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Osteoarthritis of the knee – clinical assessments and inflammatory markers[1]

Stefanie S. Brenner MD†*, Ulrich Klotz PhD†, Dominik M. Alscher MD‡, Anna Mais PhD student§, Gereon Lauer PhD§, Horst Schweer PhD¶, Hannsjörg W. Seyberth MD¶, Peter Fritz MD‡ and Uwe Bierbach MD‡

† *Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany*
‡ *Robert Bosch Hospital, Departments of Surgery, Pathology and Internal Medicine, Stuttgart, Germany*
§ *ALTANA Pharma AG, Konstanz, Germany*
¶ *Department of Pediatrics, Phillips University Marburg, Marburg, Germany*

Summary

Objective: The present cross sectional study was performed to test the hypothesis that in osteoarthritis (OA) of the knee severity of this disease is related to local levels of inflammatory metabolites and their corresponding enzymes.

Methods: From 41 patients with OA of the knee (age range 45–79 years) undergoing arthroscopy blood, synovial fluid (SF) and synovial membrane (SM) were collected. Clinical conditions were primarily assessed by the WOMAC-index and radiographic grading (K&L-grade). Concentrations of PGE_2, TxB_2 and $NO_{2/3}$ and that of IL-6, IL-1α, IL-1β, TNFα, COX-2 and iNOS were determined in SF and SM, respectively.

Results: With advancing age K&L-grade and COX-2 in SM increased significantly (P=0.005 and P=0.01, respectively). TNFα and IL-1α were not detectable in SM samples. Apart from a correlation between PGE_2 and WOMAC-index (r=0.36, P=0.035) no significant relationships could be found between the various inflammatory parameters and any of the assessed clinical signs.

Conclusions: Apparently no direct relationships exist between the measured markers of inflammation (e.g. PGE_2, $NO_{2/3}$) or the involved enzymes (e.g. COX-2, iNOS) and the severity of OA of the knee. The degenerative condition of this disease might be due to the more local, mainly mechanical injury with little systemic upset. However, further longitudinal studies are needed to clarify whether the assessed biochemical markers could serve as predictors for the progression of OA.

Key words: Osteoarthritis, Eicosanoids, Inflammatory markers, Nitric oxide.

Introduction

Osteoarthritis (OA) is characterized by progressive degenerative changes of joints. OA of the knee is one of the most common forms of arthritis in synovial joints and it is more frequently present in the elderly[1–3]. Synovitis in arthritic joints is thought to play a dominant role in the development of pain, joint inflammation and cartilage destruction[4,5]. Furthermore, prostaglandin E_2 (PGE_2) and nitric oxide (NO) have been described as mediators of inflammation and cartilage destruction[6–8]. Proinflammatory cytokines, e.g. interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα), induce the expression of cyclooxygenase-2 (COX-2) and nitric oxide synthase (NOS), especially the inducible isoform of NOS (iNOS)[9]. COX-2 and iNOS are responsible for increased levels of PGE_2 and NO which both play important roles in inflammation and pain[10–13]. Therefore it could be anticipated that severity of OA will be related to local prostanoid levels and/or other proinflammatory messengers.

While production of proinflammatory cytokines and other inflammatory mediators in synovial fluid of joints is well documented in OA[14,15], the local concentrations of inflammatory parameters in OA of the knee and especially their relation to clinical and radiographic signs have been rarely investigated. Thus, the present cross sectional study was performed in order to test the hypothesis that in patients with OA severity of this disease assessed by the most useful tools, such as the WOMAC-index[16,17] and radiographic grading[18], is associated with the levels of local markers of inflammation, such as PGE_2, NO, COX-2 or iNOS. Furthermore, we wanted to find out whether such putative associations were influenced by the age of the patient.

Materials and methods

PATIENTS

Forty-one Caucasian patients (16 males and 25 females) with OA of the knee were included in the present cross sectional study. Arthroscopy of the knee was performed for

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*Address for correspondence and reprint requests: Stefanie Brenner, MD, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Auerbachstrasse 112, D-70376 Stuttgart, Germany. Tel.: +49-711-8101-3730; Fax: +49-711-85-92-95; E-mail: stefanie.brenner@ikp-stuttgart.de
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diagnostic and/or therapeutic reasons. The age of patients ranged from 45 to 79 years; 16 patients were above 65 (median 70) and 25 patients were below 65 (median 54) years. Mean body mass index (BMI) was in the upper normal range (mean±SD: 27.8±4.5; median 27.6). Diagnosis of OA was based on the criteria of the American College of Rheumatology[19,20]. In addition to knee pain at least three of the following five characteristics had to be present: morning stiffness for less than 30 min, crepitus, bony tenderness, bony enlargement, no palpable warmth. Joint effusion was observed in 10 patients. In 14 patients taking NSAIDs (*N*=11) and/or corticosteroids (*N*=4) these medications were withdrawn at least 48 h (NSAIDs) or at least 1 week (corticosteroids) prior to arthroscopy. The characteristics of the patients are summarized in Table I. Written informed consent was obtained from each patient. The study was designed as an open, explorative, cross sectional trial and it was conducted according to the Declaration of Helsinki (as revised in 1996). The protocol was approved by the local ethics board (Landesärztekammer Baden-Württemberg, Stuttgart/Germany).

ASSESSMENT OF CLINICAL SYMPTOMS AND PAIN

For the assessment of the patients' pain, stiffness and physical function the German version of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used[17,21–23]. The WOMAC-index is a questionnaire consisting of three different subscales for the assessment of pain, stiffness and function of the affected knee. The individual scores range from 0 (most troubles) to 100 (no troubles). A Visual Analog Scale (VAS) with endpoint markings of 0 = 'no pain' and 100='worst pain that can be imagined' was used to assess pain in the affected knee at rest during the week before undergoing arthroscopy. In addition, a subscale of the WOMAC-index was taken to assess "WOMAC pain" which describes pain in every day's life activity of the patient (100=no pain, 0=worst pain). According to Kellgren and Lawrence[18,24] radiographic grading was performed in preoperative radiographs of the knee joints to assess the severity of OA in the affected knee (score 0=normal; 4=most severe). In order to avoid differences in the evaluation in each patient complete radiographic grading was performed by the same physician.

BIOLOGICAL SAMPLING

Before arthroscopy venous blood samples of all patients were collected. Analysis of CRP in serum was performed using heterogeneous sandwich enzyme immunoassay testing (Vitros Products Chemistry, Johnson & Johnson Clinical Diagnostics, Inc., Neckargemünd, Germany). Immediately prior to arthroscopy samples of undiluted synovial fluid were taken by puncture of the knee joint. During the therapeutic arthroscopy samples of synovial membrane typical for this disease were obtained as 'surgical waste'. For therapeutical and ethical reasons only small parts of damaged synovial membrane could be collected. The limited amounts of this material were not sufficient for the complete biochemical measurements in all patients. Synovial fluid and synovial membranes were immediately frozen in liquid nitrogen and stored at –80°C.

BIOCHEMICAL PARAMETERS

In an isotope dilution assay concentrations of PGE_2 and TxB_2 were determined in synovial fluid by using specific

Table I
Real-time PCR Amplicons

Gene (Accession NO.)	Forward primer	Taqman-Probe	Reverse primer
iNOS (NM_000625)	GGCTCGTGCAGGACTCACA	(FAM) ACCTCAGCAAAGCCCTCAGCAGCAT (TAMRA)	GAGCCTCATGGTGAACACGTT
COX-2 (NM_000963)	ACCCGGACAGGATTCTATGGA	(FAM) AACTGCTCAACACCGGAATTTTTGACAAGA (TAMRA)	ACTGTGTTTGGAGTGGGTTTCAG
TNF-α (NM_000594)	CTTCTCCTTCCTGATCGTGGC	(FAM) CGCCACCACGCTCTTCTGCCTGCT (TAMRA)	GGGTTTGCTACAACATGGGC
18S (X03205)	CGGCTACCACATCCAAGGAA	(VIC) TGCTGGCACCAGACTTGCCCTC	(TAMRA) GCTGGAATTACCGCGGCT

gas chromatography/triple stage quadrupole mass spectrometry (GC/MS/MS) as described by Schweer *et al.*[25]. Initially synovial fluid (500 µl) was diluted with water (500 µl) and deuterated internal standards (about 1 ng each) were added. Further sample cleanup and derivatization was performed as published recently[25].

Relative expression levels of IL-6, IL-1β, IL-1α, TNFα, COX-2 and iNOS were determined using Taqman-PCR techniques with RNA isolation from synovial membrane and reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA from synovial tissue was isolated combining phenol chloroform extraction (peqGold RNA pure, Peqlab, Erlangen, Germany) and High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany). The isolation was performed according to the manufacturers' instructions. Briefly, small pieces of synovial membrane were disrupted in 600 µl phenol reagent using a mixer mill MM300 (Retsch, Hilden, Germany) and 3 mm tungsten carbide beads. After homogenization 250 µl of chloroform was added and aqueous phase containing RNA was separated by centrifugation. The RNA containing solution was diluted with ethanol and loaded on affinity columns (Roche Diagnostics, Mannheim, Germany). The RNA isolation was performed following the supplier standard protocol including the specific DNase (Quiagen) digestion step to avoid genomic DNA contamination. Finally, RNA was eluted with 50 µl DEPC treated water (Ambion Inc., Austin, Texas, USA) and used as a template for cDNA synthesis. To generate cDNA, RNA was reversed-transcribed using RAV2-reverse polymerase (Amersham, Freiburg, Germany) and random hexanucleotide primers (Roche Diagnostics, Mannheim, Germany). Equal portions of the first strand synthesis reaction were used for the following quantitative PCR-analysis.

PCR reactions, using the ABI SDS 7900 HT instrument (Applied Biosystems, Foster City, CA, USA), were performed in a total volume of 25 µl in a 96 well plate. For all genes the final reaction mix contained: TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), forward and reverse primers at final concentrations of 0.9 µM for each primer, the corresponding probe at the final concentration of 0.2 µM. TaqMan primers and probes (Applied Biosystems, Foster City, CA, USA) for iNOS, COX-2, TNF-α, 18S were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA). This program selects primer and probes sets with optimized melting temperatures, secondary structure, base composition, and amplicon lengths. Sequences of primer and probes and the flurogenic labels are listed in the Table I. Primers and probes for IL-1α, IL-1β and IL-6 were acquired as Pre-Developed TaqMan Assay Reagents (Applied Biosystems, Foster City, CA, USA) and used according to the manufacturers' instructions. For all PCRs 10 ng cDNA was added to the reaction mix. A no template control (NTC) that contained all the above reagents except cDNA was included to detect the presence of contaminating DNA. All experiments were performed in triplicate. Amplification and fluorescence detection were conducted with a standard program of 40 cycles. A result was defined negative when no amplification occurred, i.e. the threshold cycle (Ct) value was greater than 40 cycles. For standardization of the gene expression level as determined by TaqMan analysis all PCRs were performed as multiplex reactions. 18S rRNA was quantified as an internal standard. Results are presented in relation to the expression of 18S rRNA (gene expression/18S expression×10^6).

For the detection of NO_2 and NO_3 aliquots of synovial fluid were centrifuged through a 10.000D ultrafree filter (BIOMAX 10, Millipore, Eschborn, Germany) to remove any hemoglobin due to erythrocyte lysis (4°C, 30 min, 10.000 g). $NO_{2/3}$ concentrations were determined using the Griess assay[26,27]: 80 µl of ultrafiltrate were used in the assay. NO_3 was reduced to NO_2 by mix 1 consisting of 0.08 U/ml nitrate reductase (Boehringer, Mannheim, Germany), 530 µM FAD (Sigma, Munich, Germany) and 83 µM NADPH (Sigma, Munich, Germany) in a total volume of 10 µl. Incubation was carried out 15 min at 37°C in a 96 well microtiter plate. Interfering NADPH was depleted 5 min at 37°C by mix 2 consisting of 1104 U/ml lactate dehydrogenase (Sigma, Munich, Germany) and 320 mM sodium pyruvate (Sigma, Munich, Germany) in a volume of 10 µl. The reaction was abrogated by the addition of 10 µl 1% sulfanilamide (Sigma, Munich, Germany) in 0.1N HCl and 10 µl 0.1% N-(1 naphthyl)ethylenediamine (Sigma, Munich, Germany). Following a 10 min incubation at room temperature in a microplate reader (Wallac, Turku, Finland) absorbance was read at 544 nm in reference to 690 nm. $NO_{2/3}$ concentrations were calculated by using a sodium nitrate standard curve (1–32 µM) in H_2O.

HISTOLOGIC GRADING

Samples of synovial membrane were intraoperatively collected, immediately fixed in formalin and embedded in paraffin according to standard procedures. Tissue sections (3–4 µ) were prepared from each specimen and stained with hematoxyline & eosin. The inflammatory reaction (acute or chronic) was classified as absent (=0), low (=1), moderate (=2) or strong (=3) as described previously[28,29]. A Histo score was formed by adding of the two values (acute+chronic inflammatory reaction).

STATISTICAL ANALYSIS

Results are presented as medians and arithmetic means±standard deviations (SD). Groups of patients were compared by using the Mann–Whitney U test. Correlations between the various biochemical markers were tested for significance by Spearman rank correlation test. Associations between clinical features (WOMAC-index, K&L grade) and markers of inflammation (PGE_2, $NO_{2/3}$ in SF; COX-2, iNOS in SM) were evaluated by multiple linear regression analysis taking into account the potential confounding factors/variables age and BMI. A result was considered to be significant if P was less than 0.05. All statistical analysis was performed using either GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego/California, USA) or statistical language R version 1.8.0 (http://www.r-project.org), respectively.

Results

CLINICAL PARAMETERS

The results of the clinical assessment prior to arthroscopy are summarized in Table II. Radiographic grading of OA correlated significantly (P=0.005) with the age of the patients. There were no significant differences in pain scores when patients were divided into drug-free subjects and patients in whom medication was withdrawn prior to arthroscopy. In 33 of the 41 examined knees (80.5%) concomitant meniscopathy could be observed during arthroscopy.

Table II
Baseline characteristics of the studied patients with OA

	age [years]	BMI	K&L-grade	WOMAC-index*	WOMAC-pain*	VAS-pain*	CRP [μg/ml]	Histo score
Adult patients	63	33.6	3	35.4	35	40	1.1	
(*N*=25)	48	29.8	3	90.6	70	0		2
	54	43.3	2	20.8	30	27	0.7	1
	54	27.1	3	62.5	65	12	0.6	1
	50	23.5	1	71.9	55	0		2
	49	25.2	3	57.3	40	0	0.45	1
	62	23.7	2	65.6	70	0	0.9	4
	63	30.4	2	51.0	55	0	0.7	3
	61	28.7	1	75.0	90	0	0.45	1
	62	21.9	1	61.5	60	50		1
	45	23.6	3	57.3	65	0	0.45	1
	52	30.7	2	81.3	75	10	0.6	1
	46	29.3	1	71.9	65	40	0.9	
	47	23.4	2	62.5	100	0	0.8	3
	49	23.8	0	43.8	50	10	0.45	2
	52	27.3	2	53.1	45	30	2.2	
	46	23.2	1	41.7	60	25	0.7	1
	63	28.4	1	68.8	45	0	0.45	2
	64	28.3	2	68.8	65	15	0.45	3
	61	35.6	1	58.3	65	25	0.45	1
	51	25.0	1	61.5	75	0	0.45	1
	48	30.0	3	40.6	65	0	0.45	0
	59	30.0	3	41.7	45	0	0.45	1
	59	25.6	3	40.6	60	25	0.45	
	60	25.9	1	31.3	35	20	0.6	1
Median	54	27.3	2	58.3	60	10	0.53	1.00
Mean		27.9	1.9**	56.6	59.4	13.2	0.67	1.57
SD		4.7	0.9	16.6	16.7	15.6	0.39	0.98
Elderly patients	78	27.7	3	78.1	95	0	2.1	2
(*N*=16)	78	23.8	3	70.8	60	0	3.4	3
	78	23.4	4	43.8	50	32	0.9	2
	69	27.6	2	50	65	0	1.1	1
	66	35.8	3	32.3	35	0	1.4	1
	67	26.0	1	57.3	60	0		2
	67	32.2	3	79.2	80	0	0.45	1
	66	29.2	3	52.1	40	50	0.45	1
	70	23.4	2	32.3	30	25	0.45	2
	70	27.2	3	66.7	75	0	0.8	2
	68	35.8	3	29.2	40	50	0.45	0
	74	27.8	3	43.8	35	0	5.9	5
	75	28.2	3	45.8	75	0	0.5	2
	66	30.0	3	53.1	45	0	0.45	1
	71	24.9	4	67.7	65	45		3
	79	19.6	3	71.9	65	0	0.45	1
Median	70	27.6	3	52.6	60	0	0.55	1.00
Mean		27.7	2.9**	54.6	57.2	12.6	0.93	1.68
SD		4.4	0.7	16.4	18.8	20.2	1.05	1.06

*For scoring see Methods (page 2).
** P=0.0023.

MARKERS OF INFLAMMATION

Concentrations of PGE_2, TxB_2 or $NO_{2/3}$ in synovial fluid and relative expressions of IL-6, IL-1β, COX-2 and iNOS in synovial membranes are summarized in Table III. TNFα and IL-1α were not detectable in any sample of the synovial membranes. When patients were divided according to their age (limit 65 years) into two groups no significant differences could be observed and only expression of COX-2 in synovial membrane correlated significantly with the age of patients (P=0.01). For the various biochemical markers of inflammation several linear correlations were noted: between PGE_2 and TxB_2, iNOS, IL-1β, CRP or IL-6 (r=0.60, P=0.0001; r=–0.53, P=0.04; r=0.69, P=0.005; r=0.39, P=0.03 or r=0.69, P=0.003, respectively); between TxB_2 and IL-6 or IL-1β (r=0.65, P=0.007 or r=0.57, P=0.03, respectively); between IL-6 and IL-1β (r=0.73, P=0.001) or CRP (r=0.60, P=0.04).

When concentrating on the most relevant clinical tools (e.g. K&L grade and WOMAC-index) and endogenous metabolites of inflammation (e.g. PGE_2 and $NO_{2/3}$ in synovial fluid) or the corresponding synthesizing enzymes (COX-2 and iNOS in synovial membranes), respectively, multiple regression analysis revealed a significant (P=0.035) linear correlation only between the WOMAC-index and PGE_2 if corrected for age and BMI of the patients (Pearson's partial correlation coefficient r=0.36). If the variables BMI and age were not included into the multivariate analysis the correlation was of borderline significance (Pearson's correlation coefficient r=0.30; P=0.075).

Table III
Biochemical parameters of studied patients with OA

		PGE_2 [ng/ml]	TxB_2 [ng/ml]	$NO_{2/3}$ [μM]	iNOS	COX-2	IL-1β [rel. expr. X/18S]	IL-6
		in synovial fluid			in synovial membrane			
Age		*N*=37	*N*=37	*N*=34	*N*=16	*N*=16	*N*=16	*N*=17
All patients	Median	0.13	0.14	2.69	0.70	0.25	0.39	0.36
	Mean	0.46	1.12	3.42	0.79	0.35	0.44	2.41
	SD	1.10	3.25	2.00	0.39	0.30	0.53	6.01
Below 65 years	Median	0.11	0.17	3.05	0.77	0.18	0.21	0.47
	Mean	0.34	1.61	3.63	0.87	0.22*	0.48	0.76
	SD	0.54	4.00	1.93	0.30	0.10	0.66	0.75
Above 65 years	Median	0.13	0.14	2.37	0.57	0.64	0.40	0.36
	Mean	0.70	0.21	3.02	0.64	0.58*	0.38	5.44
	SD	1.73	0.23	2.16	0.51	0.39	0.26	9.87

* *P*=0.07. PGE_2=Prostaglandin E_2; TxB_2=Thromboxan B_2; $NO_{2/3}$=nitric oxide; iNOS=inducible nitric oxide synthase; COX-2=cyclooxygenase-2; IL-1β=interleukin-1β; IL-6=interleukin-6; the two age groups were compared by the nonparametric Mann–Whitney-test.

Discussion

In the present cross sectional study we have tested the hypothesis whether in OA inflammation plays a substantial role in the development of pain and whether the clinical state of this disorder is related to biochemical alterations at the affected site.

Eicosanoids, such as PGE_2 or TxB_2 are regarded as markers of (local) inflammatory processes[30,31]. According to literature data[32] levels of PGE_2 in synovial fluid of patients with OA vary widely (22–2280 pg/ml) which could be confirmed by our measurements (38–6380 pg/ml) applying a highly selective GC/MS/MS – assay. There is some discrepancy to other authors[30,33,34] which might be due to differences in study design, sampling techniques or less specific analytical methods. Furthermore, low (20–260 pg/ml) or even undetectable concentrations of TxB_2 have been reported[15,31]. Similarly in our NSAID-free patients those levels were in general low (23–943 pg/ml) except for three patients exhibiting concentrations above this range (7230; 11400 and 15500 pg/ml) which could not be explained by special clinical, histological or radiological findings.

COX, especially inducible COX-2 represents a key enzyme for inflammation[35]. COX-2 has been semiquantified by immunostaining in synovial blood vessels, lining cells and sublining layer in different populations including patients with OA[36]. In synovial tissue of knees from patients with OA we found a relative COX-2 expression of 0.35±0.30 (mean±SD).

NO represents another marker of inflammation and cartilage destruction[7,37]. In synovial fluid of patients with OA higher levels of NO compared to normal joints have been described[14,38,39]. In general, mean concentrations of 9.1 μM and 4.7 μM have been reported[40,41] which are in good agreement to our data (3.4±2.0 μM). Likewise, elevated levels of iNOS protein have been found in joints of patients with OA, even though sometimes iNOS could not be identified in all assessed samples[40,42]. In our investigations we detected iNOS in all assessed synovial membranes. NO production and expression of NOS were absent in normal cartilage because both depend on stimulation by cytokines[8]. It has been proposed that inhibition of NO synthases will decrease the severity of OA[7,43]. However, in animal models arthritis can be worsened by NOS-inhibitors[44] questioning the potential of this therapeutic option.

Proinflammatory cytokines have been measured primarily in synovial fluid and not in synovial membranes. As different methods have been used literature data are quite variable[5,45,46]. We could not detect TNFα and IL-1α in any synovial membrane sample. Similarly, in synovial fluid TNFα was identified only in 2 of 30 patients with OA[30]. In contrast, IL-1β was detectable in 14 from 16 synovial membranes and IL-6 was present in all samples tested. According to Smith et al[5]. IL-1α, IL-1β and TNFα were present in synovial membranes and the most marked changes were seen in late states of OA.

As prevalence and severity of OA is higher in elderly patients[1–3] we divided our patients into two subgroups (below/above 65 years) and found a trend (*P*=0.07) for a higher relative expression of COX-2 in advanced age (see Table III). Therefore it was not too surprising that between both variables a significant (*P*=0.01) linear correlation existed. Thus, ageing might be a confounding factor when this parameter is compared in different populations. Likewise, a significant (*P*=0.005) linear correlation between age and K&L-grade could be observed. As the radiographic scoring can be used for characterizing a more severe OA the age-dependent increase of the K&L-grade is in line with the above epidemiological observations.

Independent of the age of the studied patients we and others found quite variable concentrations of the assessed biochemical parameters in the various biological samples. In addition, when dividing patients according to the median WOMAC-score of 57 or according to the K&L grade below and above three into two categories of disease severity no marked differences in the assessed inflammatory markers were obvious. Likewise, K&L score did not significantly correlate with clinical findings in another study[47].

In respect to our working hypothesis we could find a significant (*P*=0.035) relationship only between the WOMAC-index and PGE_2 if putative confounding factors such as age and BMI were taken into considerations. Otherwise no significant linear correlations could be found between local biomarkers (e.g. PGE_2, COX-2, $NO_{2/3}$, iNOS) and clinical parameters (e.g. WOMAC-index, K&L-grade).

In accordance with our findings Nishimura et al.[48] did not observe a significant correlation between concentrations of IL-1β, IL-6 or TNFα in synovial fluid and degeneration of articular cartilage or internal derangements in temporomandibular joints of patients with OA. Furthermore, in other

studies no correlation could be demonstrated between concentrations of IL-1β, IL-6 or TNFα in synovial fluid of temporomandibular joints and age, radiographic findings or width of mouth opening[46,49]. Borderie *et al.*[42] could not find in OA of the knee any correlations between iNOS, IL-1ß, TNFα in synovial fluid and clinical signs. From all this data one could conclude that apparently no direct relationships exist between the local levels of the various biochemical parameters and the severity/ symptoms in OA of the knee which probably is due to the more local, mainly mechanical injury with little systemic upset.

It has been postulated that prostaglandins provoke hyperalgesia by sensitizing pain receptors[50–52]. Our study indicates no significant associations between pain, other clinical or radiographic signs and PGE_2 or cytokines. This would suggest that pain in OA is not provoked primarily by inflammation but by different mechanisms such as degenerative processes, osteophyte growth with stretching of periosteum, patella cartilage volume, raised introsseous pressure, microfractures, ligament damage, capsular tension and meniscal injury[53,54]. However, based on the cross sectional character of our study we could not address the interesting question whether the assessed markers of inflammation are predictors for the progression of OA. In addition, due to the restricted sample sizes especially in the case of the synovial membranes it might be possible that we have missed some weak associations.

In conclusion, the present study explored in a limited number of patients with OA different biochemical changes in joints and their relation to clinical and radiographic signs. For the first time a large variety of biochemical markers has been determined concomitantly. From these extensive assessments it is obvious that in OA of the knee no direct relationships exist between the local levels of typical mediators of inflammation and the clinical state of the disorder. The degenerative condition of this joint disease is apparently not associated with alterations of the monitored markers of inflammatory processes. However, it remains to be proven by a longitudinal study whether inflammatory markers could serve as a predictor of a more rapid progression of OA.

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