Is pro-matrix metalloproteinase-3 a marker for posttraumatic cartilage degradation?

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Summary

Objective: Since the development of posttraumatic osteoarthritis (OA) is a relatively slow process, estimation of OA risk would be of value with regard to chondroprotective measures and medication. In this study we investigated the significance of pro-matrix metalloproteinase-3 (proMMP-3) for this purpose.

Design: Synovial fluid (SF) and serum samples were collected from 259 patients of our trauma clinic at the time of arthroscopy. The extent of cartilage damage was assessed according to the Outerbridge-score. ProMMP-3 levels in SF and serum were determined by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody. Additionally we determined SF and serum levels of total MMP-3 and COMP levels as well as TIMP-1 and -2 concentrations in 40 randomly selected patients by ELISA.

Results: Serum proMMP-3 levels of the total cohort were markedly increased compared to healthy controls \((P<0.007)\). The comparison of serum and SF lavage proMMP-3 concentrations showed a significant correlation \((r_s=0.41, P<0.0001)\), however, only 26% of the investigated samples were increased above normal ranges. The grade of cartilage damage did not correlate with enzyme concentration neither in patients’ serum nor in SF samples. ProMMP-3 SF concentration was increased early after trauma. Furthermore, proMMP-3 correlated significantly with total MMP-3 serum and SF levels as well as COMP SF levels.

Conclusions: The measurement of proMMP-3 in serum or SF did not reflect the present cartilage damage and thus appears to have only minor potential for clinical use, but it should be considered for longitudinal studies, since it may reflect a risk for cartilage degradation in a subset of patients.

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established inflammatory joint disease, such as rheumatoid arthritis, were excluded from the study. None of the patients had undergone arthroscopy before or had been treated with intraarticular steroids. The condition of cartilage was graded by findings at arthroscopy following the Outerbridge-score from 0–4: 0=no cartilage damage, 1=weakened cartilage without superficial fibrillation, 2=superficial fibrillation, 3=deep cartilage clefts, 4=bony erosion. The diagnostic findings included anterior cruciate ligament (ACL) rupture with or without concomitant lesions of the medial collateral ligament, isolated meniscal tears (MEN), and other lesions including plica syndrome, luxation of patella and isolated cartilage damage (other).

The serum control group consisted of 20 volunteers (hospital workers) with no history of knee symptoms. Nine control SF samples were obtained post mortem from organ donors without a documented joint disease, with macroscopically intact articular cartilage. The taking of SF samples from organ donors was approved by the Ethics Committee of the University of Vienna.

ACQUISITION OF SERUM AND SF SAMPLES

Blood samples were collected in sterile tubes without additives before arthroscopy was carried out. Samples were allowed to clot at room temperature and centrifuged. Thereafter serum was stored in aliquots at −70°C.

Aspiration of knee joint fluid was done using the procedure described by Geborek et al. Before arthroscopy, an arthrocentesis of the supralateral pouch was performed. The native joint fluid was microscopically evaluated and specimens with signs of inflammation (>1000 cells/mm³) were excluded. After aspiration to dryness, 20 ml of physiological saline was injected. After moving the joint five times between 0 and 90° to assure sufficient distribution of the fluid in the whole joint compartment, 3 ml of lavage of fluid was aspirated through a new puncture-needle.

The joint-lavage samples were centrifuged at 3,000g for 15 min to remove cells and debris. The supernatants were stored in aliquots at −70°C.

Acquisition of serum and SF samples was performed as described above. The SF samples were centrifuged at 3,000g for 15 min to remove cells and debris. The supernatants were stored in aliquots at −70°C.

MARKER ASSAYS

ProMMP-3 in serum and joint-lavage was quantified by ELISA (BindAzyme™, The Binding Site Limited, Birmingham, UK), according to the manufacturer’s manual.

In 40 randomly selected patients, as selected by a computerized randomization program, total MMP-3 and COMP levels were measured in serum and SF lavage, using the Quantikine human MMP-3 (total) ELISA kit (R&D Systems Inc., Minneapolis, MN, USA). COMP concentrations were assessed using an ELISA kit (COMP ELISA, AnaMar Medical AB, Uppsala, Sweden). Since it was proposed earlier for OA cartilage that an imbalance between MMPs and its inhibitors may contribute a possible pathomechanism in cartilage destruction we additionally determined Tissue inhibitor of metalloproteinases (TIMP)-1 and -2 levels in the same 40 patients by ELISA (Quantikine human TIMP-1 and Quantikine human TIMP-2 ELISA kit, R&D Systems Inc.).

Highly sensitive C-reactive protein (hs-CRP) measurements were available from 86% of the patients using an immunoturbidimetric assay (Dade-Bering, Vienna, Austria).

STATISTICAL ANALYSIS

The statistical significance of differences in the median values among the groups was determined by Kruskal–Wallis one way analysis of variance on ranks, to isolate the groups that differ from others the pairwise multiple comparison procedures (Dunn’s method) was used. Mann–Whitney rank sum test was used to determine significant differences between groups. To examine relationships between serum
and joint-lavage proMMP-3 levels the Spearman’s rank order correlation was used. A $P$ value less than 0.05 was considered significant.

**Results**

**SERUM AND SF LAVAGE LEVELS OF PROMMP-3**

Serum proMMP-3 levels (median (range): 26 (2–674) ng/ml) were increased compared to healthy controls (67.4 (42.4–91.9) ng/ml; $P<0.007$), whereas there was no detectable statistical difference of proMMP-3 levels in SF lavage (400 (6–8000) ng/ml) compared to nine organ donors (442.8 (282.8–1202.5) ng/ml; $P=0.8$). Moreover, we found a significant correlation between serum and SF lavage proMMP-3 concentration of all patients, as calculated by Spearman’s test ($r_s=0.41$, $P<0.0001$) [Fig. 1], however if values within the normal ranges (74% of 259 patients) were excluded, no correlation was seen.

Furthermore we investigated the influence of patient age and sex on proMMP-3 levels. The patients’ age ranged from 11 to 69 years with an average of 31.2 years. There was no independent influence of patients’ age, neither on serum, nor on SF lavage proMMP-3 concentrations (data not shown).

Sex was almost equally distributed including 56.8% male and 43.2% female individuals. We found no significant difference between the male and the female patients group in serum (male: 30 (2–674) ng/ml and female: 20 (2–664) ng/ml) as well as in SF lavage proMMP-3 levels (male 480 (12–8000) ng/ml and female 363 (6–8000) ng/ml).

**TEMPORAL PATTERN OF PROMMP-3 CONCENTRATIONS IN SF LAVAGE AFTER KNEE INJURY**

To evaluate a relation between time after injury and proMMP-3 levels we determined three subgroups: (1) up to 1 week after trauma, (2) 2–8 weeks and (3) >8 weeks after injury and compared serum and SF lavage proMMP-3 levels. In SF lavage, a significant difference was found for group 1 (740 (20–8000) ng/ml) compared to groups 2 (327.8 (6–8000) ng/ml; $P=0.01$) and 3 (270 (20–3820) ng/ml; $P=0.001$) [Fig. 2(A)]. There was no statistically significant difference between serum proMMP-3 concentrations within these groups (Fig. 2(B)).
To test a possible relation between serum proMMP-3 levels and the extent of a possible inflammatory reaction within the joint, we measured serum levels of highly sensitive-CRP concentrations of 222 patients. We found no significant correlation ($r_s = 0.048$, $P < 0.5$) and furthermore, there was no correlation between hs-CRP and MMP-3 ($r_s = 0.25$, $P < 0.1$) as well as between hs-CRP and COMP ($r_s = 0.2$, $P < 0.9$).

SERUM AND SF LAVAGE PROMMP-3 CONCENTRATIONS IN RELATION TO THE TYPE OF KNEE INJURY

Since knee joint injury involves damage of different joint structures, we compared the serum and SF lavage proMMP-3 levels with regard to ruptures of the anterior cruciate ligament with or without concomitant lesions of the medial collateral ligament (ACL), isolated meniscal tears (MEN) and other lesions (other), found at arthroscopy. Synovial fluid lavage proMMP-3 concentrations were highly increased in patients with ruptures of the anterior cruciate ligament (600 (6–8000) ng/ml) compared to meniscal lesions (280 (12–3820) ng/ml; $P < 0.01$) and others (340 (20–5000) ng/ml; $P < 0.05$). Serum proMMP-3 levels after knee injury that involved ACL (32 (2–674) ng/ml), MEN (23 (2–664) ng/ml) and others (20 (2–500) ng/ml) were not significantly different. It is noteworthy that correlations between serum and SF were significant for all conditions.

SERUM AND SF LAVAGE PROMMP-3 CONCENTRATIONS IN RELATION WITH THE GRADE OF CARTILAGE DAMAGE

Depending on the grade of cartilage damage, patients were divided into five study groups (grades 0–4, according to the Outerbridge-score). We found no statistical differences in SF lavage proMMP-3 concentrations between these five groups [Fig. 3(A)].

Fig. 3. Pro-matrix metalloproteinase-3 (proMMP-3) concentrations in (A) synovial fluid (SF) and (B) serum in relation to the grade of cartilage damage. The degree of cartilage damage was evaluated at arthroscopy and graded following the Outerbridge-score: 0=no cartilage damage ($n=153$), 1=weakened cartilage without superficial fibrillation ($n=26$), 2=superficial fibrillation ($n=44$), 3=deep cartilage clefts ($n=29$) and 4=bony erosion ($n=7$). There was no statistical significance in proMMP-3 levels, when groups were compared, either in SF lavage, or in serum. Horizontal lines represent the median for each patients group.
LEVELS OF TOTAL MMP-3 AND COMP IN 40 RANDOMLY SELECTED PATIENTS

We additionally evaluated the levels of total MMP-3 and COMP in parallel in 40 patients. As shown for proMMP-3, we found no dependence of the grade of cartilage damage on MMP-3 and COMP concentrations in the tested individuals (data not shown). Furthermore, our data revealed no correlation between serum and SF lavage levels for total MMP-3 (rs = 0.27, P < 0.09) as well as for COMP (rs = 0.27, P < 0.08). Matrix metalloproteinase-3 (MMP-3) correlated significantly with its pro-form on both SF (rs = 0.54, P < 0.0004) and serum levels (rs = 0.38, P < 0.02) [Fig. 4(A, B)]. When we correlated SF and serum COMP levels with that of proMMP-3 we found a significant correlation for SF (rs = 0.47, P = 0.003) [Fig. 4(C, D)]. While the concentration of total MMP-3 and COMP were within the normal ranges in the 40 posttraumatic patients, proMMP-3 levels were elevated in 55% of these patients.

BALANCE BETWEEN PROMMP-3, MMP-3 AND TIMP-1 AND TIMP-2

In order to account for the role of the inhibitors of matrix metalloproteinases, we measured TIMP-1 and TIMP-2 levels in SF and serum. Synovial Fluid-TIMP levels are shown in Fig. 5. While not statistically significant, there was a trend towards elevated TIMP-1 in posttraumatic SF lavage. The calculation of ratios between proMMP-3 and MMP-3 to TIMP-1 and TIMP-2 respectively, shows that the elevation of proMMP-3 levels in posttraumatic joints is sustained even when considering TIMP levels [Fig. 5]. Serum levels of TIMP-1 (median (range) 158 (111–241) ng/ml) and TIMP-2 (141 (93–524) ng/ml) of the posttraumatic group (n=40) were within the range observed in the controls (TIMP-1: 177 (143–229) ng/ml; TIMP-2: 138 (105–172) ng/ml). The ratio of proMMP-3 vs TIMP-1 and -2 was elevated in 50% of the patients, whereas the ratio of MMP-3 vs TIMP-1 and -2 was comparable to controls.

Discussion

MMP-3 (also termed stromelysin-1) is a 57/45 kDa (latent/active) enzyme that hydrolyzes a number of extracellular matrix components, including aggrecan, fibronectin, laminin, collagens III, IV, IX and X. Moreover, MMP-3 may contribute indirectly to collagen II breakdown by activating other MMPs. The involvement of MMP-3 in cartilage extracellular matrix degradation is well established. MMP-3 has been localized in regions of cartilage...
degradation\textsuperscript{27,28} and its endogenous expression by articular chondrocytes, as well as synovial tissue derived from OA and rheumatoid arthritis (RA) joints has also been demonstrated\textsuperscript{29–34}. Much attention has been attributed to MMP-3 as a marker in inflammatory/ degenerative joint diseases. Increased levels of MMP-3 were found in SF from OA and RA joints\textsuperscript{35–38}. Measurement of plasma MMP-3 concentrations in OA patients showed a significant increase compared to controls\textsuperscript{39}.

ProMMP-3 is the inactive form of MMP-3. In chronic inflammatory joint diseases such as RA serum levels of proMMP-3 were increased to a high extent\textsuperscript{40}. Furthermore, Walakovits et al. demonstrated that within the SF the majority of the enzyme is present as proMMP-3 after trauma\textsuperscript{14}. Since, the affinity of proMMP-3 to inhibitor proteins, such as tissue inhibitors of metalloproteinase, may be much less than that of the active form,\textsuperscript{15,16} its higher stability may provide a longer half life in the circulation.

We report a significant correlation of proMMP-3 levels in SF and serum after knee trauma. This finding parallels the results of the study of Taylor and colleagues, who demonstrated a correlation between proMMP-3 SF levels and its concentration in the circulation in patients with chronic inflammatory joint disease\textsuperscript{40}. It is of note that serum proMMP-3 levels of only 68 out of 259 patients (26\%) were above normal ranges, indicating a minor clinical significance of this marker. However, when we compared proMMP-3 with established biochemical markers, MMP-3 and COMP, we found a significant correlation between proMMP-3 and MMP-3 in serum and SF as well as between proMMP-3 and COMP in SF. Furthermore, our data show that proMMP-3 serum concentrations were elevated above normal levels in 55\% of the patients with normal serum MMP-3 or COMP ranges. After accounting for the concentration of metalloproteinase inhibitors, TIMP-1 and TIMP-2, the elevation of proMMP-3 remained apparent.

These data suggest a potential role for proMMP-3 as a marker for cartilage degradation in longitudinal studies. The question arises which condition may lead to an increase in proMMP-3 and/or MMP-3 expression. It was proposed originally, that the separate determination of the precursor and active forms of MMP-3 may reflect different processes, proMMP-3 being associated with synovial inflammation, and active MMP-3 reflecting cartilage destruction\textsuperscript{37}. However, both the latent and the active form are commonly believed to reflect synovial inflammation\textsuperscript{40–44}.

We thus investigated whether an inflammatory reaction in joints after injury may be associated with proMMP-3 concentrations. While no macroscopically detectable synovitis was described in any of our arthroscopy reports and the leucocyte numbers in the SF were below 1000 cells/mm\textsuperscript{3}, we measured hs-CRP serum levels as an ultra-sensitive marker for ongoing inflammation. Our data reveal no significant correlation neither with serum proMMP-3, nor with MMP-3 or COMP, making a major cytokine release from the joints very unlikely. While formally a local inflammatory-like process cannot be excluded, hs-CRP seems not to be a relevant marker for this question.

A positive relationship between the expression of MMP-3 and different stages of OA of the knee was reported earlier.
using immunohistochemistry on cartilage biopsies. This data suggested a connection between the amounts of expressed MMP-3 with the extent of cartilage damage. When we examined the influence of the degree of cartilage damage after knee injury on SF and serum proMMP-3 levels, as well as MMP-3 and COMP levels, we detected no relation between SF or serum enzyme levels and the grade of cartilage damage. These findings are supported by the data of Naito and co-workers, who consistently showed no influence of the grade of knee OA on plasma MMP-3, MMP-9 and TIMP-1 levels.

In summary we showed that serum levels of proMMP-3 were elevated in 26% of the postraumatic patients without a significant correlation with SF lavage levels. ProMMP-3 was elevated in SF lavage early after trauma, but proMMP-3 did not indicate the present cartilage damage. To answer the original question if proMMP-3 can predict cartilage damage, we suggest that the measurement of proMMP-3 in serum or SF has only minor clinical relevance, but should not be omitted in longitudinal studies, since it may be an additional marker for the evaluation of the risk for OA development in a subset of patients.

References


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