two were male and 13 were female, ranging in age from 62 to 79 years old (mean 67.7 years old). X-ray images of the medial compartment were grade IV on the K/L scale for all of the patients, whereas three lateral compartments were grade I and 12 were grade II. BME was detected in the MFC and the medial tibial plateau. No BME was detected in the LFC, as determined in the inclusion criteria. These results implied minimal, if any, arthritic changes in the lateral compartment. Thus, all of the patients in this series were considered to have medial-type OA.

Histological evaluation

H&E staining revealed that, in all of the cases, the articular cartilage in the weight-bearing portion of the MFC was worn and part of the surface was covered with fibrous tissue (Fig. 1). Representative H&E staining of the subchondral bone of the MFC and the LFC is shown in Fig. 2. Cystic lesions consisting of fibrous tissue were found in the subchondral plate, both in the MFC and in the LFC. The number of cystic lesions was 2.2±0.10/10 mm in the MFC and 0.47±0.22/10 mm in the LFC. The average cell number for each cyst was 94.6±34.2 in the MFC and 55.2±24.0 in the LFC. The frequency of cystic lesions was significantly higher in the MFCs than the LFCs. TRAP staining to identify osteoclasts and antibodies against CD34 to identify vascular endothelial cells clearly showed the existence of both cells (Fig. 3). The proportion of mononuclear cells in a cystic lesion was 78.7% in the MFC and 77.2% in the LFC. The proportions of osteoclasts and endothelial cells in a cystic lesion were 1.2% and 21.2% in the MFC and 0.6% and 22.4% in the LFC, respectively. No significant difference in the types of cells in the cystic lesions was found between the MFC and the LFC (Table 1).

Immunohistochemical examination revealed that certain cells or interstitial tissue in the cystic lesions in the subchondral bone plate in the MFC stained positive for Cox-2 (Fig. 4g, k), TNF-α (Fig. 4h, l), TUJ1 (Fig. 4i, m), and substance P (Fig. 4j, n). The proportion of antibody-positive specimens was as follows: Cox-1, four out of 15 cases; Cox-2, 15 out of 15 cases; and TNF-α, 13 out of 15 cases. Substance P-positive and TUJ1-positive fibers were found in the MFC (15 out of 15). No antibody-positive specimens or fibers were found in the LFC (Table 2). As for nerves in the Haversian canal, TUJ1-positive fibers were detected in both the LFC and MFC. Certain cells in the synovium also stained positively for Cox-1, Cox-2, and TNF-α. The proportion of antibody-positive synovial specimens was as follows: Cox-1, one out of 15 cases; Cox-2, 15 out of 15 cases; TNF-α, 12 out of 15 cases; substance P, 15 out of 15 cases; and TUJ1, 15 out of 15 cases (Table 2).

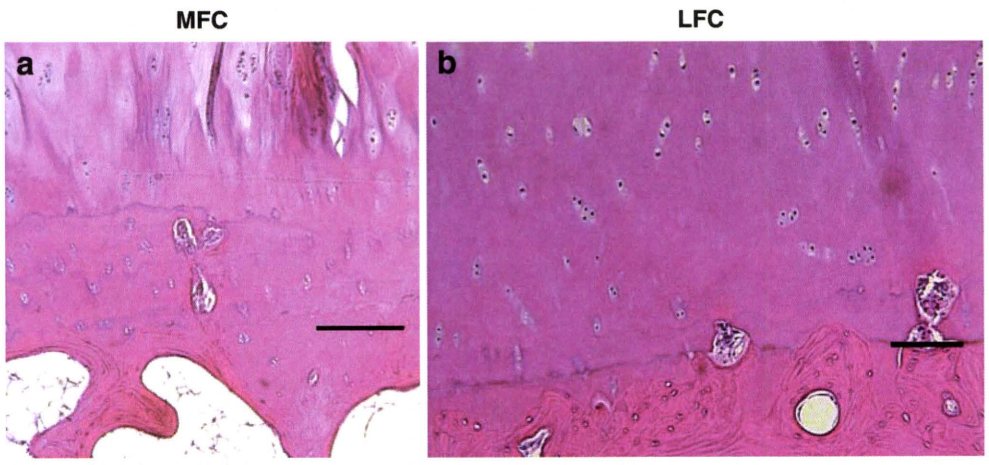

Fig. 2 Cystic lesion in the MFC. The *left photo* (**a**) shows a typical cystic lesion at the boundary of the bone and cartilage in the MFC. The lesions were also found in the LFC (*right photo*, **b**). In this specimen, the surface of the cartilage was fibrillated in the MFC and cell cloning was observed in the LFC. Scale bar denotes 100μm

Discussion

Relationship between knee pain and TUJ1, substance P, Cox-2, and TNF-α

In this study, we selected medial-type OA patients with minimal involvement of the lateral compartment based on X-ray and MRI examinations and found exclusive expression of TUJ1, substance P, Cox-2, and TNF-α in the MFC but not in the LFC. By setting the criteria such that those with BME in the lateral compartment were excluded, comparisons between affected compartments and minimally affected or unaffected compartments were possible since even low-grade X-ray examination of the knee will detect BME as an indication of early osteoarthritic changes [17]. The detection of substance P, Cox-2, TNF-α, and TUJ1 indicated that pathological changes in the subchondral plate that occurred in the affected knee compartment can be a source of knee pain, although the main source of these molecules has been thought to be the synovium [18–21]. Although the biological activities of substance P, Cox-2, TNF-α are pleiotropic, and simple detection of their existence does not necessarily indicate pain provocation, they are still considered to be pain-related or inflammatory molecules [12, 18, 19, 22–24].

Substance P is a neurotransmitter that causes reflex neurogenic inflammation in the joint after being released from afferent nerve fibers [25, 26]. Administration of substance P into the knee joint has been reported to increase the severity of arthritis in an experimental rat model [25]. Cox-2 is rapidly induced in instances of tissue injury and inflammation [27]. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) to inhibit Cox-2 activity is one of the most common treatments for OA knee pain, and the relatively high efficacy of NSAIDs in relieving knee pain suggests the involvement of Cox-2 in the generation of knee pain [28, 29]. TNF-α has been reported to play a pivotal role in the development of inflammatory hyperalgesia [23], and downregulation of TNF-α has been reported to be one of the mechanisms for pain reduction obtained by high-molecular-weight hyaluronan [30]. Thus, substance P, Cox-2, and TNF-α are among the key molecules involved in OA knee pain.

Although TUJ1 is a pan-neuronal marker and does not differentiate sensory neurons from other neurons, the exclusive, positive immunoreactivity of TUJ1 in the subchondral bone of the MFC implies the occurrence of nerve ingrowth only in the MFC. The relatively immediate reduction in pain obtained by TKA might account for the involvement of the subchondral bone in knee pain because

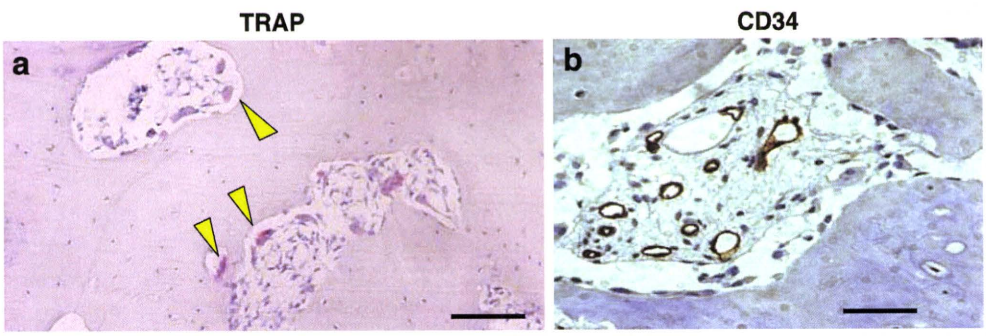

Fig. 3 Cells in a cystic lesion. Multinucleated, TRAP-positive cells were considered to be osteoclasts (*left*, *arrows*, **a**), and CD34-positive cells were regarded as vascular endothelial cells (*right*, **b**)