TNF-α and MMP-8 as Biomarkers for Diagnosing Knee Cartilage Lesions — Preliminary Results

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Objective: To analyse the correlations between the levels of Tumor Necrosis Factor α, Matrix Metalloproteinase 8, Interleukin 6 and the presence of cartilage lesions in the knee.
Methods: We studied 79 individuals divided in three groups – a group with cartilage lesions, a group with meniscal lesions and a control group. All patients underwent arthroscopic surgical interventions – either diagnostic or therapeutic. Venous blood samples and synovial fluid samples were obtained and we determined the levels of TNF-α, MMP-8 and IL-6 respectively. All study participants filled out the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Score questionnaire, based on which the IKDC score was calculated.
Results: we found higher levels of MMP-8 in both serum and synovial fluid for groups 1 and 2 compared to the control group, but no correlation between the serum and synovial fluid levels of MMP-8. The serum MMP-8 levels showed a significant negative correlation with the highest level of activity without pain and significant giving way. The synovial MMP-8 could be correlated to the IKDC score. Serum levels and synovial levels of TNF-α were in strong correlation. We found no association between serum and/or synovial TNF-α and MMP-8 levels.
Conclusions: We found that synovial MMP-8 concentrations showed a reverse correlation with the IKDC scores (an activity-based score) – thus MMP-8 might be a diagnostic and prognostic marker in knee osteoarthritis.

Keywords: TNF-α, MMP-8, biomarkers, cartilage, knee

Introduction

Many recent studies have investigated the role of the synovial membrane in the development and natural history of knee osteoarthritis – and it is believed that a rise of the cytokine and growth factors concentration in the inflamed synovial membrane can influence the production of enzymes that degrade articular cartilage [1,2,3].

Inflammatory cytokines produced by the synovial macrophages stimulate the production of metalloproteinases which destroy the cartilage's extracellular matrix [4,5,6,7].

In our study we aimed to analyse how the levels of Tumor Necrosis Factor α (TNF-α), Matrix Metalloproteinase 8 (MMP-8) and Interleukin 6 (IL-6) correlate with the presence of cartilage lesions of the knee.

Methods

Our study was conducted on a number of 79 individuals that were divided in three groups:

- group 1 with knee cartilage lesions (n=28, 45.21±2.6 years)
- group 2 with meniscal lesions (n=26, 37±2.87 years)
- group 3, a control group (n=25, 40.2±2.34 years).

We had 21 male patients in group 1, 19 in group 2 and 16 in group 3.

Oral informed consent was obtained from all study participants, and our study followed the principles outlined in the Declaration of Helsinki.

All patients underwent arthroscopic surgical interventions – either diagnostic or therapeutic. The cartilage lesions diagnosed in patients included in group 3 were of grade III and IV according to the criteria described by the International Cartilage Repair Society (ICRS) [8].

Venous blood samples were obtained preoperatively and synovial fluid samples were taken during the arthroscopic procedures. These were used to determine the serum and synovial fluid levels of TNF-α, MMP-8 and IL-6 respectively. The methods used for these determinations were as follows:

- Sandwich ELISA assays developed applying 2 commercially available antibody pairs (DY908, DY206, R&D Systems) – for MMP-8 and IL-6
- Pharmingen OPTEIA ELISA kit for TNF-α.

All study participants filled out the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Score questionnaire, based on which the IKDC score was calculated. This is an activity-based score that offers a subjective assessment of the knee from a functional and symptomatic point-of-view, and it is part of the ICRS Cartilage Injury Evaluation Package [8]. A higher score represents a better result (the maximum score value is 100 points).

Results

The values obtained for the 3 soluble factors determined from both synovial fluid and venous blood samples are shown in Table I.

The correlations of TNF-α, MMP-8 and the IKDC score are presented in Tables II, III and IV.

We observed higher levels of MMP-8 in both serum and synovial fluid for groups 1 and 2 compared to the control group, as shown in Figure 1. Despite this, we found no correlation between the serum and synovial fluid levels of MMP-8.

As for the serum MMP-8, the levels of this showed a significant negative correlation with questions Q1 and

Table I. Levels of MMP-8 and, TNF-α and IL-6 in the serum and synovial fluid samples

	Meniscal lesions (n=26)	Cartilage lesions (n=28)	Controls (n=25)
MMP-8 syn (mean±SE, pg/ml)	889.71±30.52	729.31 ± 261.0	–
MMP-8 serum (mean±SE, pg/ ml)	2054.47±168.01*	1897.68±217.85*	1182.61±173.95
TNF syn (mean±SE, pg/ ml)	8.41±2.65	3.86±1.74	–
TNF serum (mean±SE, pg/ ml)	12.11±3.16	8.19±2.97	4.24±0.68
IL-6 syn (mean±SE, pg/ ml)	–	256.88±93.87	–
IL-6 serum (mean±SE, pg/ ml)	58.47±18.01**	82.15±43.81**	11.28±1.06

values marked with asterisk, significant difference vs. controls, *p<0.01, **p<0.001

Table II. Correlations of TNF-α

Correlation	Spearman R	p-level	Correlation	Spearman R	p-level
TNF syn & Q1	-0.070	0.705	TNF serum & Q1	0.021	0.911
TNF syn & Q2	0.066	0.719	TNF serum & Q2	0.121	0.508
TNF syn & Q3	0.075	0.684	TNF serum & Q3	0.257	0.156
TNF syn & Q4	0.238	0.190	TNF serum & Q4	0.225	0.215
TNF syn & Q5	0.123	0.501	TNF serum & Q5	0.170	0.352
TNF syn & Q6	0.305	0.090	TNF serum & Q6	0.158	0.386
TNF syn & Q7	0.001	0.996	TNF serum & Q7	0.077	0.674
TNF syn & Q8	0.131	0.474	TNF serum & Q8	-0.280	0.121
TNF syn & Q9	0.360	**0.043**	TNF serum & Q9	0.397	**0.025**
TNF syn & IKDC	0.275	0.128	TNF serum & IKDC	0.332	0.063
TNF syn & age	-0.395	**0.025**	TNF serum & age	-0.227	0.211
TNF syn & BMI	-0.155	0.406	TNF serum & BMI	-0.015	0.935
			TNF serum & TNF syn	0.537	**0.002**

Q7 from the IKDC questionnaire (highest level of activity without pain and significant giving way respectively, with p values of 0.037 and 0.041).

The synovial MMP-8 could be correlated to questions Q3 (pain severity, p=0.029) and Q6 (level of joint blockage, p=0.040), as well as to the IKDC score (p=0.048). This can be seen in Figure 2.

For TNF-α, we found that serum levels of the cytokine were somewhat higher in patients from groups 1 and 2 compared to controls, and the serum levels were also in strong correlation with the synovial levels of the cytokine (R=0.53, p=0.002).

Besides these, the serum TNF-α could be correlated to question Q9 (impairment related to combined physical activity, p=0.025).

IL-6 levels were increased both in serum and synovial fluid, but we found no significant correlation with the functional score.

We found no association between serum and / or synovial TNF-α and MMP-8 levels.

Discussion

Studies have shown that some members of the matrix metalloproteinase family like MMP-1 and MMP-13 play an important role in the development of osteoarthritis. Also, some of the pro-inflammatory cytokines, like TNF-α and IL-1 have been identified as triggers for MMP production in the inflamed joint [9] and might be of prognostic interest in conjunction with the metalloproteinase levels.

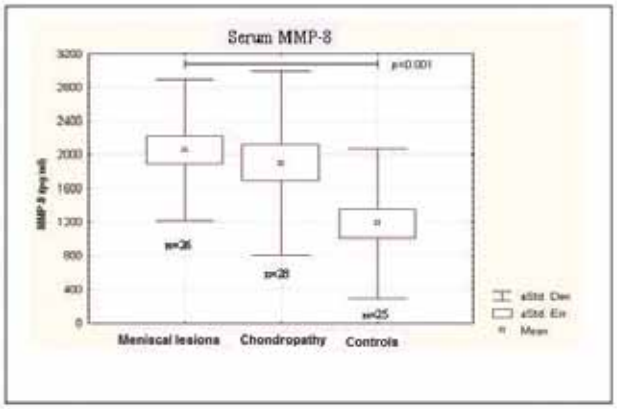

Fig. 1. Serum levels of MMP-8 – comparison between groups

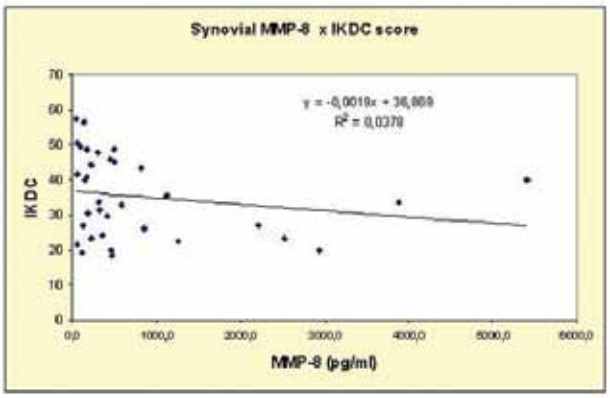

Fig. 2. Correlations of the synovial levels of MMP-8 and the IKDC score

Table III. Correlations of MMP-8

Correlation	Spearman R	p-level	Correlation	Spearman R	p-level
MMP-8 syn & Q1	-0.166	0.349	MMP-8 serum & Q1	-0.296	**0.037**
MMP-8 syn & Q2	-0.243	0.167	MMP-8 serum & Q2	0.192	0.182
MMP-8 syn & Q3	-0.374	**0.029**	MMP-8 serum & Q3	-0.036	0.806
MMP-8 syn & Q4	-0.227	0.197	MMP-8 serum & Q4	0.119	0.409
MMP-8 syn & Q5	-0.309	0.075	MMP-8 serum & Q5	-0.240	0.094
MMP-8 syn & Q6	-0.354	**0.040**	MMP-8 serum & Q6	-0.104	0.471
MMP-8 syn & Q7	-0.119	0.502	MMP-8 serum & Q7	-0.290	**0.041**
MMP-8 syn & Q8	0.063	0.725	MMP-8 serum & Q8	-0.040	0.782
MMP-8 syn & Q9	-0.256	0.144	MMP-8 serum & Q9	-0.119	0.410
MMP-8 syn & IKDC	-0.342	**0.047**	MMP-8 serum & IKDC	-0.127	0.381
MMP-8 syn & age	-0.042	0.813	MMP-8 serum & age	-0.009	0.949
MMP-8 syn & BMI	0.216	0.228	MMP-8 serum & BMI	0.137	0.347
MMP-8 syn & TNF syn	-0.315	0.445	MMP-8 serum & MMP-8 syn	0.149	0.416
MMP-8 syn & TNF serum	-0.264	0.144	MMP-8 serum & TNF syn	0.069	0.708
MMP-8 syn & IL-6 serum	0.261	0.163	MMP-8 serum & TNF serum	0.032	0.827
MMP-8 syn & IL-6 syn	0.318	0.160	MMP-8 serum & IL-6 serum	-0.011	0.948
			MMP-8 serum & IL-6 syn	-0.349	0.121

Table IV. Correlations of the IKDC score

Correlation	Spearman R	p-level
IKDC & age	-0.459	**0.000**
IKDC & BMI	-0.272	**0.049**
IKDC & MMP-8 syn*	-0.342	**0.047**
IKDC & MMP-8 serum	-0.127	0.381
IKDC & TNF syn*	0.272	0.119
IKDC & TNF serum	0.153	0.289
IKDC & IL-6 syn **	0.005	0.983
IKDC & IL-6 serum	-0.061	0.708

values marked with asterisk, significant difference vs. controls, *p<0.01, **p<0.001

MMP-8 is a proteinase predominantly synthetized by neutrophil granulocytes and has a variety of biological effects (it acts as a collagenase, aggrecanase, chemokine activator). Early studies regarding MMP-8 expression in human joints predicted an important pathogenic role for the enzyme [10,11].

But other studies questioned the central role of MMP-8 in osteoarthritis. Stremme et al. [12] found only a little amount of MMP-8 m-RNA and a slight staining for MMP-8 in normal and osteoarthritic cartilage. Furthermore, using an osteoarthritic transgenic mouse model, Salmin et al. found that increase of MMP-13 m-RNA is much superior to those of MMP-2, 3, 8, 9 [13]. According to these published data, the participation of MMP-8 in the cartilage degradation process is ambiguous.

The higher levels of MMP-8 in the blood and synovial fluid samples of groups 1 and 2 of our study – as compared to those of the 3rd, control group – support the concept of this soluble factor being involved in the intra-articular pathological processes of the knee.

The correlation between MMP-8 levels and the functional score of the knee could mean that this metalloproteinase has a role in the degradation of articular cartilage.

Of course further studies are needed to assess the role of MMP-8 in these processes, and although the current study is part of a larger project, it unfortunately has the inherent economic limitations that determine the number of soluble factors that can be evaluated.

Conclusions

We found that synovial MMP-8 concentrations showed a reverse correlation with the IKDC scores (an activity-based score) – thus MMP-8 might be a diagnostic and prognostic marker in knee osteoarthritis.

TNF-α levels were slightly increased in the majority of cases. TNF-α in serum and synovial fluid correlated positively, and could be associated to the functional knee score, making TNF-α a possibly valuable biomarker of cartilage lesions as well.

A future „biological staging" of knee cartilage lesions and osteoarthritis could become available, with therapeutic importance – for instance the development of MMP inhibitor drugs.

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Appendix

2000 IKDC Subjective Knee Evaluation Form

Patients Part:

Your Full Name_____

Today's Date: _____/_____/_____ (Day / Month / Year)

Date of Injury: _____/_____/_____ (Day / Month / Year)

SYMPTOMS*:

*Grade symptoms at the highest activity level at which you think you could function without significant symptoms, even if you are not actually performing activities at this level.

1. **What is the highest level of activity that you can perform without significant knee pain?**

   ❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   ❑Strenuous activities like heavy physical work, skiing or tennis
   ❑Moderate activities like moderate physical work, running or jogging
   ❑Light activities like walking, housework or yard work
   ❑Unable to perform any of the above activities due to knee pain

2. **During the <u>past 4 weeks</u>, or since your injury, how often have you had pain?**

	0	1	2	3	4	5	6	7	8	9	10	
Never	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	Constant

3. **If you have pain, how severe is it?**

	0	1	2	3	4	5	6	7	8	9	10	
No pain	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	Worst pain imaginable

4. **During the past 4 weeks, or since your injury, how stiff or swollen was your knee?**

   ❑Not at all
   ❑Mildly
   ❑Moderately
   ❑Very
   ❑Extremely

5. **What is the highest level of activity you can perform without significant swelling in your knee?**

   ❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   ❑Strenuous activities like heavy physical work, skiing or tennis
   ❑Moderate activities like moderate physical work, running or jogging
   ❑Light activities like walking, housework, or yard work
   ❑Unable to perform any of the above activities due to knee swelling

6. **During the <u>past 4 weeks</u>, or since your injury, did your knee lock or catch?**

   ❑Yes ❑No

7. **What is the highest level of activity you can perform without significant giving way in your knee?**

   ❑Very strenuous activities like jumping or pivoting as in basketball or soccer
   ❑Strenuous activities like heavy physical work, skiing or tennis
   ❑Moderate activities like moderate physical work, running or jogging
   ❑Light activities like walking, housework or yard work
   ❑Unable to perform any of the above activities due to giving way of the knee

SPORTS ACTIVITIES:

8. **What is the highest level of activity you can participate in on a regular basis?**

❑Very strenuous activities like jumping or pivoting as in basketball or soccer
❑Strenuous activities like heavy physical work, skiing or tennis
❑Moderate activities like moderate physical work, running or jogging
❑Light activities like walking, housework or yard work
❑Unable to perform any of the above activities due to knee

9. **How does your knee affect your ability to:**

		Not difficult at all	Minimally difficult	Moderately Difficult	Extremely difficult	Unable to do
a.	Go up stairs	❑	❑	❑	❑	❑
b.	Go down stairs	❑	❑	❑	❑	❑
c.	Kneel on the front of your knee	❑	❑	❑	❑	❑
d.	Squat	❑	❑	❑	❑	❑
e.	Sit with your knee bent	❑	❑	❑	❑	❑
f.	Rise from a chair	❑	❑	❑	❑	❑
g.	Run straight ahead	❑	❑	❑	❑	❑
h.	Jump and land on your involved leg	❑	❑	❑	❑	❑
i.	Stop and start quickly	❑	❑	❑	❑	❑

FUNCTION:

10. **How would you rate the function of your knee on a scale of 0 to 10 with 10 being normal, excellent function and 0 being the inability to perform any of your usual daily activities which may include sports?**

FUNCTION PRIOR TO YOUR KNEE INJURY:

Cannot perform daily activities — No limitation

0 ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8 ❑ 9 ❑ 10 ❑

CURRENT FUNCTION OF YOUR KNEE:

Cannot perform daily activities — No limitation

0 ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8 ❑ 9 ❑ 10 ❑

Scoring Instructions for the 2000 IKDC Subjective Knee Evaluation Form

Several methods of scoring the IKDC Subjective Knee Evaluation Form were investigated. The results indicated that summing the scores for each item performed as well as more sophisticated scoring methods.

The responses to each item are scored using an ordinal method such that a score of 1 is given to responses that represent the lowest level of function or highest level of symptoms. For example, item 1, which is related to the highest level of activity without significant pain is scored by assigning a score of 1 to the response "Unable to Perform Any of the Above Activities Due to Knee" and a score of 5 to the response "Very strenuous activities like jumping or pivoting as in basketball or soccer". For item 2, which is related to the frequency of pain over the past 4 weeks, the response "Constant" is assigned a score of 1 and "Never" is assigned a score of 11.

The IKDC Subjective Knee Evaluation Form is scored by summing the scores for the individual items and then transforming the score to a scale that ranges from 0 to 100. **Note:** The response to item 10 "Function Prior to Knee Injury" is not included in the overall score. The steps to score the IKDC Subjective Knee Evaluation Form are as follows:

1. Assign a score to the individual's response for each item, such that lowest score represents the lowest level of function or highest level of symptoms.
2. Calculate the raw score by summing the responses to all items with the exception of the response to item 10 "Function Prior to Your Knee Injury"
3. Transform the raw score to a 0 to 100 scale as follows:

$$\text{IKDC Score} = \left[\frac{\text{Raw Score} - \text{Lowest Possible Score}}{\text{Range of Scores}}\right] \times 100$$

Where the lowest possible score is 18 and the range of possible scores is 87. Thus, if the sum of scores for the 18 items is 60, the IKDC Score would be calculated as follows:

$$\text{IKDC Score} = \left[\frac{60 - 18}{87}\right] \times 100$$

$$\text{IKDC Score} = 48.3$$

The transformed score is interpreted as a measure of function such that higher scores represent higher levels of function and lower levels of symptoms. A score of 100 is interpreted to mean no limitation with activities of daily living or sports activities and the absence of symptoms.

The IKDC Subjective Knee Score can still be calculated if there are missing data, as long as there are responses to at least 90% of the items (i.e. responses have been provided for at least 16 items). To calculate the raw IKDC score when there are missing data, substitute the average score of the items that have been answered for the missing item score(s). Once the raw IKDC score has been calculated, it is transformed to the IKDC Subjective Knee Score as described above.