Biochemical markers in the diagnosis of chondral defects following anterior cruciate ligament insufficiency

Nikolaus A. Streich · David Zimmermann ·
Holger Schmitt · Gerrit Bode

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Abstract

Purpose The aim of this study was to determine the value of systemic biochemical markers of bone turnover—urine levels of cross-linked C-terminal telopeptide I (uCTX-I), urinary C-terminal telopeptide II (uCTX-II) and serum cartilage oligomeric matrix protein (sCOMP)—in the diagnosis of chondral defects after anterior cruciate ligament (ACL) rupture. Thirty-eight patients with previous ACL rupture were included.

Methods Magnetic resonance imaging (MRI) of the injured and the intact knee joint was performed with volumetric measurement of volume and area of cartilage (VC/AC), area of subchondral bone (cAB), and area of subchondral bone denuded and eroded (dAB). Biochemical markers were measured using commercially available enzyme-linked immunoassays.

Results MRI-based volumetric cartilage measurement showed significant differences between the injured and the intact knees. uCTX-I, sCOMP and in parts uCTX-II correlated well with MRI parameters. CTX-I showed a significant correlation with VC and AC of the whole knee joint.

Conclusions The results suggest that uCTX-I, uCTX-II and sCOMP could identify patients with focal cartilage lesions from an early stage of osteoarthritis of the knee.

Introduction

Osteoarthritis (OA) of the knee joint with the resulting morphological changes in cartilage, bone and soft tissue is one of the most common diseases. Symptomatic OA is a progressive disorder often leading to permanent disability. Several risk factors are known for OA of the knee. Increasing age, overweight, male gender and in particular traumatic lesions such as rupture of the anterior cruciate ligament (ACL) predispose to later OA [1].

Clinical examination and measurement of joint-space width (JSW) used to be the gold standard in the diagnosis of OA. These methods suffer from a lack of sensitivity, especially in the early stages of knee OA, as cartilage damage has to be severe before it can be detected on radiographs [2, 3]. In these early stages, however, therapeutic intervention is still possible. Therefore new diagnostic instruments for the early stages of knee OA are needed. In recent years biochemical markers of bone and cartilage turnover have been shown to be useful tools in identifying patients with high risk of joint degradation in OA [4]. Furthermore, their correlation with the findings of magnetic resonance imaging (MRI) has been demonstrated [5, 6]. However, the capacity of such markers for early detection of OA has not been exhaustively investigated. There is evidence that OA of the knee is distinguished not only by a change in cartilage surface but also by specific forms of damage in bone and surrounding soft tissue. Therefore different combinations of markers may play an important role in prognosis of the disease.

As degenerative damage begins with cartilage degradation we postulated that the systemic expression of biochemical markers of bone and cartilage turnover correlates with cartilage volume as assessed by means of MRI.

N. A. Streich (✉) · D. Zimmermann · H. Schmitt
Department of Orthopaedic Sports Medicine,
University of Heidelberg,
Schlierbacher Landstrasse 200a,
69118 Heidelberg, Germany
e-mail: Nikolaus.Streich@med.uni-heidelberg.de

G. Bode
Department of Trauma Surgery, University of Freiburg,
Freiburg, Germany

In this study we chose to focus on markers of bone turnover because their role in the diagnosis of OA has not yet been clearly established. Garnero et al. pointed out that C-terminal telopeptide of collagen type I (CTX-I) is an important risk factor for OA progression and showed that CTX-I is released into blood earlier than markers of cartilage turnover. They came to the conclusion that this order of release reflects the progress of the destruction in OA, which begins in the bone and continues in cartilage [7]. Bettica et al. demonstrated that CTX-I is increased in patients with progressive OA and not in those with non-progressive OA [8]. To our knowledge the correlation of urinary CTX-I to MRI-based cartilage volumetry has not yet been examined. Therefore this paper investigates the potential correlation of chondral degeneration measured by MRI-based cartilage volumetry and markers of cartilage turnover such as C-terminal telopeptide of collagen type I (CTX-I), type II (CTX-II) and COMP (cartilage oligomeric matrix protein).

Patients and methods

Patients

As it is thought that insufficiency of the ACL leads to progressive joint degradation, patients undergoing conservative treatment for a unilateral untreated ACL rupture were enrolled. The study cohort comprised 38 patients (11 female, 27 male) with a previous diagnosis of ruptured ACL. Beyond taking medical history, all patients underwent a specific orthopaedic examination to exclude potential osteoarthritis in any other joint than the knee. Individuals with fractures, metabolic bone or rheumatoid diseases, Paget's disease, endocrine or gastrointestinal disorders, renal dysfunction, a history of bone tumour or a history of osteoanabolic therapy were excluded. None of the female patients was taking hormone replacement therapy. Patients with contraindications for MRI, such as cardiac implants, pregnancy, any kind of metal implants or claustrophobia, were also not involved. Apart from clinical examination each patient underwent a MRI of both knees and collection of blood and urine specimens.

Methods

Measurement of biochemical markers

Urine samples were collected on second morning voiding. The samples were aliquoted and stored at -20°C until all patients had been recruited. Blood samples were collected at the end of the clinical examination and centrifuged

within two hours of examination to avoid haemolysis. Each sample was aliquoted and centrifuged at 6000 rpm for ten minutes. All patients had at least three (max. 5) samples. According to the manufacturer's instructions the samples were stored at -20°C or -80°C . Before analysis, they were thawed to room temperature. Haemolysis was assessed by using direct spectrophotometry to calculate the semiquantitative haemolysis index. Only non-haemolysed samples were processed. No individual had to be excluded. CTX-I and CTX-II as urinary markers of bone and cartilage degradation were measured using commercially available enzyme-linked immunosorbent assays (ELISA) (CTX-I: Cross-laps TM, Osteometer Biotech, Herlev, Denmark; CTX-II: Urine CartiLaps, Nordic Bioscience, Denmark). Urinary creatinine was measured by an automatic assay based on the Jaffe method. The concentration of the markers was standardised to total urinary creatinine.

Serum levels of cartilage oligomeric matrix protein (COMP) were also measured using commercially available ELISA (Wieslisa[®] hCOMP, Lund, Sweden).

MRI acquisition

Magnetic resonance images of both knees of each patient were acquired at 1.5 T with a circular knee coil. In each case the healthy knee was used as a reference value for the injured knee. Following imaging, segmentation of cartilage on a section by section basis was accomplished, then cartilage surfaces were reconstructed three-dimensionally. Finally, cartilage volumes were calculated according to the standardised method described by Hudelmaier et al. [9].

Statistical analysis

The results were analysed by descriptive and interference statistical methods using Graph Pad Prism 5. Quantitative variables at baseline were expressed as mean $\pm$ standard deviation (SD), with qualitative variables as frequencies. All data were tested for normal distribution. The comparison of biochemical markers with their normal values was accomplished by means of a *t*-test. The MRI data of each patient's intact knee and injured knee were also compared using a paired *t*-test. Relationships between biochemical markers and MRI values were assessed by the calculation of Pearson's correlation coefficient and by linear regression analysis. Age, sex and BMI were taken as predictors for linear regression. All tests were two-sided and a *p*-value ≤ 0.05 was considered to indicate a significant difference.

This study received the approval of the local ethics committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of

Helsinki. All participants gave their informed consent prior to their inclusion in the study.

Results

Thirty-eight patients could be included with a follow-up time of mean (SD) 19.96 years (2.6) after suffering from ACL insufficiency. Eleven females with a mean age of 42.18 (3.7) years and 27 males aged 46.4 (8.5) were enrolled. The mean BMI was 26.1 (2.8) for male patients and 24.2 (5.2) for female patients considering a BMI of 18–25 as normal value.

MRI-based volumetry showed significant differences between the injured and intact knee joints of all patients. We observed significant changes in the area of subchondral bone covered with cartilage (cAB) ($p=0.024$).

In particular, the area of subchondral bone denuded, eroded, full-thickness defect (dAB), with 37.02 (84.9) cm² on the injured versus 4.33 (17.2) cm² on the intact side, expressed the cartilage loss in the injured knees. This parameter describes focal lesions of cartilage. There were significant differences in the area of full-thickness defect in the whole knee and in the individual segments. There were no comparable defects in the intact knee joints. Even though VC was lower in the injured knees we did not find significant changes in the volume of cartilage (VC).

Urinary CTX-I

CTX-I levels in our patients' urine samples were in the upper range of normal. We measured a CTX-I level of 277.3 (156.6) considering 220.0 (86.6) nMBCE/nM creatinine a normal value. Normal values were offered by Nordic Bioscience Diagnostics, Herlev Denmark. CTX-I was the only biochemical marker to correlate with the volume of cartilage ($r=-0.359$; $p=0.044$) of the whole knee joint and the area of cartilage surface ($r=-0.336$; $p=0.030$).

Furthermore, correlations of CTX-I with the volume of cartilage could be found in the lateral tibia ($r=0.450$, $p=0.010$). Correlations with the area of cartilage were seen in the medial tibia ($r=0.338$; $p=0.029$) and the medial and lateral femur (cLF.AC $r=-0.364$, $p=0.040$, cMF.AC $r=-0.304$, $p=0.045$).

CTX-II and COMP

The mean CTX-II levels were higher than normal (325.9 [171] ng/mmol vs. 218 [253] ng/mmol). Normal values were offered by Nordic Bioscience Diagnostics, Herlev, Denmark. We found significant correlations of CTX-II with dAB of the medial tibia ($r=0.303$; $p=0.046$).

Values of COMP were within normal ranges, with a value of 1.11 (0.2) mg/ml.

Discussion

Our data suggest that the measured cartilage markers could be indicators for volumetric changes and focal cartilage lesions in the early stages of OA after long-term ACL insufficiency. Although the MRI measurements did not reflect overall loss of cartilage volume, focal lesions and cartilage degradation in the injured knee joints in comparison to the intact joints were clearly demonstrated.

This accords with an investigation published by Gandy et al. They came to the conclusion that loss of cartilage volume is less sensitive than the presence of focal lesions for the diagnosis of early OA [10].

Concentrations of CTX-I and CTX-II in urine samples and of COMP in serum samples correlated with the degenerative damage even though we could only demonstrate weak correlation coefficients. CTX-I in urine is highly specific for type I cartilage. We therefore suggest that it reflects the increase in local production of inflammatory cytokines resulting in uncoupling of bone turnover and finally cartilage degradation.

Bettica et al. reported increased bone turnover in patients with progressive OA and demonstrated that elevated CTX-I levels indicated increased bone resorption in patients with progressive OA [8]. In earlier studies, however, urinary CTX-I levels were lower in patients with knee OA [11]. This contradiction reflects the difficulties of using biochemical markers in OA diagnosis. On the other hand, it could be shown that high baseline levels of urinary CTX-I and CTX-II independently predict an increased risk of radiological progression over four years in patients with early rheumatoid arthritis (RA) [12]. In patients with hip OA, urinary CTX-I levels indicated higher bone turnover and led to the conclusion that higher cartilage and bone turnover are associated in patients with OA [13]. Similarly, Garnero et al. demonstrated that expression of CTX-I takes place after expression of CTX-II just as bone degradation follows cartilage degradation [14]. Finally, Davis et al. showed correlations of CTX-I with patients' Kellgren/Lawrence scores, bone density and changes in JSW. They concluded that CTX-I reflects general pathological changes in knee OA [15].

Our results corroborate the hypothesis that CTX-I can reflect focal cartilage lesions in the diagnosis of knee OA. Urinary CTX-I levels correlated not only with MRI parameters of bone turnover such as the area of subchondral bone (tAB) in the lateral tibia and femur but also with parameters of cartilage turnover such as volume of cartilage

(VC) in the whole knee and the lateral tibia. Therefore urinary CTX-I identifies pathological changes in the complete joint.

CTX-II has shown a consistent association with the symptoms and joint damage of OA. Several studies have measured higher levels of CTX-II in patients with OA and RA than in controls [11, 16, 17]. Jordan et al. found a strong association between urinary CTX-II and both disease severity and the presence of OA at the tibiofemoral and patellofemoral joints in men [18]. Sharif and colleagues pointed out that progression of knee OA is associated with alterations of systemic levels of biological markers of type II collagen metabolism [19]. Alterations of CTX-II values in comparison with baseline values seemed to be of special interest and importance. It was shown that subjects with a CTX-II level in the highest quartile had a 6.0-fold risk for progression of radiographic OA at the knee and that CTX-II is associated with both the prevalence and the progression of radiographic OA at the knee and hip [17]. A recent study clearly demonstrated firstly, that short-term changes in CTX-II identified patients with the highest risk of losing tibial cartilage after one year and secondly, that there is a significant association between CTX-II and thickness loss. In our study only baseline urinary CTX-II levels were recorded, so it is not possible to assess OA progression. The correlations measured for CTX-II were quite weak, which may be due to the very slight cartilage changes. On the other hand, Garnero et al. assumed that expression of CTX-II directly indicates cartilage destruction. Thus, the CTX-II levels in the study might also indicate focal cartilage loss seen on the MRI [14].

In addition to the CTX-I findings, this study shows focal cartilage lesions in early OA after cruciate ligament insufficiency. Combination of these two biochemical markers might constitute a useful new diagnostic tool.

COMP is a pentameric protein. Its synthesis is increased in chondrocytes and in synovial cells on activation by proinflammatory cytokines [20]. Several studies have suggested that COMP might be useful in the diagnosis of OA even though it is not a specific marker for cartilage degradation but represents the complex pathways of OA [13, 21–23]. In our study we found no correlations of COMP with cartilage volume as assessed by means of MRI. We did see instead a correlation of COMP with the area of cartilage in the medial femur of male patients. Hence we found an association of COMP with knee OA although it is arguable whether this protein is related to cartilage degradation or inflammation.

Overall it must be admitted that all correlation coefficients were quite weak. This was a pilot study to investigate the use of biochemical markers in OA diagnosis. We were able to demonstrate the possible use of biochemical markers in the

diagnosis of focal cartilage lesions. Future studies with longitudinal data and a healthy control group are needed.

Conclusion

These results demonstrate the expected cartilage degradation in the post-traumatic knee joints in comparison with the intact joint after almost 20 years of conservative treatment after ACL rupture.

Considering our hypothesis, we conclude that measurement of urinary CTX-I, CTX-II and serum COMP could be a useful additional parameter in the early detection of OA. Bearing in mind that there were only weak correlations between cartilage correlations and the markers used, we believe that further studies with longitudinal data with a healthy control group without any cartilage changes are necessary.

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